final report

Project code: B.NBP.0659

Prepared by:
Michael McGowan
Laercio Portoneto
Gry Boehansen
Gabriel Bo
Sophia Edwards (Butler)
Kerri Chandra
Brian Burns
Peter Atkinson
Di Vankan
Nana Satake
Geoff Fordyce
Ian Braithwaite

Date published: November 2015

ISBN: 9781741919899

PUBLISHED BY:
Meat and Livestock Australia Limited
Locked Bag 1961
NORTH SYDNEY NSW 2059

Strategies to increase the adoption of AI in northern Australian tropical beef genotype herds

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government to support the research and development detailed in this publication.

Abstract

Artificial insemination (AI) provides a practical means by which improved genetics can be disseminated rapidly and efficiently throughout a beef cattle population. The problem in northern Australia is that AI is estimated to be utilised in less than 1% of extensively managed bull breeding herds. The objectives of this project were to develop optimum methods of synchronising ovulation in rising 2–year-old Brahman heifers, investigate practical methods of increasing the proportion of heifers cycling at the commencement of an AI programme, and improve the ability of producers to select heifers likely to become pregnant to fixed-time AI (FTAI). Replacing oestradiol benzoate with gonadotrophin releasing hormone to synchronise follicular wave emergence and ovulation resulted in poorer pregnancy rates to FTAI. Key factors affecting the likelihood of heifers becoming pregnant to FTAI were presence of a corpus luteum on the ovaries at the time of commencement of treatments to synchronise ovulation and the inherent fertility of the heifers. Becoming pregnant to FTAI was shown to be a heritable trait.

Executive Summary

Artificial insemination (AI) provides a practical means by which improved genetics can be disseminated rapidly and efficiently throughout a beef cattle population. The problem in northern Australia is that AI is estimated to be utilised in less than 1% of extensively managed bull breeding herds. The main reasons being historically poor outcomes, management of programs over the wet season, provision of fodder in extensive areas and poor fertility rates in young lactating breeders. A strategy to increase adoption of AI in these extensive herds is routine utilisation of technology to synchronise ovulation to enable fixed-time AI (FTAI) in maiden heifers, as it eliminates the requirement for oestrous detection, alleviates issues of sourcing suitable young cows with calves at foot, and decreases generation interval.

The objectives of this project were to develop optimum methods of synchronising ovulation in rising 2–year-old Brahman heifers, investigate practical methods of increasing the proportion of heifers cycling at the commencement of an AI programme, and improve the ability of producers to select heifers likely to become pregnant to FTAI. Heifers were studied because they are the group of cattle in most herds that are separately managed and control mated even in continuously joined herds. Further, Brahman heifers were studied as previous research has demonstrated that 100% *Bos indicus* heifers have the highest risk of adverse responses to hormonal treatments to synchronise ovulation, and thus if improved protocols could be developed for this genotype then these would likely result in improved pregnancy rates to FTAI in other *Bos indicus* derived cattle. A series of studies were conducted on co-operating commercial beef cattle properties across southern, central and north Queensland and at one research station.

Although replacement of oestradiol benzoate (ODB) with gonadotrophin releasing hormone (GnRH) in a standard intravaginal progesterone releasing device (IPRD) protocol to synchronise ovulation resulted in a similar pattern of follicular growth and ovulation, the pregnancy rate to FTAI in the GnRH treated heifers was significantly lower than that in the ODB treated heifers (29.5% v's 40.7%, respectively). A new more practical ODB-IPRD protocol was developed and field evaluation demonstrated that the pregnancy rate to FTAI was at least as good if not better than the standard protocol (standard v's modified 37.6 v's 40.0% respectively). The new protocol employed a shorter period of IPRD insertion (6 rather than 8 days) and the timing of the ODB treatment after IPRD removal enables AI to be done in the early morning.

Heifers that had a corpus luteum (CL) on their ovaries at the commencement of treatments to synchronise ovulation achieved significantly higher pregnancy rates to FTAI than those heifers that did not have a CL (36.7% v's 29.0%). The use of a biostimulation treatment or yard-handling of the heifers prior to commencement of treatment to synchronise ovulation did not improve pregnancy rate to FTAI. Further, despite there being significant variation in live-weight of heifers at the time of commencement of treatments to synchronise ovulation (range 280 to 440+ kgs) there was no significant relationship between live-weight and pregnancy rate to FTAI.

Of critical importance was the demonstration that the pregnancy rate during the first 6 weeks of bull mating of heifers accurately predicted the pregnancy rate to FTAI; i.e., the inherent fertility of the heifers is likely to influence to a degree the outcome of AI. Using the data from this project the estimated heritability of becoming pregnant to FTAI was 18%. The finding that this new trait is heritable supports selection of heifers conceiving to FTAI into a property's bull breeding herd.

Overall, this project evaluated a range of modifications to the existing standard protocol for synchronisation of ovulation in Brahman heifers and identified important factors affecting pregnancy rate after FTAI. In herds with a history of selection for fertility, application of the FTAI programme protocols recommended from the findings of this project will consistently result in pregnancy rates to FTAI of 40 to 43%. Further, it was demonstrated that producers can conduct FTAI of large numbers of heifers (400-600) on a single day with relatively simple modifications to existing yard infrastructure that enables two experienced technicians to AI cattle simultaneously. Finally, during the course of the project there was opportunity to evaluate the outcome of FTAI of lactating Brahman cows on three commercial properties. Pregnancy rates to FTAI ranged between 48% to 55% demonstrating that where producers can access and manage lactating cows during the wet season, very acceptable pregnancy rates to FTAI can be achieved. Selection of replacement bulls from these cows is likely to improve herd fertility over the long term.

Table of Contents

1 Background

The northern Australian beef industry is currently facing a series of environmental, social, and economic challenges that require significant fundamental change in the industry. Poor reproductive performance in extensively managed tropically adapted herds has been identified as an important contributing factor (McCosker et al., 2010) to poor financial performance. Research has demonstrated large genetic variation in reproduction traits of Brahman genotypes, which implies substantial opportunity for improvement through genetic selection (Johnston et al., 2009).The challenge for the northern beef industry is to disseminate identified superior genetics as efficiently and as expediently as possible so that the industry can deal with the economic challenges it is facing.

The northern female breeding herd predominantly consists of Brahman and Brahman-cross cattle (Burrow et al., 2003), due to their inherent ability to withstand extreme environmental temperatures and parasites, and their ability to utilise poor quality pastures (Burns et al., 2010). However, the *Bos indicus* adaptive advantage is often accompanied by later onset of puberty and prolonged post-partum anoestrus—characteristics that are detrimental to reproductive performance (Burns et al., 2010, Sartori et al., 2010). Commercial producers are increasingly seeking Brahman sires with high genetic merit for female fertility and carcass traits. There is also an increasing demand for lower *Bos indicus* content, 'flat-back' cattle to improve market access, as well as polled genotypes to address welfare and occupational health and safety concerns. Genetic tools are currently available or will become available in the near future to enable selection for polledness, selection for improved fertility, and selection for carcass traits in tropically adapted cattle (Mariasegaram et al., 2012).

Artificial insemination (AI) provides a practical means by which these improved genetics can be disseminated rapidly and efficiently throughout the population (Edwards et al., 2015, Edwards et al., 2013a). It has recently been estimated that the improvement in genetic merit in a Brahman herd of average genetic merit (Jap Ox Index of $20) was $1275 vs. $237 (Sire Jap Ox Index – $20/2 = Calf Genetic Improvement) where semen from a bull in the top 10% for Brahman Jap Ox Index was used for fixed-time AI (FTAI) versus use of this bull for natural mating, respectively (Edwards et al., 2013a). The problem in Northern Australia is that AI is estimated to be utilised in less than 1% of extensively managed northern breeder herds.

Cattle in northern Australia are generally extensively managed on large properties in remote locations that have limited access to skilled labour, 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. A strategy to increase adoption of AI in northern beef herds is FTAI, as it eliminates the requirement of oestrus detection and enables AI of large numbers of females (400-600) on a single day; therefore, the overall production of calves is greater than typical oestrus detection AI (ODAI) programmes (Edwards et al., 2012). The success of an AI program is often judged on the number of females pregnant after one insemination and the cost per calf produced. A central QLD study involving rising 2-year-old Brahman heifers demonstrated that the cost per calf by FTAI was $23.70 lower compared to that by ODAI (Edwards et al., 2015). Nevertheless, the adoption of FTAI in the northern beef industry is lagging and this is largely due to the lower than expected pregnancy rates (mean 40%) in *Bos indicus* heifers after FTAI. Natural mating conception rates per oestrous cycle can assist in benchmarking expectations of pregnancy rates from FTAI. Fordyce et al. (2005) calculated per oestrous cycle pregnancy rates in 2-year-old Brahman heifers at several sites in QLD to be between 40 and 70%. It is important for producers to understand that use of FTAI will not increase the probability of conception and that generally pregnancy rates per AI will be higher when performed following detection

of oestrus compared to FTAI. However, the major advantage of FTAI is that all female are inseminated rather than only those detected on heat, and thus the total number of calves sired by AI is often higher following use of FTAI (Edwards et al., 2015). In some cases, a combination of heat detection and then FTAI of all those females not detected on heat can be used (Phillips et al., 2006).

To enable FTAI, females must be treated with a series of hormones to synchronise and induce ovulation. *Bos indicus* cattle have a unique reproductive physiology compared with their *Bos taurus* counterparts, which needs to be considered when recommending the most appropriate ovulation synchronisation protocol (Carvalho et al., 2008). For example, Brahman cattle commonly attain puberty at a later age than *Bos taurus* genotypes (Chenoweth, 1994, Johnston et al., 2009). As a result, the proportion of 2-year-old Brahman heifers in northern Australia that have a corpus luteum (CL) at commencement of breeding has been reported to be 43% compared with 63% in tropical composites (50% *Bos taurus*, 50% *Bos indicus*) (Johnston et al., 2009). 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 $PGF_{2\alpha}$ alone for oestrus synchronisation is ineffective in pre-pubertal heifers as a prerequisite for its use is the presence of a responsive CL (Aguer, 1991).

Heifers are generally preferred for FTAI in northern Australia as they are often controlled mated and issues with managing a cow and calf unit are avoided. Current best practice protocols to synchronise ovulation in *Bos indicus* heifers in Australia incorporate the use of an intravaginal progesterone (P_4) releasing device (IPRD), usually for 8 days, and two oestradiol benzoate (ODB) treatments (Bo et al., 2003), one at the time of IPRD insertion to synchronise follicular wave emergence and a second after IPRD removal to induce synchronised ovulation of developing follicles. Typically the second ODB treatment is administered at 24 h post IPRD removal and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) treatment, and FTAI is conducted 54 h after IPRD removal (Bo et al., 2003). The reported pregnancy rate to FTAI of Brahman heifers treated with this protocol in northern Australia ranges between 31 to 44% (Butler et al., 2011a, Phillips et al., 2010, Cavalieri et al., 2002). Historically, treatment of Brahman heifers with Norgestomet subcutaneous implants instead of IPRD's has been shown to result in very similar pregnancy rates to FTAI, however these implants are no longer commercially available in Australia. Treatment of heifers with progesterone (P_4) has been shown to advance the onset of puberty (Polat et al., 2009); therefore, pre-pubertal 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. Overall, these synchronisation protocols result in about 75% of Brahman heifers synchronously ovulating and developing a normal CL (Butler et al., 2011c).

A contributor to the poor pregnancy rates to FTAI in *Bos indicus* heifers is the failure to grow a dominant follicle that will result in a fertile ovulation after ovulation synchronisation (Carvalho et al., 2008, Butler et al., 2011c). A series of studies conducted in northern Australia (Butler et al., 2011c, Edwards et al., 2013b), Brazil and Argentina (Carvalho et al., 2008), and the United States (Bridges et al., 2008) have concluded that *Bos indicus* heifers are more sensitive to hormone treatments, and the timing and type of hormonal treatment needs to be modified to increase the proportion of heifers that ovulate a well-developed follicle to enable satisfactory rates of conception.

One strategy to improve follicular growth could be to increase the interval from IPRD removal to ovulation (IPO). Holstein heifers treated with a gonadotrophin releasing hormone (GnRH) and $PGF_{2\alpha}$ -based synchronisation protocol achieved 80.1% maximum probability of pregnancy to FTAI when the IPO interval was 3 days as compared to approximately 65%

when the interval was only 2 days (Colazo and Ambrose, 2011). The interval from ODB treatment to LH surge has been reported to be 19.8 ± 1.5 h in Brahman (*Bos indicus*) heifers (Rhodes et al., 1978), indicating an approximate IPO interval of 44 h, but there is currently limited information for *Bos indicus* heifers on the use of ODB or IPRD ovulation synchronisation protocols with an extended IPO. Current ovulation synchronisation protocols may be inducing ovulation too early and consequently not permitting the ovulatory follicle to reach maximum growth and maturation prior to induction of ovulation. A further limitation to current ovulation synchronisation protocols for *Bos indicus* heifers is that there is evidence that treatment with IPRDs and ODB can cause ovarian dysfunction in some heifers (Butler et al., 2011c). In Brahman heifers the combination of IPRD and ODB treatment at the time of IPRD insertion markedly decreases endogenous follicle stimulating hormone (FSH) secretion (Edwards et al., 2013b).

Administration of ODB in conjunction with IPRDs induces follicular atresia, which in turn stimulates the emergence of a new follicular wave, resulting in a newly emerged developing follicle at the time of IPRD removal (Bo et al., 1995a, Adams, 1998). Although not significant, a general trend towards a larger diameter dominant follicle and lower prevalence of ovarian dysfunction was observed when Brahman heifers were treated with a double $PGF_{2\alpha}$ protocol (Butler et al., 2011c). These findings suggest that a further modification to the current 'best practice' ovulation synchronisation protocols mat be warranted. For example, omitting the ODB treatment at the time of IPRD insertion may reduce the magnitude of FSH suppression, and thus improve follicular development and reduce the risk of ovarian dysfunction. A shortened duration of IPRD insertion may also be necessary to reduce the risk of ovulating an aged oocyte (Bo et al., 1995a). Alternatively, administration of exogenous FSH close to the time of follicular wave emergence (which has been shown in Brahman heifers to be 4.3 days after ODB and IPRD insertion (Bo et al., 2003, Butler et al., 2011c)) may be effective in improving follicular diameter at ovulation.

A global increase in public awareness of the use of oestrogenic compounds in food producing animals has led to the ban of oestradiol for ovulation synchronisation of cattle in Europe and New Zealand (Lane et al., 2008). In Australia, the use of ODB for ovulation synchronisation has been electively banned in the dairy industry in lactating cows. Although use of ODB is currently available for use in beef cattle, it is realistic to assume that a ban could be imposed in the future. The most suitable alternative to ODB in ovulation synchronisation protocols is gonadotrophin releasing hormone (GnRH). Although GnRH has been routinely used in *Bos taurus* beef and dairy genotypes, resulting in acceptable pregnancy rates to FTAI, there is limited published information on the ovarian response of *Bos indicus* genotypes to GnRH-based ovulation synchronisation protocols, or on how GnRH compares to ODB in ovulation synchronisation protocols. Therefore, it is difficult to assess whether a ban on the use of ODB for ovulation synchronisation in north Australian beef cattle will negatively impact the success of FTAI programs.

Treatment of cattle with GnRH stimulates a surge in luteinising hormone (LH) to facilitate the emergence of a new follicular wave or to induce ovulation in LH receptive follicles. Studies investigating GnRH-based ovulation synchronisation protocols have found that response to the first GnRH treatment at IPRD insertion did not affect the pregnancy outcome in Holstein heifers (Colazo and Ambrose, 2011). However, the endocrine response to GnRH differs between genotypes, with increasing *Bos indicus* content resulting in decreasing concentrations of LH and a reduction in the interval from the peak in LH to ovulation. In one study, Angus (*Bos taurus*) heifers secreted a mean peak LH concentration of 7.0 ± 0.8 ng/ml as compared to 4.6 ± 0.8 ng/ml and 2.9 ± 0.8 ng/ml in Brangus (5/8 Angus) and Brahman x Angus heifers ($P<0.05$), respectively (Portillo et al, 2008). Dias *et al*. (2010) found that increasing doses of GnRH from 100 to 200 µg altered the concentration of the induced LH

surge (9.8 ± 1.4 and 14.8 ± 2.1 ng/ml; P≤0.01, respectively), but there was no change in ovulation rate (8/8 and 7/8; P=0.4, respectively). In addition to this physiological difference, Saldarriaga *et al*. (2007) reported that 40% of Brahman x Hereford cows failed to develop a synchronised follicular wave after treatment with a GnRH + IPRD ovulation synchronisation programme, resulting in poorer than expected pregnancy rates to FTAI (39%). The literature suggests that further information is required to categorise the ovarian and endocrine response of *Bos indicus* heifers to GnRH-based ovulation synchronisation programmes. Specifically, the time of ovulation in a GnRH protocol needs to be determined so that the timing of ovulation induction is optimal to reduce the prevalence of ovulation of inadequately grown follicles (Sá Filho et al., 2010, Sa Filho et al., 2009).

In the dry tropical rangelands of northern Australia—where seasonal rainfall can vary greatly—ensuring that the majority of heifers are sufficiently well-grown to have reached puberty prior to commencement of treatments to synchronise ovulation can be challenging. Biostimulation—defined as the stimulatory effects of males on female cyclic activity through genital stimulation, pheromones or other less defined external cues (Fiol and Ungerfield, 2012)—has been found to reduce the age at which heifers attain puberty (Fiol et al., 2010) and improve pregnancy rates after treatment to synchronise oestrus/ovulation (Fiol and Ungerfield, 2012). Positive responses to biostimulation appear to be more consistently observed in post-partum cows than prepubertal heifers and reported biostimulation protocols vary greatly. The efficacy of biostimulation for improving pregnancy rates in extensively managed *Bos indicus* heifers in northern Australia has not been investigated, but presents a viable alternative strategy for stimulating the onset of puberty prior to FTAI in these animals.

Stressors are also known to adversely affect ovulation through suppression of lutenising hormone secretion (Dobson and Smith, 2000). In northern Australia replacement heifers are handled at the time of weaning and then may only be handled one more time before they are submitted for mating. To use FTAI in heifers requires handling the females through the cattle yards and crush 4 to 5 times over a 2 week period, which can be stressful for animals not used to being handled. One potentially practical way of reducing the risk of handling-induced stress and the subsequent negative impact on ovulation is to expose replacement heifers to low stress yard handling at weaning and/or handle them through the yards using ‘low stress handling’ techniques several weeks prior to commencement of treatments to synchronise ovulation.

In addition to protocol modification and strategies to induce puberty and reduce stress, the success of FTAI in Brahman cattle could also be improved through the development of selection criteria that can predict those heifers most likely to become pregnant after FTAI and therefore help with the selection of females to submit to this type of program. Age at puberty is known to impact reproductive performance in *Bos indicus* cattle and current strategies to decrease age at puberty in heifers include improved nutritional management (Gasser et al., 2006) and genetic selection for earlier age at puberty (Nogueira, 2004). Genome wide association studies (GWAS) offer a valuable tool to gain insight into trait architecture or candidate loci for any phenotypic trait that can be defined and scored across many individuals. GWAS are particularly valuable for detecting genetic variants associated with complex traits. With recent developments in platforms for high density genotyping and the commercial availability of high density SNP panels, GWAS are increasingly used to understand the genetics contributing to phenotypic traits of economic importance. Reproductive traits that have been investigated in *Bos indicus* females include post-partum anoestrus, post-partum ovulation, and age at puberty (Hawken et al., 2012, Fortes et al., 2012a, Fortes et al., 2014). The identification of genetic markers linked to these traits is an important strategy for genetic improvement of cow reproductive performance. GWAS analysis of FTAI outcomes have not been undertaken to date; such studies could help

unravel the genetic architecture of this trait and inform attempts to select animals for improved FTAI outcomes.

1.1 Economic evaluation of FTAI for genetic improvement

An in-depth assessment of the economic impact of using FTAI in a genetic improvement program for northern Australian beef herds was undertaken as a preliminary justification to underpin the project objectives (Edwards et al., 2013a; Appendix I).

The potential return on investment of implementing a genetic improvement program in a self-replacing commercial Brahman breeding herd was evaluated using three different selection and breeding strategies: Natural mating with no genetic improvement (NATM-G), Natural mating with genetic improvement (NATM+G), and Fixed-time AI (FTAI) with genetic improvement (FTAI+G). For each strategy, the Jap Ox Index was used to quantify the genetic merit of sires and genetic gain (ABRI, 2013). Genetic improvements were made using a Brahman sire with a top 10% Jap Ox Index ($45). In each strategy, sires were selected from the progeny generated in Year 1, and then used in Year 3 for natural mating in a multiplier herd. The cows mated in each strategy were all assumed to have a breed average Jap Ox Index ($20). Assumptions for purchase of sire and frozen semen, pregnancy rate to FTAI, overall weaning rates, and costs of FTAI in a 200 cow breeding herd were based on realistic industry averages.

A partial budget was used to calculate the cost per calf weaned. The costs per calf weaned in Year 1 were calculated to be $46.83, $371.42 and $173.76 for NATM-G, NATM+G and FTAI+G, respectively. The Jap Ox Index for the progeny was calculated to be $20.00, $32.50 and $32.50 for NATM-G, NATM+G and FTAI+G, respectively. However, when progeny from Year 1 were used in Year 3 for breeding, the costs per calf weaned in Year 3 were calculated to be $46.83, $10.27 and $4.35 for NATM-G, NATM+G and FTAI+G, respectively. In Year 3, total genetic profit was calculated to be $0, $237.25 and $1275.00 for NATM-G, NATM+G and FTAI+G, respectively.

This model supports the return on investment from using FTAI for genetic improvement in Brahman cattle in northern Australia. By demonstrating the value of FTAI for both disseminating improved genetics and improving rate of genetic gain, this model justifies further research into improving FTAI protocols for *Bos indicus* cattle in northern Australia, and selecting females likely to succeed in FTAI programs.

2 Project objectives

The overall aims of the project were to develop practical, effective, low-cost strategies to increase the adoption of AI in northern Australia, and thus facilitate rapid dissemination of superior genetics for genetic improvement in northern Australian beef herds.

The three main objectives of the project—which targeted improving ovarian function, reducing the age of puberty, and identifying selection criteria for heifers likely to succeed in fixed-time AI (FTAI) programs—were as follows:

Objective 1: Develop modifications to ovulation synchronisation protocols to improve the growth and development of the pre-ovulatory follicle and subsequent growth and function of the corpus luteum (CL).

Objective 2: Investigate the role of practical management tools to improve conception rates in *Bos indicus* maiden heifers such as exposure to intact bulls prior to, and during FTAI programs and low stress stock handling prior to commencement of the program.

Objective 3: Investigate whether the use of genetic markers for fertility and evaluation of flight speed can be used to identify *Bos indicus* heifers more likely to conceive to FTAI.

2.1 Studies to achieve project objectives

Five separate studies were originally planned to satisfy the objectives of the project. Brahman heifers were studied as previous research has demonstrated that 100% *Bos indicus* heifers have the highest risk of adverse responses to hormonal treatments to synchronise ovulation, and thus if improved protocols could be developed for this genotype then these would likely result in improved pregnancy rates to FTAI in other *Bos indicus* derived cattle.

Objective 1: Develop new synchronisation protocols to improve development and growth of pre-ovulatory follicles

Study 1: Conduct a pilot Study to investigate the following:

a) The ability of low-dose follicle stimulating hormone (FSH) to override the suppression of natural FSH secretion from treatment with an intravaginal progesterone releasing device (IPRD).
b) The follicular dynamics in Brahman heifers treated with various combinations of gonadotrophin releasing hormone (GnRH) and/or oestradiol benzoate (ODB) in conjunction with an IPRD, without treatment to induce ovulation.

The results of Study 1 were expected to inform the design of Study 2.

Study 2: Compare the effects of GnRH as a replacement for ODB in IPRD-based ovulation synchronisation programs on pregnancy rates in *Bos indicus* heifers.

This study also aimed to compare pregnancy rates in FTAI heifers and naturally mated heifers on the same properties, and to measure flight speed.

The results of Study 2 were expected to inform the design of Study 3.

Study 3: Investigate whether a reduction in the duration of IPRD insertion and an increase in interval from $PGF_{2\alpha}$ treatment to time of AI will improve development of the pre-ovulatory follicle and pregnancy rate in *Bos indicus* heifers. The planned control group for this experiment was the 7 day IPRD exposure, GnRH treatment schedule from Experiment 2, with a 5 day IPRD exposure as the treatment group. Both treatment and control groups were to be further divided into FTAI at 60 h or FTAI at 72 h post IPRD removal.

The results of Experiments1-3 were expected to inform the design of Experiment 4.

Objective 2: Biostimulation as a management strategy to improve pregnancy rates to FTAI

Study 4: Investigate the effects of biostimulation for 21 days prior to commencement of oestrus synchronisation treatments on pregnancy rates following FTAI in *Bos indicus* heifers.

Collect concurrent data in experimental animals on factors that are known to affect pregnancy rates to FTAI—such as liveweight and body condition score of heifers at commencement of an AI programme, and changes in these traits between commencement of the programme and pregnancy diagnosis—as well as factors contributing to calf loss from pregnancy diagnosis to weaning (Burns et al., 2010).

Objective 3: Develop new criteria for selection of heifers for FTAI

Study 5: Examine genetic markers and physical parameters to identify selection criteria for Brahman heifers that are likely to conceive to FTAI.

Conduct a genome-wide association study (GWAS) on selected heifers that were enrolled in studies 2 and 3 (those that were also part of the Beef CRC Polled Gene Discovery project) to investigate whether heifers that conceive to FTAI are genetically different to those that fail to conceive. Genetic markers developed by the Beef CRC for fertility and age at puberty as well as other new markers will be investigated.

Examine data collected as part of this project (flight speed, presence of CL, diameter and presence of ovulatory follicle, liveweight, and BCS at commencement of oestrus synchronisation) for select criteria that might predict Brahman heifers more likely to conceive to FTAI.

2.2 Modifications to planned studies to achieve project objectives

Study 1: As experiment 1 failed to demonstrate a benefit from treatment with FSH, the design of experiment 2 was altered to include an additional pilot study. This study examined whether one less treatment could be used in a synchronisation protocol by utilising GnRH at the end of a typical ODB + IPRD protocol. This would reduce the number of treatments and therefore cattle handling occasions from 4 to 3 in a typical ovulation synchronisation and FTAI programme.

Study 2: Flight speed could not be recorded on the heifers enrolled in this study as originally indicated due to limitations of handling facilities on cooperating properties. Also, a natural mating comparison was not able to be undertaken as planned because cooperating properties were interested in enrolling entire heifer mobs and only using natural mating after FTAI. Therefore, this study only validated the best protocol from study 1 in a large field trial.

Study 3: Taking into account the findings of study 2 (substituting GnRH for ODB in the synchronisation protocol resulted in significantly lower pregnancy rates to FTAI), a series of modifications to the ODB + IPRD protocol were developed and initially investigated in an intensive study (Study 3a) followed by a large field trial to compare the outcome of treatment with either the modified protocol or the current best practice protocol (Study 3b). Further, as flight speed and a natural mating comparison could not be investigated in Study 2, these were included in the experimental design for study 3b.

Study 3a investigated the following four changes to the current best practice oestrus synchronisation protocol:

- Omit ODB treatment at IPRD insertion to reduce the suppression of endogenous gonadotrophins
- Reduce the duration of IPRD insertion from 8 days to 6 days
- Increase the time from IPRD removal to ODB treatment from 24 h to 36 h
- Increase the time from IPRD removal to FTAI from 54 h to 72 h.

Study 4: The original recommendation to identify the effects of biostimulation on pregnancy rates to AI was to determine whether this would successfully stimulate the onset of puberty in pre-pubertal heifers (Fiol et al., 2010). These authors in their review of biostimulation in cattle concluded that androgenised cows may be just as effective as teaser bulls in inducing a biostimulatory effect. Using androgenised cows instead of teaser bulls would also improve animal welfare, reduce expense, and be more practical for industry to implement. In addition

to the original methodology, the effects of low stress pre-handling were also investigated in this study.

3 Methodology

Ethical approval for all studies was granted by The University of Queensland's Animal Ethics Committee – approval number SVS/210/11/MLA.

3.1 Study 1

3.1.1 Experimental design and animals

This study was conducted at the Commonwealth Scientific and Industrial Research Organisations (CSIRO) Belmont Research Station, Rockhampton, QLD, and involved two separate experiments. Heifers used in the study were born and raised on the property where the study was conducted. Heifers were managed in a rotationally grazed irrigated Callide Rhodes grass (*Chloris gayana*) pasture and were fed ad libitum Pangola (*Digitaria eriantha*) haylage when heifers were held in the yard for observations and treatments.

Rising 2 year old Brahman heifers (n = 60) were enrolled in the study. The same heifers were used in experiment 1 and experiment 2. Experiment 2 commenced 27 days after the completion of experiment 1 to ensure that all cycling heifers had had a full oestrous cycle and had developed a corpus luteum.

At the commencement of each experiment (day -10), heifers were weighed, body condition scored (BCS; 1 = poor to 5 = fat (Jephcott and Norman, 2004, Entwistle and Fordyce, 2003a)) and examined by transrectal ultrasonography to assess reproductive function.

On Day 0, in both experiments all heifers underwent another ovarian examination to determine the proportion cycling in each group. Thereafter, reproductive evaluation was performed at the following times:

In experiment 1 (**Fig. 1**):

- daily from days 1 to 7
- twice daily (12 ± 1 h intervals) from day 8 until the heifer ovulated
- on day 23 to confirm ovulation and the subsequent development of a CL

In experiment 2 (**Fig. 2**):

- twice daily (12 ± 1 h intervals) from day 8 until the heifer ovulated
- at 54 h post IPRD removal (the recommended time of FTAI)
- on day 15 to confirm ovulation and the subsequent development of a CL.

3.1.2 Treatment allocation

Heifers in both experiments were allocated to one of three treatment groups based on the presence of a corpus luteum (CL). Heifers were randomly allocated to control or treatment groups based on this data: only those heifers that had a liveweight (LW) ≥ 280 kg and a BCS ≥ 2.5 (scale 1 = thin to 5 = fat) were included, and approximately equal numbers of cycling and non-cycling heifers were assigned to each group.

Heifers were assigned to the following three treatment groups after the collection of data (CL, LW, BCS) on day -10, and treatments were commenced on day 0 (Fig 1 and Fig. 2):

Experiment 1:
OP = ODB + IPRD + $PGF_{2\alpha}$,
GP = GnRH + IPRD + $PGF_{2\alpha}$
OPF = ODB + IPRD + FSH + $PGF_{2\alpha}$.

Experiment 2
OPO = ODB + IPRD + $PGF_{2\alpha}$ + ODB
GPG = GnRH + IPRD + $PGF_{2\alpha}$ + GnRH
OPG = ODB + IPRD + $PGF_{2\alpha}$ + GnRH

3.1.3 Ovulation synchronisation treatments

All heifers in both experiments received a half dose intravaginal progesterone releasing device for 7 days, prepared according to (Butler et al., 2011c) (IPRD; Cue-Mate®; 0.78g progesterone; Bioniche Animal Health A/Asia Pty Ltd, Sydney, NSW, Australia, now Vetoquinol), and 500μg $PGF_{2\alpha}$ (cloprostenol; Ovuprost™, Bayer Sydney, Australia) by intramuscular injection at the time of IPRD removal. As typical GNRH and P_4-based (GPG) protocols utilise an IPRD insertion period of 7 days (Wiltbank and Pursley, 2014), a 7 day insertion was also used for the ODB and P_4-based (OPO) treatment group as an 8 or 7 day insertion does not significantly affect pregnancy rate in an OPO protocol (Balla et al., 2006, Chesta et al., 2003).

In addition, heifers received differing combinations and/or timing of the following intramuscular injections: 1 mg oestradiol benzoate (ODB; Bomerol™, Bayer Australia, Sydney, Australia); 100 μg gonadotrophin releasing hormone (GnRH; Ovurellin™, Bayer Australia, Sydney, Australia); 10 mg follicle stimulating hormone (FSH; Folltropin-V™, Bioniche Animal Health A/Asia Pty Ltd, Sydney, Austalia, now Vetoquinol), diluted in MAP-5 (Tríbulo et al., 2011, Tríbulo et al., 2012) (Sodium Hyaluronate, Bioniche Animal Health, Belleville, Canada).

The synchronisation protocols for experiment 1 are illustrated in Fig. 1, and for experiment 2 are illustrated in **Fig. 2**.

3.1.4 Evaluation of follicular dynamics

Transrectal ultrasonography was used to monitor ovarian function. Ultrasonography was performed using a SonoSite M-Turbo ultrasound machine equipped with a L52X/10-5 mHz linear array transrectal transducer (SonoSite Inc., Bothel, WA, USA).

Ovarian examinations were performed according to Edwards et al. (2014). At each examination, an ovarian map was drawn for each ovary, recording the location and diameter of any antral follicles, the diameter of the largest (dominant) follicle, the presence of a CL, and pregnancies or abnormalities of the reproductive tract. The presence of a CL was identified by the echogenic appearance of luteal tissue (Kastelic et al., 1990, Veronesi et al., 2002).

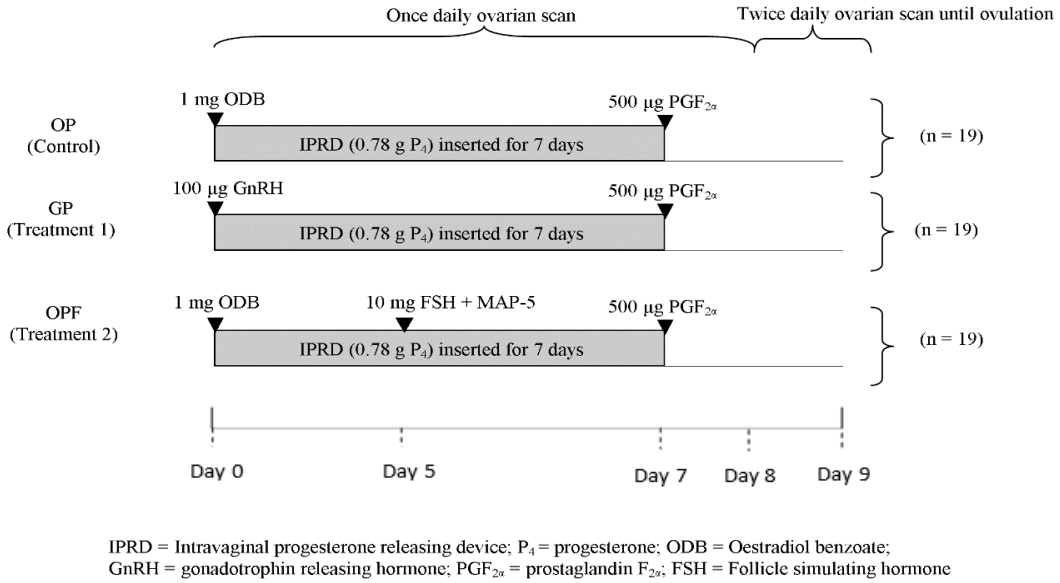
At the same time, a 10 s video clip (mp4 file) was recorded for retrospective analysis of ovarian function using Image J 1.46r (Wayne Rasband National Institutes of Health, USA). The accuracy of every ovarian map was checked by retrospective analysis of the video image.

3.1.5 Data analysis

The day of emergence of the ovulatory follicle was retrospectively determined. The day of follicular wave emergence (FWE) was defined as the first day that the diameter of the dominant follicle was 4–5 mm. If the follicle was not detected until it was 6–7 mm, the day prior to this was recorded as the day of FWE (Ginther et al., 1989c). Ovulation was defined by the disappearance of a dominant follicle (DF) of ≥ 7 mm diameter (Gimenes et al., 2008). Data for follicular development in heifers that were cycling and received the OP protocol was normalised and plotted from FWE to ovulation. The growth and the static phases of follicular

development were determined using published methods (Adams et al., 1994, Ginther et al., 1989a).

Analysis for all variables in Experiments 1 and 2 were compared between treatment groups with respect to all heifers, only cycling heifers, and only non-cycling heifers. Data analysis for the continuous variables—day of FWE, diameter of the dominant follicle at 54 h post IPRD removal, and diameter of the dominant follicle prior to ovulation—were performed using a one-way ANOVA (StataCorp, 2007). Data analysis for the binomial variables—proportion ovulated and the proportion with synchronous ovulations—were performed using a one-tailed Fishers Exact test (Abramson, 2004).



IPRD = Intravaginal progesterone releasing device; P_4 = progesterone; ODB = Oestradiol benzoate; GnRH = gonadotrophin releasing hormone; $PGF_{2\alpha}$ = prostaglandin $F_{2\alpha}$; FSH = Follicle simulating hormone

Fig. 1: Ovulation synchronisation protocols for experiment 1, involving 57 Brahman heifers allocated to either the control group (OP) or one of two treatment groups (GP, OPF). All heifers received an intravaginal progesterone releasing device for 7 days and prostaglandin $F_{2\alpha}$ at the time of IPRD removal. Groups OP and OPF received oestradiol benzoate and group GP received gonadotrophin releasing hormone at the time of IPRD insertion. On day 5, the OPF group also received long acting follicle stimulating hormone (FSH).

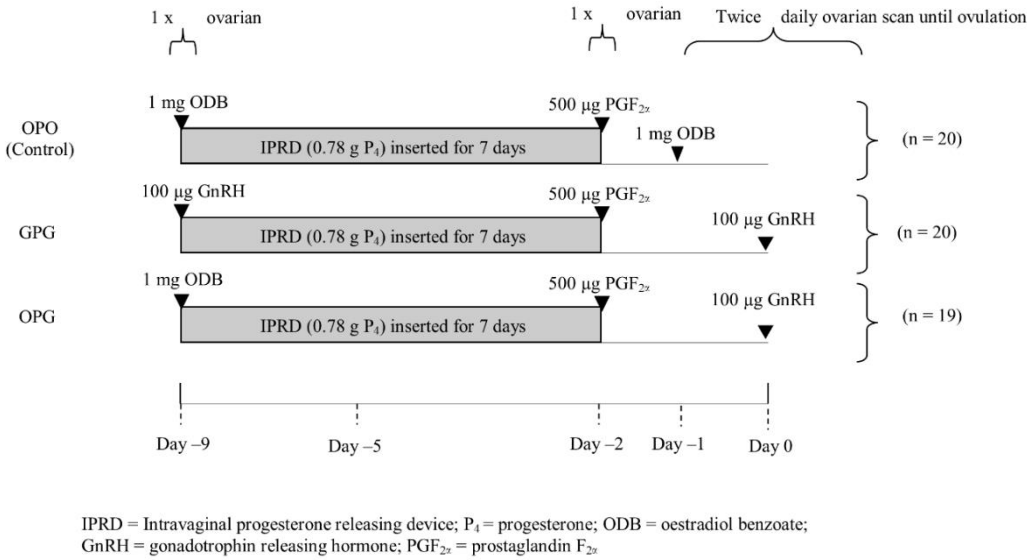


Fig. 2: Ovulation synchronisation protocols for experiment 2, involving 59 Brahman heifers allocated to either the control group (OPO) or one of two treatment groups (GPG, OPG). All heifers received an intravaginal progesterone releasing device (IPRD) for 7 days and prostaglandin $F_{2\alpha}$ at the time of IPRD removal. At the time of IPRD insertion (day 0), groups OPO and OPG received oestradiol benzoate (ODB) and group GPG received gonadotrophin releasing hormone (GnRH). On day 8, the OPO group also received ODB and on day 9 the GPG and OPG groups also received GnRH.

3.2 Study 2

3.2.1 Experimental design and animals

This study was conducted during summer (January to February, 2012) on four commercial beef cattle properties in QLD. Property A was located in the central QLD region (25°01'44.42"S, 150°26'06.21"E), and Properties B (20°28'43.54"S, 140°34'52.66"E), C (20°12'27.25"S, 140°22'59.30"E) and D (20°59'38.18"S, 141°00'44.16"E) were located in north QLD. 1143 rising 2 year old Brahman heifers that were born and raised on each property were selected for the study: Property A (n = 408); Property B (n = 73); Property C (n = 450) and D (n = 213). All heifers were typical of Brahman heifers that are annually mated in northern Australia. All were vaccinated for clostridial diseases and leptospirosis prior to the trial as part of routine property health management practice.

The study commenced on Property A first, and commenced at Properties B, C, and D 19, 27, and 31 days, respectively, after Property A.

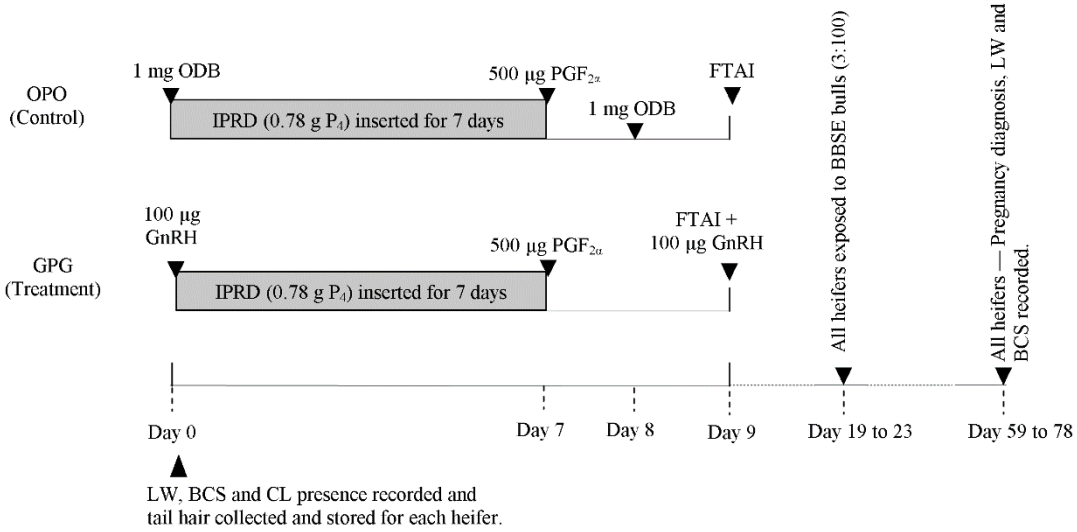
At the commencement of this study (day 0), all heifers were weighed, body condition scored (BCS; 1 = poor to 5 = fat (Jephcott and Norman, 2004, Entwistle and Fordyce, 2003a)), and examined by transrectal ultrasonography (procedure detailed in 3.1.4) immediately prior to starting ovulation synchronisation treatment. Heifers that had a liveweight ≤ 280 kg (n = 3), a BCS < 2.5 (n = 0), were pregnant (n = 62), had an immature reproductive tract, or other abnormalities (n = 11) were excluded from the study.

Included heifers were randomly allocated to one of two treatment groups: OPO (control) or GPG (treatment). Treatments were administered as described in Section 3.1.3. FTAI protocols for the OPO and GPG groups are summarised in **Fig. 3**.

On day 8, when heifers in the OPO group received treatment (with ODB), heifers in the GPG group were also handled but received no treatment. Between 54 and 58 h after IPRD removal, (day 9) all heifers were artificially inseminated. At the time of AI, every heifer in the GPG group received their final GnRH treatment.

3.2.2 Treatment allocation

The allocation procedure used in this study was similar to previous studies published by this group (Butler et al., 2011b, Edwards et al., 2012). On Day 0 heifers were allocated to a treatment group as they presented to the handling facility. Heifers were allocated alternately to either the control or the treatment groups. To ensure all heifers could be inseminated between 54 and 58 h after removal of the IPRD on each property, heifers on Property A, C, and D were drafted into 2, 3, and 2 AI groups, respectively, that were estimated visually to be even with respect LW and BCS. The numbers of heifers in each AI group on each property was dependent on the property yards and labour availability. On these properties (where multiple AI groups were allocated) treatments to synchronise ovulation commenced on Day 0, 1, and 2 for AI groups 1, 2, and 3, respectively.



IPRD = Intravaginal progesterone releasing device; P_4 = progesterone; ODB = Oestradiol benzoate; GnRH = gonadotrophin releasing hormone; FTAI = fixed-time artificial insemination; $PGF_{2\alpha}$ = prostaglandin $F_{2\alpha}$

Fig. 3: Ovulation synchronisation protocols for study 2, involving Brahman heifers allocated to either the control group (OPO) or the treatment group (GPG). All heifers received an intravaginal progesterone releasing device (IPRD) for 7 days and prostaglandin $F_{2\alpha}$ at the time of IPRD removal. At the time of IPRD insertion (day 0), OPO heifers received oestradiol benzoate (ODB) and GPG heifers received gonadotrophin releasing hormone (GnRH). On day 8, the OPO group also received ODB and on day 9 the GPG group also received GnRH.

3.2.3 Artificial insemination and pregnancy diagnosis

All AI occurred on Day 9 of the synchronisation treatment schedule regardless of treatment group. On Properties A, C, and D where multiple AI groups were allocated, AI commenced on Day 9, 10 and 11 for AI groups 1, 2 and 3, respectively. On Property A, two crushes operated simultaneously and technicians 4 to 7 inseminated on a rotational basis, changing when each technician became fatigued. On Properties B and C, two crushes operated simultaneously with technicians 1 and 2 inseminating until fatigued, and technician 3

relieving them. On Property D, only one crush operated and thus technician 2 did the majority of inseminations, relieved by technician 3 when fatigued.

Semen was thawed at 35°C for 15 to 30 sec in a water bath prior to insemination. 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 x 10^8 sperm/ml; > 35% live and > 35% progressively motile).

Heifers were exposed to bulls (3 bulls per 100 heifers) from days 19 to 23. All bulls had passed a bull breeding soundness examination.

Pregnancy diagnosis was by transrectal ultrasonography between 8.5 and 12 weeks after FTAI (Property A: Day 95 to 96; B: Day 79; C: Day 72 to 74 and D: Day 69 to 70). Any heifer that did not present for pregnancy diagnosis was removed from the data set (Property A: n = 24; B: n = 0, C: n = 5 and D: n = 2). All pregnancies were foetal aged so that the pregnancy category could be standardised across all properties due to the differing pregnancy diagnosis dates. Pregnancy status was categorised as either pregnant to FTAI, pregnant to bulls on first natural return to oestrus (heifers must have conceived within 10 to 30 days after AI), pregnant after first return (conceived > 30 days after AI), or not detectably pregnant (NDP).

On the day of pregnancy diagnosis heifers were again weighed and body condition scored.

3.2.4 Sire allocation

Heifers on Property A, which were part of a large scale Brahman sire genetic evaluation project, were allocated to sires (n = 31) on presentation for AI to the handling facility. Sires were used in numerical order until semen stores were depleted. Heifers on Properties B and C were allocated to sires (n = 2 and n = 12, respectively) using stratified randomisation according to treatment group (OPO or GPG), presence of a CL, LW, and AI group. This was to minimise any confounding effects of sire, CL and LW on response to treatment. Heifers on Property D were also part of a genetic selection programme. Heifers were allocated to sires (n = 19) to reduce the risk of inbreeding, structural or temperament faults.

Sires 5, 20, 7, 27 and 29 were used on more than one herd.

3.2.5 Data analysis

The sire allocation procedure at each property was retrospectively analysed to verify that there was no allocation bias between the OPO and GPG groups. The pregnancy rate (PR) was defined as the proportion of treated heifers diagnosed pregnant according to mating criteria.

Data were analysed using GenStat 14th edition (GenStat, 2013). The LW and BCS at allocation were analysed by fitting general linear mixed models using residual maximum likelihood (REML) methods. Presence of CL at allocation and the PR to FTAI were analysed by fitting general linear models with a binomial distribution and logit link. Analyses were first performed separately for properties to incorporate the difference in design and management structure and then pooled for an overall analysis to test factors common to all four properties.

Allocation analyses tested the difference in LW, BCS and presence of CL with respect to properties A, B, C and D. Where sires were used on more than one property, a REML analysis was performed to detect differences between batches of semen. If differences were found to be significant in either the continuous or binomial data, then pairwise least

significant differences were used to determine which levels of factors were significantly different. Significance was set at $P < 0.05$. Predicted means were back-transformed for presentation of the binomial data.

3.3 Study 3a

3.3.1 Experimental design and animals

The study was conducted during spring (Late September to October 2012) on a commercial beef cattle property in south east QLD (26°59'53.27" S, 152°20'57.11" E). Rising 2 year old high-grade Brahman heifers (n = 60), which were born and raised on the property, were used in the study. All heifers were vaccinated for clostridial diseases, leptospirosis, and bovine ephemeral fever prior to the trial as part of routine property health management practice. All heifers had ad libitum access to a dry lick (10% urea and 5% phosphorous) prior to, during, and after the trial. Heifers grazed a mixture of primarily couch grass (*Elytrigia repens*), spear grass (*Heteropogon contortus*) and Queensland bluegrass (*Dichanthium sericum*), with some common vetch (*Vicia sativa*), snail medic (*Medicago scutellata*) and lucerne (*Medicago sativa*). During the trial period (Day 0–12) heifers had access to ad libitum oaten (*Avina sativa*) hay.

Ten days prior to commencement of the study (Day -10) every heifer was weighed, body condition scored (BCS; 1 = poor to 5 = fat (Entwistle and Fordyce, 2003a, Jephcott and Norman, 2004)) and examined by transrectal ultrasonography. Procedures for evaluating the reproductive tract by ultrasonography are detailed in Section 3.1.4. All enrolled heifers had normal reproductive tracts, and adequate weight (> 280 kg) and BCS (> 2.5).

At the commencement of the study (Day 0), all heifers underwent another ovarian examination by transrectal ultrasonography. Ultrasonography was performed daily from day 0 to day 8, twice daily (12 ± 1 h intervals) from Day 9 to 12 or until ovulation was detected, and then again at the time of FTAI (at 54 h for OPO-8 heifers and at 72 h for all heifers).

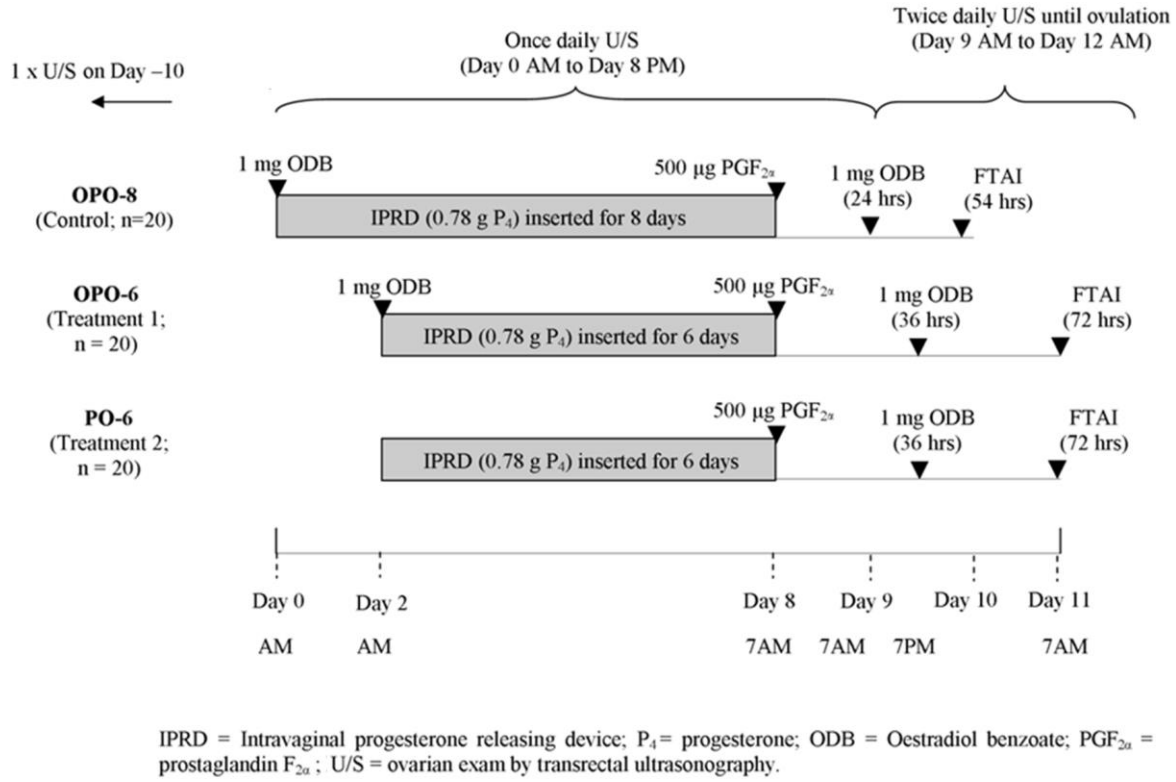
3.3.2 Treatment allocation

Liveweight, BCS, and presence of a CL recorded on Day −10 were used to allocate heifers by stratified randomisation to either the control protocol (OPO-8; current best practice for ovulation synchronisation in Brahman heifers (Butler et al., 2011b)) or one of two modified protocols: OPO-6 or PO-6.

3.3.3 Ovulation synchronisation treatments

The different ovulation synchronisation treatments investigated are outlined in **Fig. 4**, and details of those treatments (registered drug names, suppliers, dose rates, and routes of administration) are provided in Section 3.1.3.

On Day 0, every heifer treated with the OPO-8 protocol and on Day 2 every heifer treated with the OPO-6 and PO-6 protocols had a half-dose IPRD inserted. At the same time, heifers receiving the OPO-8 and OPO-6 protocols received ODB. On Day 8, the IPRD was removed from all heifers and each received $PGF_{2\alpha}$. At 24 h after IPRD removal all heifers in the OPO-8 group received ODB, and 36 h after IPRD removal all heifers in the OPO-6 and PO-6 group received ODB. Artificial insemination was performed on day 10, (54 h after IPRD removal) for heifers in the OPO-8 group, and on day 11 (72 h after IPRD removal) for heifers in the OPO-6 and PO-6 groups.



IPRD = Intravaginal progesterone releasing device; P_4 = progesterone; ODB = Oestradiol benzoate; $PGF_{2\alpha}$ = prostaglandin $F_{2\alpha}$; U/S = ovarian exam by transrectal ultrasonography.

Fig. 4: Treatment and observation schedules for Brahman heifers enrolled in the OPO-8, OPO-6 or PO-6 treatment groups. All heifers received an intravaginal progesterone releasing device (IPRD) for 6 days (OPO6, PO6) or 8 days (OPO8) and prostaglandin F2α at the time of IPRD removal. At the time of IPRD insertion (day 0), OPO8 and OPO6 heifers received oestradiol benzoate (ODB). All groups received ODB after IPRD removal, either at 24 h (OPO8) or 36 h (OPO6, PO6) post removal.

3.3.4 Oestrus detection, FTAI, and pregnancy diagnosis

On the morning of Day 9, every heifer had an oestrus detection aid applied to the sacral area (Estrotect™ heat detector, Rockway Inc.,Spring Valley, Wisconsin, USA). Heifers were observed for 45 min intervals twice daily from the morning of Day 9 to the afternoon of Day 11, looking for signs of oestrus (standing heat, mucus and degree of rubbing on heat mount detectors).

One Brahman sire was used in this study. Frozen-thawed semen from the same batch was thawed at 35ºC for 30 s prior to loading the insemination gun. Semen was tested for post-thaw quality and found to be acceptable (concentration > 1 x 10^8 sperm/ml; > 35% live and > 35% progressively motile).

Heifers were pregnancy tested at 27 (OPO-6 and PO-6) and 28 days (OPO-8) after FTAI by transrectal ultrasonography.

3.3.5 Data analysis

The day of emergence of the ovulatory follicle, the day of follicular wave emergence (FWE), and ovulation were defined and determined as outlined in Section 3.1.5.

Data analysis was carried out using Statistica for Windows (version 7, StatSoft Inc., Tulsa, OK, USA). Analysis of the ovarian response to experimental variables was tested by Mann–Whitney U test to compare two grouping variables. Categorical and non-categorical variables were otherwise analysed using a generalised linear model appropriate for comparison of more than two treatment protocols. All data expressed as proportions were subjected to arcsine transformation (Zar, 1984) to normalise data prior to ANOVA analysis.

3.4 Study 3b

3.4.1 Experimental design and animals

The study was conducted during late spring-summer on three commercial beef cattle properties. Property A (22º57'27.55"S, 149º25'39.68"E) and Property B (23º33'42.42"S, 149º3'17.91"E) were located in central QLD and Property C (20°12'27.25"S, 140°22'59.30"E) was located in north QLD. All heifers used in the study were typical of replacement Brahman heifers annually mated in northern Australia.

All animal husbandry practices were conducted in line with the model code of practice for the welfare of animals for cattle (Primary Industries Standing Committee, 2004).

Property A: All heifers (n = 453) were born and raised on the property. Heifers were managed in a 1500 ha paddock prior to the trial and a 90 ha paddock during the trial, and grazed pastures that were primarily comprised of Buffel grass (*Cenchrus ciliaris*) and Rhodes grass (*Chloris gayana*). Animal handling facilities were equipped with water, hay feeders and shade and had appropriately fenced holding paddocks. Heifers did not experience withdrawal from feed or water throughout the study. Prior to the study, heifers were managed as two separate cohorts (Stud; n = 160 and Commercial; n = 293).

Property B: All heifers (n = 271) at this farm were born and raised on the property. Heifers were managed in a 607 ha paddock prior to and during the trial and grazed pastures that were primarily comprised of Buffel grass (*Cenchrus ciliaris*). Animal handling facilities did not have water, hay feeders, or shade, and as a consequence heifers experienced periods (up to 12 h) without feed or water when brought in for hormonal treatments and AI. All heifers were managed as one cohort.

Property C: Heifers (n = 393) were either born on the property (homebred; n = 343) or purchased as yearlings (purchased; n = 156). Heifers were managed in a 365 ha paddock prior to the study and a 102 ha paddock during the study and grazed pastures that were primarily comprised of Mitchell grass (*Astrebla spp.*) and Blue grass (*Dichanthium sericium*). Animal handling facilities were equipped with water, hay feeders, and shade, and had appropriately fenced holding paddocks. Heifers did not experience withdrawal from feed or water throughout the study. All heifers were managed as one cohort.

At the commencement of the study (day 0), all heifers were weighed, body condition scored (BCS; 1 = poor to 5 = fat (Jephcott and Norman, 2004, Entwistle and Fordyce, 2003a)), and examined by transrectal ultrasonography (procedure detailed in 3.1.4) immediately prior to selection and ovulation synchronisation treatment. Heifers were rejected from the study if they had a liveweight ≤ 280 kg (Property A: n = 1, Property B: n = 1, and Property C: n = 16), a BCS < 2.0 (Property C: n = 3), were pregnant (Property A: n = 30, Property B: n = 16 and Property C: n = 8), lactating (Property A: n = 2)), had an immature reproductive tract (Property A: n = 6, Property B: n = 2 and Property C: n = 1), an abnormal reproductive tract (Property A: n = 2 and Property C: n = 2) or had poor temperament (Fordyce et al., 1988) (Property B: n = 1 and Property C: n = 2).

Enrolled heifers were randomly allocated to one of two treatment groups: OPO-8 (control) or OPO-6 (treatment). At properties B (n = 108) and C (n = 106) additional heifers were allocated to a natural mating group (NATM) for comparison with a subset of OPO-8 heifers on property B (n = 96) and all OPO-8 heifers on property C (n = 196). Heifers in the NATM groups were managed under the same conditions as those in the FTAI groups. All heifers in the NATM groups and those OPO-8 heifers available for comparison on properties B and C also received two additional examinations by rectal ultrasonography at days -24 and -10 to

determine the proportion of heifers that were cycling. Liveweight and BCS were also recorded at day -24.

The details of FTAI treatments administered (registered drug names, suppliers, dose rates, and routes of administration) were the same as those detailed in Section 3.1.3, except for the registered name and supplier of ODB (Cidirol®, Genetics Australia, Bacchus Marsh, VIC, Australia). FTAI protocols for OPO-8 and OPO-6 groups are described in Section 3.3.3 and summarised in **Fig. 5**.

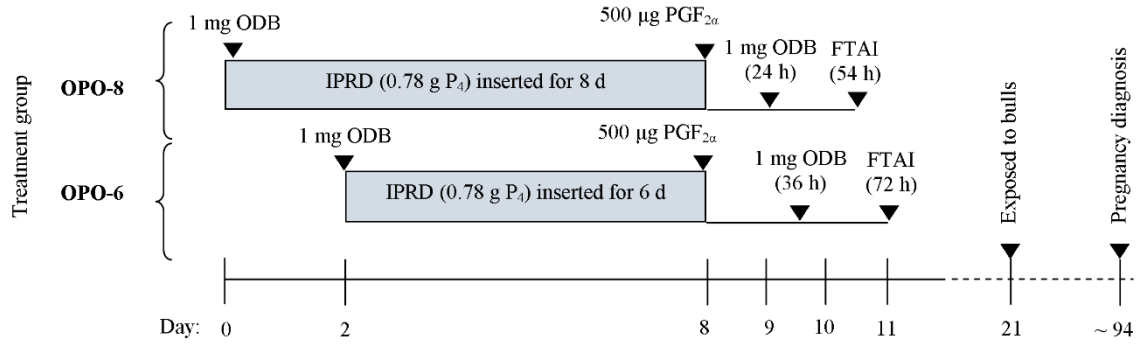


Fig. 5: Treatment and observation schedule for Brahman heifers treated to synchronise ovulation for fixed-time artificial insemination (FTAI) with intravaginal progesterone (P_4) releasing devices (IPRD), oestradiol benzoate (ODB) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). Heifers were enrolled in either the OPO-8 or OPO-6 treatment groups.

3.4.2 Treatment allocation

The allocation procedure used in this study was similar to previous studies published by this group (Butler et al., 2011b, Edwards et al., 2012). On Day 0, heifers that were suitable for the study were allocated alternately as they presented to the handling facility.

All heifers allocated to the OPO-8 and OPO-6 groups on Properties B and C were inseminated on one day. To ensure all heifers in a treatment group were inseminated within a 4 hour window, heifers on property A were drafted into two similar size groups prior to allocation (A1: n = 238 and A2: n = 215), allowing treatment and insemination to be conducted on two consecutive days.

3.4.3 Artificial insemination, exposure to bulls, and pregnancy diagnosis

AI was performed at 54 h or 72 h after IPRD removal for OPO-8 and OPO-6 heifers, respectively. A varying number of AI sires were used on property A (n = 24), B (n = 10) and C (n = 10). At each property, sires were allocated alternately as heifers presented for FTAI. Every heifer was inseminated using frozen-thawed semen. Semen was thawed at 35ºC for 30 s prior to loading the insemination gun, and the entire semen dose (0.25 ml) was placed in the body of the uterus. Inseminations were performed by experienced technicians. At property A, inseminations were performed using one crush, with technician 1 inseminating and technicians 2 and 3 relieving technician 1 when fatigued. At property B, all inseminations were performed by technician 4 using one crush. At property C, two crushes operated simultaneously and inseminations were performed by technicians 4 and 5. All semen used was analysed prior to commencement of the study and was found to be of acceptable post-thaw quality (concentration > 1 x 10^8 sperm/ml; > 25% progressively motile) (Barth, 1993).

All heifers receiving FTAI protocols were exposed to bulls on day 21. Bulls at each property had all passed a breeding soundness examination, including sperm morphology assessment (Entwistle and Fordyce, 2003b), and were well familiarised with each other prior to the trial to reduce fighting during mating. Heifers in the NATM groups on properties B and C were exposed to bulls 2 days prior to the day of AI for the FTAI heifers. Heifers in the NATM groups on each property were exposed to the same bulls as the FTAI heifers on those properties, all at a bull to female ratio of 2.5:100. The FTAI heifers were placed in the same paddock with the NATM heifers 10 days after FTAI, and additional bulls were placed with the heifers to maintain a 2.5:100 bull to female ratio.

Pregnancy diagnosis was performed by transrectal ultrasonography at 12 weeks after the day of FTAI. Foetal aging was performed to determine whether females were pregnant to FTAI or NATM.

3.4.4 Flight speed observations

Flight speed equipment was set up according to Burrow et al. (1988) on each property at the exit of the crush. The flight speed of each enrolled heifer on these properties was recorded on 3 separate occasions: at time of IPRD removal, at time of second ODB injection, and at the time of pregnancy diagnosis. At each recording, heifers were ranked by quartiles from fastest to slowest (1 to 4) within each property and management group. An average rank was then calculated for each heifer.

3.4.5 Data analysis

Heifers in the NATM group were diagnosed pregnant to the first oestrous cycle if they were pregnant between Day 0 and 21 of the mating season. The second oestrous cycle conception rate for the NATM group was defined as heifers that were pregnant from Day 22 to 42 of the mating season. The first oestrous cycle conception rate of the FTAI heifers was calculated as heifers pregnant on Day 0 as they were treated for ovulation synchronisation. The second oestrous cycle conception rate for the FTAI heifers was calculated from Day 14 to 35, as this is the period in which heifers return to oestrus after ovulation synchronisation if they fail to become pregnant to AI. The six week pregnancy rate was defined for both groups as the proportion of females that were pregnant after 6 weeks of mating, including heifers pregnant to AI from the FTAI group.

Data were analysed using GenStat 14th edition (GenStat, 2013). The LW and BCS at allocation were analysed by fitting general linear mixed models using residual maximum likelihood (REML) methods. CL at allocation and pregnant to FTAI were analysed by fitting general linear models with a binomial distribution and logit link. Analyses were first performed separately for each property to incorporate the difference in design and management structure, and then pooled for an overall analysis to test factors common to all three properties.

Allocation analyses tested the difference in LW, BCS, and presence of CL with respect to commercial or stud heifers and day of AI (A1 or A2) at property A, purchased or homebred heifers at property C, technician at properties A and C, and OPO protocol and sire at all properties. Analyses of the pregnancy rates to FTAI tested the effects of the factors listed above as well as presence of CL, LW category (280 to 300 kg; 300 to 325 kg; 325 to 350 kg; 350 to 375 kg; 375 to 400 kg and > 400 kg) and BCS. All 3-way interactions between stud and commercial heifers at property A, homebred and purchased at property C, day of AI at property A, OPO protocol and presence of CL were initially included with any non-significant interaction dropped from the model via backward selection.

If the differences were found to be significant in either the continuous or binomial data, then pairwise least significant differences were used to determine which levels of factors were significantly different. Significance was set at $P < 0.05$. Predicted means were back-transformed for presentation of the binomial data.

3.5 Study 4

3.5.1 Experimental design and animals

The study was conducted during late summer (January to March 2015) on a commercial beef cattle property in north Queensland (20°12'27.25"S, 140°22'59.30"E). All heifers used in the study were typical of rising 2–year-old replacement heifers annually mated in northern Australia. The majority were Romagnola/Brahman cross or Brahman breed heifers with a small number of Senepol/Brahman and Angus/Brahman cross. The property was in the third year of three failed wet seasons and had largely been destocked except for the heifers used in this study. Due to the adverse seasonal conditions the threshold weight for inclusion in the study was reduced from 280kg to 250 kg.

All animal husbandry practices were conducted in line with the model code of practice for the welfare of animals for cattle (Primary Industries Standing Committee, 2004).

Thirty days prior to FTAI, all heifers were weighed, body condition scored (BCS; 1 = poor to 5 = fat (Jephcott and Norman, 2004, Entwistle and Fordyce, 2003a)), and examined by transrectal ultrasonography (procedure detailed in 3.1.4) to determine the presence of a CL and diameter of the dominant follicle. Heifers were rejected from the study if they had a live weight ≤ 250 kg (n=112), were pregnant (n=7), or had any other abnormality likely to affect future performance (n=1).

At the commencement of the treatments to synchronise ovulation (11 days prior to FTAI), enrolled heifers were again weighed, body condition scored and examined by transrectal ultrasonography. Enrolled heifers were alternatively allocated using the method of Butler et al. (2011b) to one of three treatment groups as they presented to the handling facility: biostimulation (BT), pre-handling (PH) or control.

3.5.2 Treatment groups

Biostimulation prior to FTAI (BT, n = 91). Five non-pregnant mature cows that had been treated with testosterone propionate according to published protocols (Wolfe, 1986) were placed with these heifers between 11 to 18 days prior to commencement of treatments to synchronise ovulation (22 to 29 days before FTAI). Briefly, the teaser cows were treated with 0.2 mg of testosterone propionate intramuscularly (Testoprop 50mg/ml testosterone propionate, Jurox Pty Ltd) for 6 days and then on the last day of treatment they received 1gm. The impact of biostimulation was assessed by comparing the proportion of heifers that had a CL before and after biostimulation and by comparing pregnancy rates to FTAI with that achieved by the control group.

Pre-handling prior to FTAI (PH, n = 99). Between 16 to 19 days prior to commencement of treatments to synchronise ovulation (27 to 30 days before FTAI) these heifers were held in the cattle yards that would be used for all treatments to synchronise ovulation and FTAI. The heifers were handled through the drafting pens, race, crush and holding pens once a day for 3 days by two very experienced stock persons using the principles of low stress handling. On the second day of pre-handling the flight speed of each heifer in this group was determined according to the method described by Burrow et al. (1988). The assessment of flight speed was conducted in the handling facilities that would subsequently be used for FTAI. On the day before FTAI the flight speed of all heifers enrolled in the study was

determined. The impact of pre-handling was assessed by comparing the mean flight speeds before and after pre-handling, and by comparing the pregnancy rate to FTAI of the pre-handling group with that achieved by the control group.

Control (n=92). These heifers were allowed to graze as per normal for the 20 day period prior to commencement of treatments to synchronise ovulation. Between 11 to 17 days prior to commencement of treatments to synchronise ovulation the BT and PH heifers co-grazed with the control heifers.

To ensure the control heifers were not exposed to a biostimulatory effect there was one paddock between them and the paddock where the biostimulation group grazed, a separating distance of 1.5km. Also during the period when the teaser cows were run with the biostimulation heifers the prehandling group of heifers were held in the cattle yards to be used for FTAI, where they underwent low stress prehandling.

3.5.3 Ovulation synchronisation, FTAI, and pregnancy diagnosis

Ovulation was synchronised following the OPO-6 protocol detailed in Section 3.4. FTAI was performed on day 11, 72 hours after IPRD removal using frozen-thawed semen from a number of AI sires. Sires were allocated alternately as heifers presented for FTAI.

All semen used was analysed prior to commencement of the study and was found to be of acceptable post-thaw quality (concentration > 1 x 10^8 sperm/ml; > 25% progressively motile) (Barth, 1993). Semen was thawed at 35ºC for 30 s prior to loading the insemination gun, and the entire semen dose (0.25 ml) was placed in the body of the uterus. Inseminations were conducted by two experienced technicians operating simultaneously in two crushes.

Nine days after FTAI, nine bulls (5 x 4 yr old bulls and 4 x 5 yr old bulls) which had passed a breeding soundness examination and had been vaccinated against vibriosis were placed with the heifers.

Pregnancy diagnosis was performed by transrectal ultrasonography approximately 10 weeks after FTAI. All pregnancies were foetal aged to determine if the heifers were pregnant to FTAI (i.e were 10 weeks pregnant) or to the bulls in the first 3 weeks of mating (i.e., weeks 2 to 5 after FTAI).

3.5.4 Data analysis

The sire allocation procedure was retrospectively analysed to verify that there was no allocation bias between the treatment groups.

Data cleaning procedures were carried out before analysis. There were 17 animal records with unknown conception status at FTAI, which were subsequently removed from the dataset. Descriptive analysis of key variables (live weight, BCS, flight speed, proportion of heifers with a CL) was initially conducted. Change in live weight and BCS from induction to time of pregnancy diagnosis was calculated as Liveweight (or BCS) at date of pregnancy diagnosis minus live weight (BCS) at induction. Negative values represented losses and positive values represented gains in live weight or BCS. Continuous variables (Live weight, BCS, changes in live weight and BCS) were treated as categorical variables taking a value of 1 if a given variable's value was greater than the median or zero. Breed was treated as a categorical variable with two levels (Romagnola/Brahman cross and others), and horn status, presence or absence of a CL at time of allocation to treatment group, and treatment group were treated as categorical variables.

Data were evaluated to determine the frequency of risk factors between conceiving and non-conceiving heifers at FTAI (n conceiving = 121) and when exposed to the Bull (n conceiving = 106) using two independent logistic regression models.

The association between candidate explanatory variables and conception to FTAI or Bull was assessed using univariable logistic regression models. A likelihood ratio test P value < 0.30 was used as a criterion for entry of an explanatory variable into the multivariable logistic regression model. Model parameters were estimated by forward stepwise elimination.

Variables were retained in the multivariable model if the likelihood ratio test P values were <0.05. Two-way interactions were assessed and retained in the final model if the likelihood ratio test P values were <0.05. An explanatory variable was declared as a confounder if the confounding variable influenced the coefficients estimate of other explanatory variables in the multivariable model by 15% or greater. The confounding variable was retained in the model and declared. As the objective of this study was to evaluate the effect of treatment group on conception outcome to FTAI or Bull, treatment group was forced into the final model. Over-dispersion was declared to be present if the chi-squared p-value was less than or equal to 0.05. All statistical analyses were conducted in R (R Development Core Team, 2014).

3.6 Study 5

3.6.1 Animals, phenotypes, and Single Nucleotide Polymorphisms (SNP) genotypes

DNA samples from approximately 2-year-old Brahman heifers (n = 614) that were phenotypically assessed as high-grade Brahman were analysed in this study. Samples were from heifers enrolled in Study 2 and Study 3b (OP group, study 2; OPO-8 group, study 3b). The cattle were from four commercial beef breeding properties, and were selected to have similar age (same birth year), body condition score, and weight. To avoid potential bias from the inclusion of pre-pubertal cattle on the pregnancy outcomes after FTAI, only heifers that had a corpus luteum (CL) at the start of hormonal treatment to synchronise ovulation were included in the study.

All heifers were similarly treated to synchronise ovulation; protocol details are described in detail in Sections 3.1.3 and 3.3.3. Briefly, all heifers were treated with a low dose IPRD (Cue-Mate®; one P_4 impregnated pod; 0.78 g P_4; Bioniche Animal Health Aust/Asia, now Vetoquinol) and received 1 mg of oestradiol benzoate (ODB; Bomerol®, Bayer Australia) im; 8 days later the IPRD was removed and the heifers received 500 µg cloprostenol ($PGF_{2\alpha}$; Ovuprost®, Bayer Australia) im.; 24 hrs later all heifers received 1 mg of ODB im.; FTAI occurred 54 hrs post IPRD removal. At the time of pregnancy diagnosis (approximately 10 weeks after FTAI), there were 269 pregnant and 345 non-pregnant heifers, with average weight of 356.82 Kg (SD = 42.59).

DNA was extracted from individual hair samples and heifers were genotyped using the 20,000 SNP chip from Neogen (GGP, http://www.neogen.com/Agrigenomics/). Since all animals had less than 5% missing values, quality control filter was applied to SNP only, where SNP with more than 10% no-calls were removed. After quality control, all animals had genotypes for 18,895 SNP. This dataset was imputed to 51,588 SNP using Beagle (Browning and Browning, 2011). The reference population for imputation consisted of 2,112 Australian Brahman cattle (Barwick et al., 2009) that were previously genotyped using either the BovineHD chip (Illumina, San Diego) or the BovineSNP50 chip (Matukumalli et al., 2009, Illumina, San Diego USA). The majority of these reference animals were genotyped using

the BovineSNP50 chip, therefore imputation targeted this SNP density, resulting at an average accuracy estimate (r^2) of 0.92 (SD = 0.10) across all individuals.

3.6.2 Estimation of *Bos indicus* ancestry

Brahman cattle are mainly of *Bos indicus* genetic composition. Nevertheless, during breed expansion, Brahman cattle were "graded up" via crossbreeding with *Bos taurus* cattle. A small proportion of the Brahman genome can be attributed to *Bos taurus* ancestry (Gibbs et al., 2009). The genetic composition of individual heifers (n = 614) was estimated based on maximum likelihood as implemented in the software, Admixture (Alexander et al., 2009), using the supervised mode for clustering (K = 2) and two references populations: a pure bred *Bos indicus* (Nelore, n = 91) (Porto-Neto et al., 2013) and a pure bred *Bos taurus* (Angus, n = 81). This methodology was adapted from Porto-Neto et al. (2014). The percentage of the genomic composition that can be attributed to each of the reference populations was estimated, resulting in individual records for *Bos indicus* content. This reference population was also used to explore the allelic frequency of highly associated SNP to each of the phenotypes tested.

3.6.3 Statistical analysis

The effects of property of origin, *Bos indicus* content, and live weight at start of treatment on pregnancy outcome following FTAI were estimated using SAS 9.3 (SAS Inst. Inc., Cary, NC). For numerical tractability that would allow us to employ a linear model on a binomial phenotype (pregnant vs non-pregnant), a random uniform in the 0, 1 interval was added to the codes of 1 for pregnant and 3 for non-pregnant.

Genome-wide association studies (GWAS) were performed for each trait separately using the final dataset of 614 heifers and 51,588 SNP. For pregnancy outcome after FTAI (PREG1) and then for body weight (BW), one SNP at a time was tested for significance using Qxpak.5 (Perez-Enciso and Misztal, 2011), applying a univariate linear mixed model, which included the fixed effect of property, and two covariates, the estimated *Bos indicus* content and weight at start of treatment, as well as animal as a random additive effect via estimated genomic relationship matrix calculated using Qxpak.5, the SNP genotype (coded as 0, 1, 2) as a fixed covariate and the random residual component. The GWAS for weight used an equivalent model, but weight measurements were fitted as the dependent variable instead of a covariate in the model. Manhattan plots were generated using SNPEVG1 (Wang et al., 2012). Significant genomic regions were identified and their genomic content explored using the Variant Effect Predictor (http://asia.ensembl.org/info/docs/tools/vep/index.html, McLaren et al., 2010), and the bovine genome assembly UMD3.1 (Zimin et al., 2009).

The false discovery rate was estimated following published methods (Bolormaa et al., 2013):

$$\mathrm{FDR} = \frac{P\left(1-\frac{S}{T}\right)}{\left(\frac{S}{T}\right)(1-P)}$$

Where *P* in the p-value threshold (e.g. 0.001), *S* is the number of SNP significantly associated at that p-value and *T* is the total number of SNP tested (51,588).

The percentage of the genetic variance explained by the i-th SNP was estimated according the following formula:

$$\%V_i = 100 \text{ x } \frac{2p_i q_i \hat{\alpha}_i^{\,2}}{\sigma_g^{\,2}}$$

where pi and qi are the allele frequencies for the *i*-th SNP, $\hat{\alpha}_i$ is the estimated additive effect of the *i*-th SNP on the trait under analysis, and $\sigma_g^{\,2}$ is the REML estimate of the (poly-) genetic variance for the trait.

4 Results

4.1 Study 1

The following points summarise the major findings of Study 1, and the following subsections provide the result details.

- Follicular dynamics and ovulation rates were similar for heifers treated either with ODB or GnRH in a standard IPRD ovulation synchronisation programme. The diameter of the DF was significantly larger at time of FTAI (54 hours after IPRD removal) in GnRH treated heifers compared to the ODB treated heifers.
- The pattern of ovulations for heifers treated with the standard ODB +IPRD protocol was similar to that observed in heifers where ODB was replaced with GnRH. Most ovulations occurred between 36 to 84 hrs after IPRD removal. However, the proportion of heifers ovulating synchronously (i.e between 6 to 18 hours after FTAI) tended to be higher in the ODB treated heifers.

4.1.1 Animal data

Table 1 provides a description of the heifers used in experiments 1 and 2. The mean body condition score of enrolled heifers was 3.3 (range 3-3.5) in experiment 1 and 3.6 (ranges 3-4) in experiment 2.

Table 1: Mean and range of liveweight (LW), body condition score (BCS), and number of Brahman heifers that were cycling and enrolled in treatments OP, GP and OPF in experiment 1 and treatments OPO, GPG, and OPG in experiment 2 of study 1.

Experiment	Treatment	Cycling	n	LW (Kg)	BCS (1-5)
1	OP[A]	Yes	9	356 ± 9	3.39 ± 0.22
		No	10	346 ± 10	3.35 ± 0.24
	GP[B]	Yes	11	350 ± 7	3.32 ± 0.25
		No	8	346 ± 6	3.31 ± 0.26
	OPF[C]	Yes	8	348 ± 13	3.38 ± 0.23
		No	11	349 ± 6	3.23 ± 0.26
2	OPO[D]	Yes	18	382 ± 7	3.61 ± 0.03
		No	2	361 ± 44	3.50 ± 0.00
	GPG[E]	Yes	16	384 ± 7	3.59 ± 0.03
		No	4	373 ± 7	3.69 ± 0.03
	OPG[F]	Yes	16	385 ± 22	3.59 ± 0.12
		No	3	373 ± 7	3.67 ± 0.03

[A] OP treatment group: Control – ODB + IPRD + $PGF_{2\alpha}$;
[B] GP treatment group: - GnRH + IPRD + $PGF_{2\alpha}$
[C] OPF treatment group: ODB + IPRD + FSH + $PGF_{2\alpha}$;
[D] OPO treatment group: ODB + IPRD + $PGF_{2\alpha}$ + OBD
[E] GPG treatment group: GnRH + IPRD + $PGF_{2\alpha}$ + GnRH
[F] OPG treatment group: ODB + IPRD + $PGF_{2\alpha}$ + GnRH

4.1.2 Follicular wave emergence

The day of follicular wave emergence (FWE) was detected in 17/19, 19/19 and 17/19 heifers in the OP, GP and OPF groups, respectively. The mean follicular wave emergence (FWE) did not differ between heifers treated in the OP, GP or OPF groups. Although not significant, the mean day of FWE in the OPF treatment group tended to be approximately one day later than heifers in the OP and GP groups (Table 2; *P*=0.393). However, when analysed by cycling status, the FWE occurred two days earlier in GP heifers that were not cycling compared to OP heifers (Table 3; *P* = 0.048). Interestingly, those heifers that ovulated between 0 and 132 hrs after the removal of the IPRD had a mean FWE day of 3.86 ± 0.43 as compared to a FWE day of 6.29 ± 0.58 in heifers that did not ovulate between 0 and 132 hrs (*P* = 0.002; data not shown).

Table 2: Follicular wave emergence (FWE), dominant follicle (DF) diameter and occurrence of ovulation in synchronised Brahman heifers that were either not induced to ovulate (Exp.1) or induced to ovulate (Exp.2).

		FWE (day)[1]	Ovulation DF diameter (mm)[2]	DF diameter 54 hrs[3]	Ovulated[4]	Synchronised Ovulation[5]
	Treatment Group	*Mean*	*Mean*	*Mean*	-	-
Experiment 1	OP (n = 19)	4.81 ± 0.56 (n = 17)	13.48 ± 0.39 (n=13)	-	13 (68.4%)	3 (15.8%)
	GP (n = 19)	4.73 ± 0.61 (n = 19)	12.99 ± 0.44 (n=13)	-	13 (68.4)	1 (5.3%)
	OPF (n = 19)	5.73 ± 0.58 (n = 17)	13.21 ± 0.46 (n=9)	-	9 (47.4%)	0 (0.0%)
	P – value	0.393	0.703	-	0.947	0.309
Experiment 2	OPO (n = 20)	-	13.66 ± 0.54[b] (n=19)	10.76 ± 0.56[b]	20[a] (100.0%)	15 (75%)
	GPG (n = 20)	-	16.05 ± 0.65[a] (n=19)	12.53 ± 0.68[a]	19[ab] (95.0%)	10 (50.0%)
	OPG (n = 19)	-	14.25 ± 0.70[b] (n=13)	9.91 ± 0.73[b]	13[b] (68.4%)	7 (36.8%)
	P – value	-	0.012	0.003	0.003	0.056

1 – The first day (day = number of days after commencement of synchronisation treatment) of wave emergence was defined as the first day that a 4 to 5 mm follicle was subsequently identified as a DF. If the follicle was not detected until it was 6 to 7 mm in diameter the day prior to this was recorded (Ginther et al., 1989b).
2 – The maximum diameter of a developing follicle detected prior to ovulation in those heifers where ovulation was detected.
3 – The diameter of the DF measured 54 hrs after the removal of the IPRD. This is the standard time of fixed-time AI.
4 – Heifers that ovulated between 0 and 132 h (Exp 1) and 0 and 108 h (Exp 2) after IPRD removal. Every heifer that ovulated formed a CL.
5 – Proportion of heifers that ovulated between 60 and 72 hrs after removal of the IPRD; i.e. 6-18 hours after recommended time for FTAI

Table 3: Follicular wave emergence (FWE), dominant follicle (DF) diameter and occurrence of ovulation in cycling and non-cycling Brahman heifers that were synchronised with intravaginal progesterone releasing devices and prostaglandin $F_{2\alpha}$ with oestradiol benzoate or gonadotropin releasing hormone to initiate a new follicular wave without induction of ovulation (Experiment 1: OP and GP) and with induction of ovulation (Experiment 2: OPO or GPG).

	Treatment	n	FWE (day)[1]	DF diameter 54 hrs[2]	Ovulated[3]	Ovulation DF diameter (mm)[4]	Synchronised Ovulation[5]
Experiment 1							
Cycling	OP	10	4.1 ± 0.7	-	10 (100%)	13.4 ± 0.7	6 (60%)
	GP	12	5.7 ± 0.7	-	9 (75%)	13.1 ± 0.5	4 (33%)
	P value		*0.160*	-	*0.143*	*0.727*	*0.206*
Non-cycling	OP	9	4.4 ± 0.7	-	3 (33%)	13.9 ± 1.5	3 (33%)
	GP	7	2.4 ± 0.5	-	4 (52%)	14.5 ± 0.5	3 (43%)
	P value		*0.048*	-	*0.329*	*0.715*	*0.549*
All heifers	OP	19	4.3 ± 0.5	-	13 (69%)	13.5 ± 0.6	9 (47%)
	GP	19	4.5 ± 0.6	-	13 (69%)	13.5 ± 0.6	7 (37%)
	P value		*0.821*	-	*1.000*	*0.977*	*0.372*
Experiment 2							
Cycling	OPO	18	-	12.7 ± 0.7	18 (100%)	13.7 ± 0.6	14 (78%)
	GPG	16	-	15.3 ± 0.6	16 (100%)	15.8 ± 0.5	9 (56.3%)
	P value		-	*0.007*	*1.000*	*0.016*	*0.166*
Non-cycling	OPO	2	-	12.8 ± 1.3	2 (100%)	12.3 ± 1.3	2 (100%)
	GPG	4	-	15.5 ± 1.7	3 (75%)	13.9 ± 1.9	2 (50%)
	P value		-	*0.605*	*0.667*	*0.351*	*0.400*
All heifers	OPO	20	-	12.7 ± 0.6	20 (100%)	13.6 ± 0.6	15 (75%)
	GPG	20	-	15.0 ± 0.6	19 (95%)	15.7 ± 0.4	10 (50%)
	P value		-	*0.009*	*1.000*	*0.008*	*0.191*

1 The first day (day = number of days after commencement of synchronisation treatment) of wave emergence was defined as the first day that a 4 to 5 mm follicle was subsequently identified as a DF. If the follicle was not detected until it was 6 to 7 mm in diameter the day prior to this was recorded (Ginther et al., 1989a).

2 The diameter of the DF measured 54 h after the removal of the IPRD. This is the standard time of fixed-time AI.

3 Heifers that ovulated between 0 and 132 h (Exp 1) and 0 and 108 h (Exp 2) after IPRD removal. Every heifer that ovulated formed a CL.

4 The maximum diameter if the DF detected prior to ovulation from only those heifers that ovulation was detected in.

5 Proportion of heifers that ovulated between 48 and 72 h after removal of the IPRD.

4.1.3 Dominant follicle development

The mean dominant follicle diameter observed prior to ovulation did not significantly differ between heifers treated in the OP, GP and OPF groups (Table 2; *P* = 0.703), irrespective of cycling status (Table 3). However, heifers in the GPG treatment group had a significantly larger dominant follicle prior to ovulation and at 54 hrs after IPRD removal than heifers in the OPO and OPG treatment groups (Table 2; *P* = 0.003). Heifers that ovulated had a significantly larger dominant follicle at 54 hrs after IPRD removal than heifers that did not ovulate (13.81 ± 0.32 vs. 8.33 ± 0.95, respectively; *P*<0.001). There were no significant differences between treatment groups (OP/GP/OPG; or OPO/GPG/OPG) in the mean growth rate of the dominant follicles from 0 to 60 hrs in heifers that ovulated (Fig. 6, *P* = 0.754; Fig. 7, *P*=0.373).

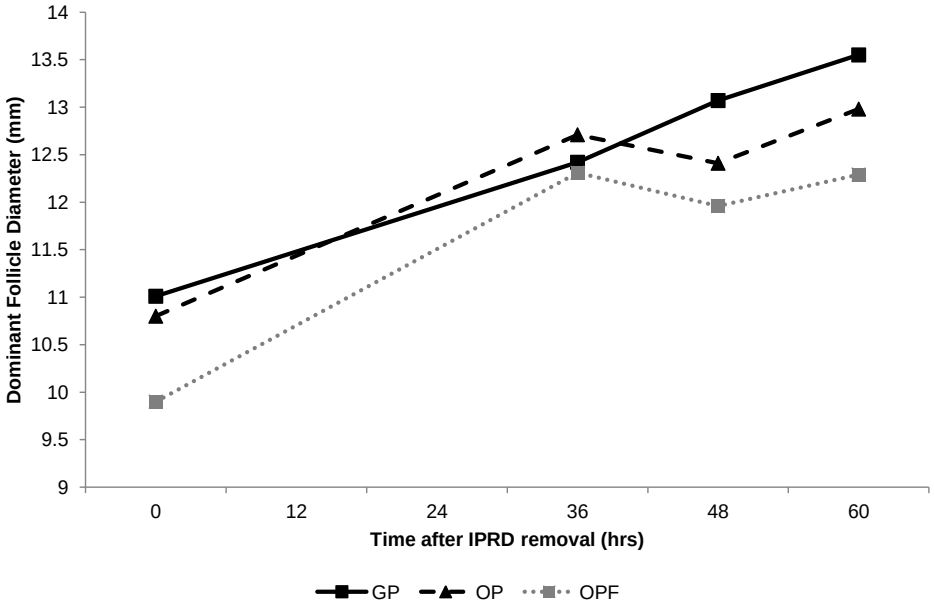


Fig. 6: Mean follicular growth after removal of IPRD in Brahman heifers treated to synchronise ovulation with OP (oestradiol benzoate (ODB) + intravaginal progesterone releasing device (IPRD) + prostaglandin ($PGF_{2\alpha}$); GP (Gonadotrophin releasing hormone + IPRD + $PGF_{2\alpha}$); or OPF (ODB + IPRD + follicle stimulating hormone + $PGF_{2\alpha}$).

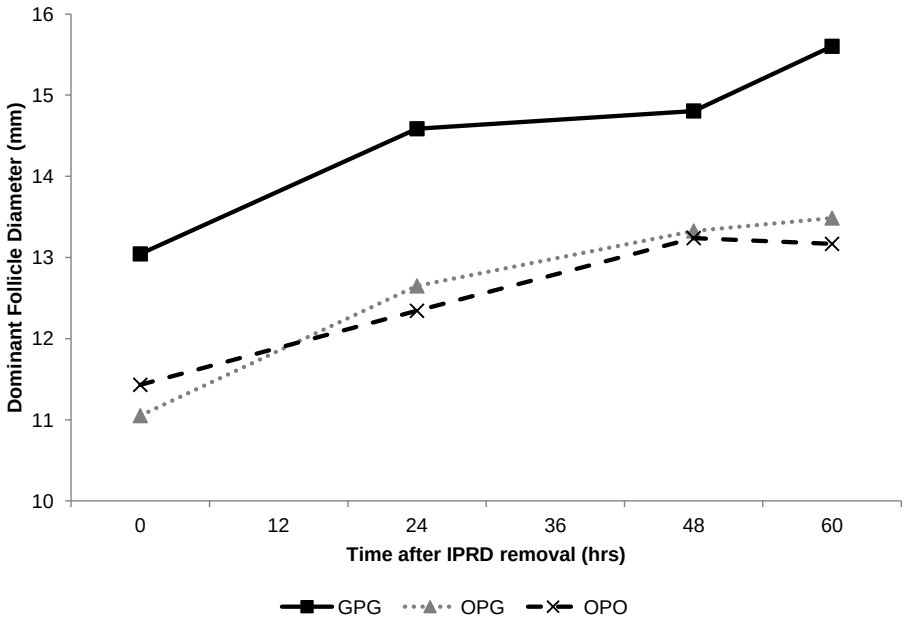


Fig. 7: Mean follicular growth after removal of IPRD in an ovulation synchronisation protocol in Brahman heifers. OPO (oestradiol benzoate (ODB) + intravaginal progesterone releasing device (IPRD) + prostaglandin ($PGF_{2\alpha}$) + ODB); GPG (Gonadotrophin releasing hormone (GnRH) + IPRD + $PGF_{2\alpha}$ + GnRH) or OPG (ODB + IPRD + $PGF_{2\alpha}$ + GnRH).

The growth and static phases of follicular development for heifers in the OP group that were cycling at the start of the experiment were normalised and plotted, as shown in **Fig. 8**. The static growth phase of the dominant follicle commenced at 5.5 d after FWE.

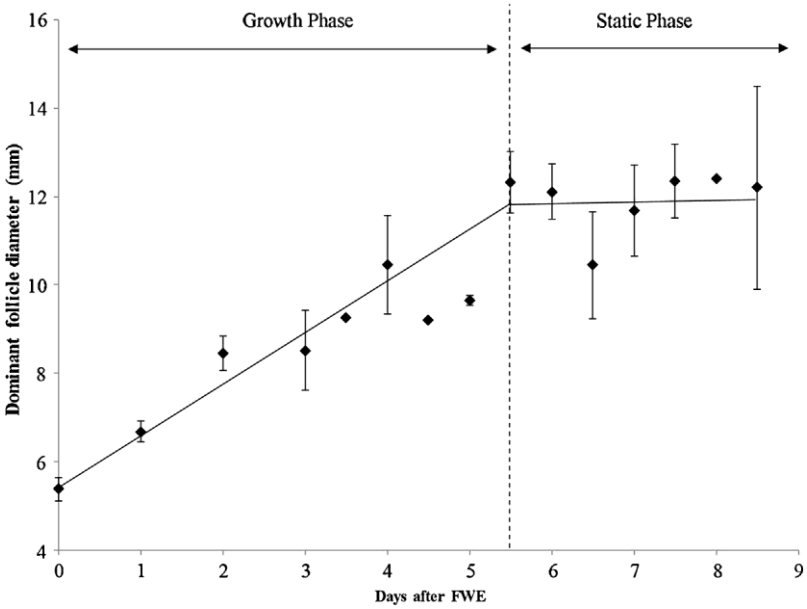


Fig. 8: The follicular growth curve from normalised first day of follicular wave emergence (FWE), including the growth and the static phase of the ovulatory follicle in those Brahman heifers where a FWE and subsequent ovulation was detected (n = 8) after treatment with intravaginal progesterone releasing devices (IPRD),oestradiol benzoate (ODB) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) but without treatment to induce ovulation.

4.1.4 Ovulation and corpus lutuem development:

The ovulation rate (heifers that ovulated between 0 and 132 hrs after the removal of the IPRD) did not significantly differ between heifers treated in the OP, GP, or OPF groups (Table 2; Fig. 9). In the OP, GP and OPF treatment groups, 1, 3 and 3 heifers, respectively, ovulated after 132 hrs. Every heifer that ovulated formed a CL by Day 23. In Experiment 2, the ovulation rate (heifers that ovulated between 0 and 108 hrs after the removal of the IPRD) was lower in the OPG group than the OPO group, but was not lower than the GPG group (Table 2; Fig. 10). There were no heifers that ovulated after 108 hrs. In the OPO group, one ovulatory heifer failed to develop a CL by Day 6.

The proportion of synchronised ovulations (proportion of heifers that ovulated between 60 and 72 hrs after the removal of the IPRD; i.e. 6-18 hours after recommended time for FTAI) did not significantly differ between OP, GP, and OPF groups, but no heifers in the OPF treatment group ovulated during this time period (Table 2). Although there were no significant differences between experiment 2 treatment groups, the OPO group had more synchronised ovulations than the GPG group or the OPG group (Table 2).

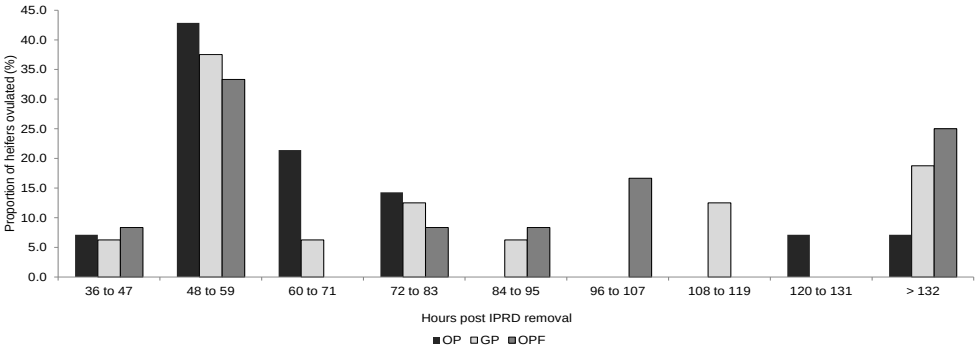


Fig. 9: Time of ovulation for Brahman heifers in experiment 1 treated with OP (oestradiol benzoate (ODB) + intravaginal progesterone releasing device (IPRD) + prostaglandin F2α (PGF2α)); GP (Gonadotrophin releasing hormone + IPRD + PGF2α) and OPF (ODB + IPRD + follicle stimulating hormone + PGF2α).

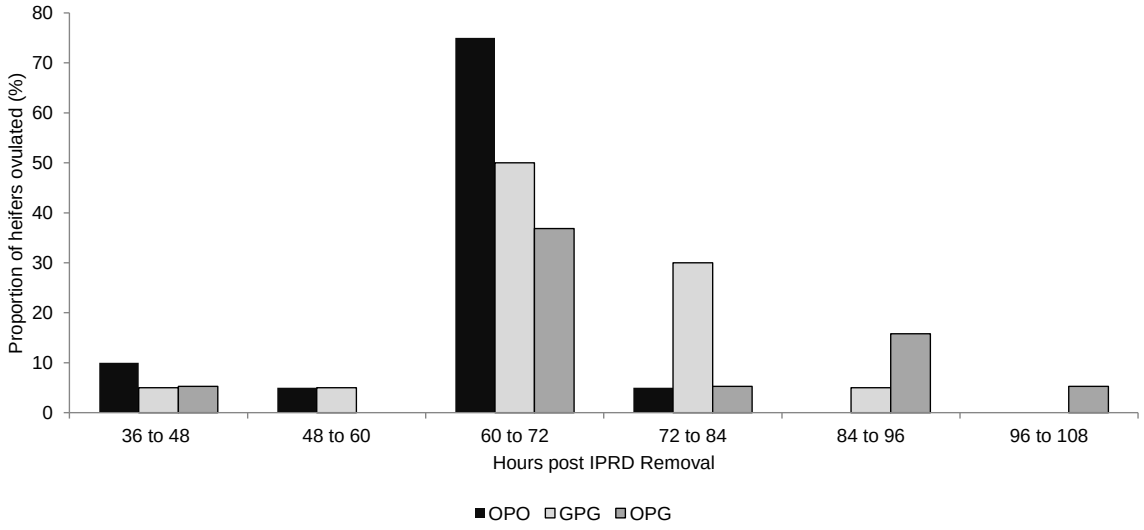


Fig. 10: Time of ovulation in Brahman heifers in experiment 2 treated with OPO (oestradiol benzoate (ODB) + intravaginal progesterone releasing device (IPRD) + prostaglandin ($PGF_{2\alpha}$) + ODB); GPG (Gonadotrophin releasing hormone (GnRH) + IPRD + $PGF_{2\alpha}$ + GnRH); or OPG (ODB + IPRD + $PGF_{2\alpha}$ + GnRH).

Analysis of the effect of the first injection of GnRH (on the day of FWE, the mean largest follicle diameter at IPRD insertion, IPRD removal and ovulation, the incidence of ovulation, and the time of ovulation) is presented in **Table 4**. In experiment 1, only 5/19 heifers responded to the first GnRH treatment (i.e. ovulated or formed a CL) despite 13/19 heifers having a follicle ≥ 10 mm at time of treatment, and only 3 of the 5 heifers that responded subsequently ovulated after IPRD removal. In experiment 2, 2/20 heifers responded to the first GnRH treatment and only one of these subsequently ovulated after IPRD removal. However, 18/18 of those heifers that did not respond to the first GnRH treatment subsequently ovulated.

Table 4: Incidence of ovulation to first GnRH injection in Brahman heifers treated to synchronise ovulation with GP (Gonadotrophin releasing hormone (GnRH) + intravaginal P_4 releasing devices (IPRD) + prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)) protocol (Experiment 1) and with GPG (GnRH + IPRD + $PGF_{2\alpha}$ + GnRH) protocols (Experiment 2). Effect on mean largest follicle diameter (LF) at IPRD insertion, mean day of follicular wave emergence (FWE), LF diameter at IPRD removal, mean LF diameter at ovulation, proportion that ovulated and the mean time of ovulation after IPRD removal.

		n	Mean LF diameter at IPRD insertion	Mean FWE	LF diameter at IPRD removal	Ovulated 2nd GnRH injection	Mean LF diameter at 54 hrs (FTAI)	Mean LF diameter at ovulation	Ovulated	Mean time of ovulation
	Ovulated to 1st GnRH treatment		*%*	*Day*	*%*		*mm*	*mm*	*%*	*Hrs (range)*
Experiment 1	Yes	5/19	11.24 ± 0.60	5.0 ± 0.8 (n = 13)	11.20 ± 0.64	-	13.37 ± 0.39 (n = 4)	12.88 ± 0.33 (n = 3)	3/5 (60.0)	68.0 ± 8.2 (36 to 132)
	No	14/19	10.56 ± 0.67	3.0 ± 0.5 (n = 5)	10.98 ± 0.96	-	12.04 ± 1.03 (n = 8)	13.68 ± 0.43 (n = 10)	10/14 (71.4)	78.4 ± 10.1 (48 to 84)
	P – value	0.004	0.583	0.149	0.894	-	0.517	0.428	0.637	0.654
Experiment 2	Yes	2/20	11.01 ± 0.56	-	11.59 ± 0.32	0/2 (0.0)	12.53 ± 3.2	16.03 ± 0.00* (n = 1)	1/2 (50.0)	84.0 ± 0.0
	No	18/20	11.07 ± 0.57	-	13.11 ± 0.68	1/18 (5.6)	15.27 ± 0.53	15.72 ± 0.47 (n = 18)	18/18 (100.0)	74.7 ± 2.5 (48 to 96)
	P – value	<0.001	0.970	-	0.477	0.732	0.158	0.880	0.002	0.401

4.2 Study 2

The following points summarise the major findings of Study 2, and the following subsections provide the result details.

- Replacing ODB with GnRH in the standard ovulation synchronisation7 day IPRD protocol consistently resulted in significantly lower pregnancy rate to FTAI (overall GnRH 29.5% v's ODB 40.7%). However, the pregnancy rate to natural mating between 10 to 30 days after FTAI was higher in the GnRH treated heifers than the ODB treated heifers (28.2% v's 20.8 %, respectively). Overall, use of FTA followed by natural mating commencing 10 days later resulted in 61.5 % of ODB treated heifers and 57.7% of GnRH treated heifers becoming pregnant within an approximately 4 week period.
- Brahman heifers that had a CL at the commencement of treatment to synchronise ovulation achieved significantly higher pregnancy rates to FTAI than those heifers which did not have a CL (36.7% v's 29.0%).
- Despite there being significant variation in liveweight of heifers at the time of commencement of treatments to synchronise ovulation (range 280 to 440+ kgs) there was no significant relationship between liveweight and pregnancy rate to FTAI.

4.2.1 Animal data

Table 5 provides a summary (mean and range of liveweights and body condition scores, and proportion of heifers with a CL) of all enrolled heifers at the commencement of this study.

Table 5: The mean ± SEM and range of liveweight (LW) and body condition scores (BCS) of selected Brahman heifers, and the proportion with a CL at the time heifers were enrolled in the study (Day 0).

Property	n	LW (kg)	BCS (1-5)	Presence of CL (%)
A	408	376.0 ± 1.5 (301 – 456)	3.25 ± 1.01 (2.50 – 4.00)	317 (77.7)
B	73	356.3 ± 3.5 (296 to 442)	3.50 ± 0.00 (3.50– 3.50)	64 (87.7)
C	450	349.7 ± 1.5 (284 to 463)	3.69 ± 0.01 (3.00 - 4.50)	344 (76.4)
D	213	394.2 ± 2.6 (294 – 526)	3.51 ± 0.01 (3.00 – 4.00)	183 (85.9)

4.2.2 Analysis of allocation procedures

The procedures used to allocate heifers to their respective treatment groups (OPO or GPG) were retrospectively analysed to determine whether there were any significant differences between the heifers in the OPO or GPG treatment groups (Appendix III, Section 11.1). No significant differences were detected.

The AI technician allocation procedure was retrospectively analysed to verify that there was no AI technician bias between the OPO and GPG groups (Appendix III, Section 11.1). The procedures used to allocate heifers to sires was retrospectively analysed to verify that there was no allocation bias between the OPO and GPG groups (Appendix III; Section 11.2). AI Sire and AI technician did not significantly affect pregnancy rates to FTAI (P = 0.133, and *P* = 0.467, respectively).

A REML analysis was performed to ensure no significant differences between batches of semen between sires (*P* = 0.659), and thus batch number was excluded from the analyses.

4.2.3 Reproductive performance analysis

The pregnancy rates to FTAI (A: 29.97%, B: 38.34%, C: 38.16% and D: 41.23%; *P* = 0.253) and to natural mating (A: 23.78%, B: 13.64%, C: 26.99% and D: 25.52%; *P* = 0.113) did not significantly differ between properties. Similarly, there was no significant difference in pregnancy rates to FTAI or natural mating between AI groups within property (**Table 6**; *P* = 0.785 and P = 0.864, respectively).

Table 6: Pregnancy rate (PR) of Brahman heifers to fixed time AI (FTAI) and natural mating (after the AI program), treated for ovulation synchronisation on Properties A, B, C and D with either oestradiol benzoate (OPO) or gonadotrophin releasing hormone (GPG) in conjunction IPRD (0.78 g P_4) and cloprostenol on pregnancy rate (PR) to fixed-time AI (FTAI) and natural mating. The PR to FTAI and natural mating did not significantly differ between properties (P = 0.785 and P = 0.864, respectively).

		PR (%)	
Property	Treatment	FTAI*	Natural Mating†
A	OPO (n = 198)	36.9	20.2
	GPG (n = 186)	24.2	27.4
B	OPO (n = 37)	48.6	8.1
	GPG (n = 36)	30.6	16.7
C	OPO (n = 221)	43.9	14.9
	GPG (n = 224)	33.5	25.0
D	OPO (n = 108)	42.9	12.4
	GPG (n = 107)	29.1	16.5

* Pregnant to FTAI

† Pregnant to natural service during the first return to oestrus (i.e. conceived within 10 to 30 days after AI).

Heifers synchronised with ODB had a significantly higher pregnancy rate to FTAI than heifers synchronised with GnRH. Conversely, heifers synchronised with ODB had a significantly lower pregnancy rate to natural mating then heifers synchronised with GnRH. The presence of a CL and the dominant follicle diameter at IPRD insertion had a significant effect on pregnancy rates to FTAI, but not to natural mating (**Table 7**).

Table 7: Effect of synchronisation treatment, CL presence, and follicle diameter at intravaginal progesterone (P_4) releasing device (IPRD) insertion in Brahman heifers treated for ovulation synchronisation with either oestradiol benzoate (OPO) or gonadotrophin releasing hormone (GPG) in conjunction IPRD (0.78 g P_4) and cloprostenol on pregnancy rate (PR) to fixed-time AI (FTAI) and natural mating. Superscripts signify significant differences (P<0.05).

		PR (%)	
		FTAI*	Natural mating†
Treatment			
	OPO	40.66[a]	20.75[b]
	GPG	29.52[b]	28.23[a]
	P-value	<0.001	0.004
CL at IPRD insertion			
	Present	36.70[a]	25.82
	Absent	28.98[b]	22.65
	P-value	0.029	0.446
DF diameter at IPRD insertion (mm)			
	≤ 5	38.60[ab]	22.80
	6 to 7	32.40[b]	29.47
	8 to 9	29.68[b]	25.04
	10 to 11	35.47[ab]	27.08
	≥ 12	44.00[a]	20.56
	P-value	0.022	0.391

* Pregnant to FTAI

† Pregnant to natural service during the first return to oestrus (i.e. conceived within 10 to 30 days after AI) – cover up bulls.

The mean LW and BCS at IPRD insertion, the mean gain (adjusted to 60 days post FTAI) in LW, and the mean change in BCS from IPRD insertion to pregnancy diagnosis did not significantly differ between heifers that were pregnant to FTAI or natural mating (**Table 8**).

Table 8: Effects of mean liveweight (LW) and body condition score (BCS) ± SEM at Day 0, and mean change in BCS and mean LW gain from Day 0 to pregnancy diagnosis (adjusted to 60 days post AI), on the overall pregnancy rate and pregnancy rate to fixed-time AI (FTAI) and natural mating in Brahman heifers treated with oestradiol benzoate (OPO) or gonadotrophin releasing hormone (GPG) based ovulation synchronisation protocols in conjunction with intravaginal progesterone releasing devices containing 0.78 g progesterone and cloprostenol.

	LW (kg)				BCS (1-5)			
	Day 0	**P-value**	**Mean Gain[d]**	**P-value**	**Day 0**	**P-value**	**Mean Change**	**P-value**
NDP[a] (n=479)	366.9	-	50.8	-	3.5	-	0.4	-
Pregnant FTAI[b] (n=389)	367.2	0.979	52.4	0.336	3.5	0.906	0.4	0.985
Pregnant Natural[c] (n=240)	369.0	0.344	49.2	0.311	3.5	0.953	0.3	0.580

[a] Not detected pregnant (NDP).
[b] Pregnant to FTAI
[c] Pregnant to natural mating during first return to oestrous cycle (conceived within 10 to 30 days after AI).
[d] Gain = the gain in LW or BCS from Day 0 to pregnancy diagnosis (adjusted to 60 days post AI).

When the pregnancy rates to FTAI and natural mating were examined between LW categories at IPRD insertion, there were no significant differences (**Table 9**).

Table 9: Predicted pregnancy rate of Brahman heifers in liveweight (LW) categories at the time of intravaginal progesterone releasing device (IPRD) insertion that were treated to synchronise ovulation with an IPRD and either oestradiol benzoate or gondotrophin releasing hormone.

		Pregnancy Rate %	
LW category (kg)	n	FTAI	Natural mating
280 to 299	21	48.10	37.14
300 to 319	71	41.16	16.78
320 to 339	176	37.69	24.78
340 to 359	217	30.81	28.73
360 to 379	246	42.19	19.06
380 to 399	196	36.52	26.10
400 to 419	112	46.41	26.26
420 to 439	73	42.29	30.02
440 +	31	29.21	19.09
	P-value	0.273	0.158

4.3 Study 3a

The following points summarise the major findings of Study 3a, and the following subsections provide the result details.

- Decreasing the duration of insertion of the IPRD by 2 days and extending the interval from IPRD removal to treatment with ODB by 12 hours resulted in similar follicular dynamics to that observed with the standard protocol; there was a tendency for improved development of the dominant follicle and a higher ovulation rate.
- From a practical perspective the modified protocol reduced the period heifers needed to be held in holding paddocks to complete all treatments to synchronise ovulation and enabled FTAI to be conducted during the cooler early morning period rather than at mid-day.

4.3.1 Animal data

The mean LW and BCSs of heifers in each treatment group are presented in Table 10. At commencement of the study all heifers had a CL and weighed an average of 374.8 ± 3.8 kg (range 309–453 kg) and had an average BCS of 3.63 ± 0.04 (range 3–4).

Table 10: Mean LW (kg) and BCS ± SEM of Brahman heifers allocated to either OPO-8, OPO-6 or PO-6 treatment groups.

Treatment group	LW (kg)	BCS (1-5)
OPO-8 (control; n = 20)	374.6 ± 6.2	3.55 ± 0.07
OPO-6 (n = 20)	374.5 ± 7.5	3.63 ± 0.08
PO-6 (n = 20)	375.4 ± 6.3	3.73 ± 0.06

4.3.2 Follicular wave emergence and dominant follicle diameter

A new follicular wave emerged after IPRD and ODB treatment in every heifer treated with the OPO-8 and OPO-6 protocols. In these groups, all follicles present at the time of IPRD insertion became atretic and were no longer present at the time of ovulation. However, in the PO-6 protocol, a new follicular wave was not detected in 5/20 heifers (P = 0.009). In these heifers, the ovulatory follicle was present at the time of IPRD insertion (three were of ovulatory capacity, ≥8.5 mm, (Gimenes et al., 2008)), and continued to grow from the day of IPRD insertion until ovulation. The day of FWE was detected in 18/20 and 17/20 heifers treated with OPO-8 and OPO-6 protocols, respectively, and did not differ between heifers in these groups. Of the 15 PO-6 treated heifers that had a follicle emerge after IPRD insertion, the day of FWE was two days earlier than heifers treated with the OPO-8 protocol (*P* = 0.008), but not significantly different to heifers treated with the OPO-6 protocol (**Table 11**). Interestingly, 9/15 of these PO-6 heifers had a follicle of ovulatory capacity at IPRD insertion, which subsequently became atretic.

Estimated follicle diameter at ovulation (13.03 ± 0.66 mm vs. 11.67 ± 0.59 mm; P = 0.188) and ovulation rate (100% vs. 86.7%; P = 1.000) did not significantly differ between PO-6 treated heifers with follicles that continued to grow and heifers that had initiated a new follicular wave. However, the diameter of the dominant follicle tended to be larger (12.51 ± 0.57 mm vs. 10.33 ± 0.57 mm; P = 0.052) at the time of FTAI in the five heifers in which a new follicular wave did not emerge after IPRD insertion. **Table 12** summarises and compares follicular growth characteristics in PO-6 heifers that did and did not initiate a new follicular wave.

The mean maximum diameter of the ovulatory follicle was not significantly different between the OPO-8, OPO-6, and PO-6 protocols. Although not significant, there was a general trend for the mean dominant follicle diameter at FTAI to be smallest in heifers treated with the OPO-8 protocol, intermediate in heifers treated with the OPO-6 protocol, and largest in heifers treated with the PO-6 protocol (**Table 11**).

Table 11: Follicular wave emergence (FWE), dominant follicle (DF) diameter and occurrence of ovulation in synchronised Brahman heifers treated to synchronise ovulation with oestradiol benzoate (ODB) and an intravaginal progesterone releasing device (IPRD) inserted for 8 (OPO-8) or 6 days (OPO-6) or and IPRD inserted for 6 days without ODB treatment (PO-6), and prostaglandin $F_{2\alpha}$ at removal.

	FWE (day)[1]	**n (%) with DF >8.5mm at induction of ovulation**	**DF diameter at ovulation (mm)[2]**	**DF diameter at FTAI (mm)[3]**	**Proportion that ovulated[4]**	**Synchronised ovulation**	**Duration of IPO (h)[5]**
Treatment Group	*Mean*	*n and %*	*Mean± SEM*	*Mean ± SEM*	*n and %*	*n and %*	*Mean± SEM*
OPO-8 (n = 20)	5.3 ± 0.4[a] (n = 18)	14 (70%)	11.13 ± 0.54	9.74 ± 0.51 (n = 18)	13 (65.0%)	12 (60%)	73.8 ± 1.9[a] (n = 13)
OPO-6 (n = 20)	4.0 ± 0.4[ab] (n = 17)	16 (80%)	11.47 ± 0.50	10.52 ± 0.51 (n = 17)	15 (75.0%)	15 (75%)	87.2 ± 1.8 [b] (n = 15)
PO-6 (n = 20)	3.4 ± 0.4[b] (n = 14)	17 (85%)	11.77 ± 0.45	11.34 ± 0.50 (n = 19)	18 (90.0%)	18 (90%)	84.8 ± 1.7[b] (n = 16)*
P – value	0.008	0.703	0.667	0.091	0.177	0.125	<0.001

1 The first day (day = number of days after commencement of synchronisation treatment) of wave emergence was defined as the first day that a 4 to 5 mm follicle was subsequently identified as a DF. If the follicle was not detected until it was 6 to 7 mm in diameter, the day prior to this was recorded (Ginther et al., 1989b).

2 The maximum diameter of the developing follicle detected prior to ovulation from only those heifers where ovulation was detected.

3 The diameter of the developing follicle measured at the time of FTAI (54 h after the removal of the IPRD in OPO-8 group or 72 h after the removal of the IPRD in OPO-6 and PO-6 group).

4 Heifers that ovulated between 0 and 96 h after IPRD removal. Every heifer that ovulated formed a CL.

5 Time (hours) from removal of IPRD and prostaglandin $F_{2\alpha}$ treatment to estimated time of ovulation (note: time of ovulation was recorded in 12 h intervals).

* Two heifers in the PO-6 group ovulated after 96 h and so specific time of ovulation was not recorded. These heifers were removed from this data set.

[a,b] superscripts differ $P< 0.05$.

Table 12: Follicular growth in heifers treated with the PO-6 (n = 20) protocol. Characterisation of follicles that grew from IPRD insertion to expected time of ovulation, and follicles that emerged after insertion of the IPRD.

	Diameter of DF at IPRD insertion[1]		**Ovulation DF diameter (mm)[2]**	**DF diameter FTAI[3]**	**Ovulated[4]**	**Synchronised Ovulation[5]**	**Pregnant to FTAI[7]**			
Follicle origin	5 – 8.4 mm	≥ 8.5 mm	-	-	-	-	5 – 8.4 mm	≥ 8.5 mm	*P-value*	***Total***
Present at IPRD insertion (n = 5)	2 (10.0%)	3 (15.0%)	13.03 ± 0.62 (n = 5)	12.87 ± 0.55 (n = 4)	5 (100.0%)	5 (100.0%)	2 (100.0%)	1 (33.3%)	*0.136*	3 (60.0%)
Emerged after IPRD insertion (n = 15)	6* (30.0%)	9* (45.0%)	11.28 ± 0.66 (n = 13)	10.38 ± 0.47 (n = 15)	13 (86.7%)	11 (73.3%)	2 (33.3%)	2 (22.2%)	*0.634*	4 (26.7%)
P – value	-	-	0.128	0.039	0.389	0.197	0.102	0.700	-	0.176

1 – Diameter of the dominant follicle at insertion of the IPRD (0.78 g progesterone). Follicles ≥ 8.5 mm are defined as having ovulatory capacity in *Bos indicus* heifers (Gimenes et al., 2008).
* - All follicles regressed.
2 - The maximum diameter if the DF detected prior to ovulation from only those heifers that ovulation was detected in.
3 – The diameter of the DF measured at the time of FTAI, 72 h after the removal of the IPRD.
4 – Heifers that ovulated between 0 and 96 h after IPRD removal. Every heifer that ovulated formed a CL.
5 – Proportion of heifers that ovulated between 12 h prior and 24 h after FTAI i.e. between 60 and 96 h after removal of the IPRD.
6 – The number and proportion of heifers in the PO-6 treatment group diagnosed pregnant to FTAI at 27 days post AI.

4.3.3 Ovulation rate and synchrony

Although the ovulation rate was highest in heifers treated with the PO-6 protocol it was not significantly different to that observed in heifers treated with the OPO-8 and OPO-6 protocols (**Table 11**). The proportion of heifers that had a synchronised ovulation (i.e. ovulated between 12 h prior and 24 h after the time of FTAI) was highest in the PO-6 and OPO-6 protocol as compared to the OPO-8 protocol, respectively, but this difference was not significant ($P = 0.125$) (**Table 11**). The time and proportion of heifers that ovulated in each group is presented in **Fig. 11**. All heifers that ovulated, irrespective of treatment group, subsequently formed a CL. The interval from IPRD removal to ovulation (IPO) was significantly ($P < 0.001$) shorter in heifers treated with the OPO-8 protocol as compared to the OPO-6 and PO-6 protocols (**Table 11**).

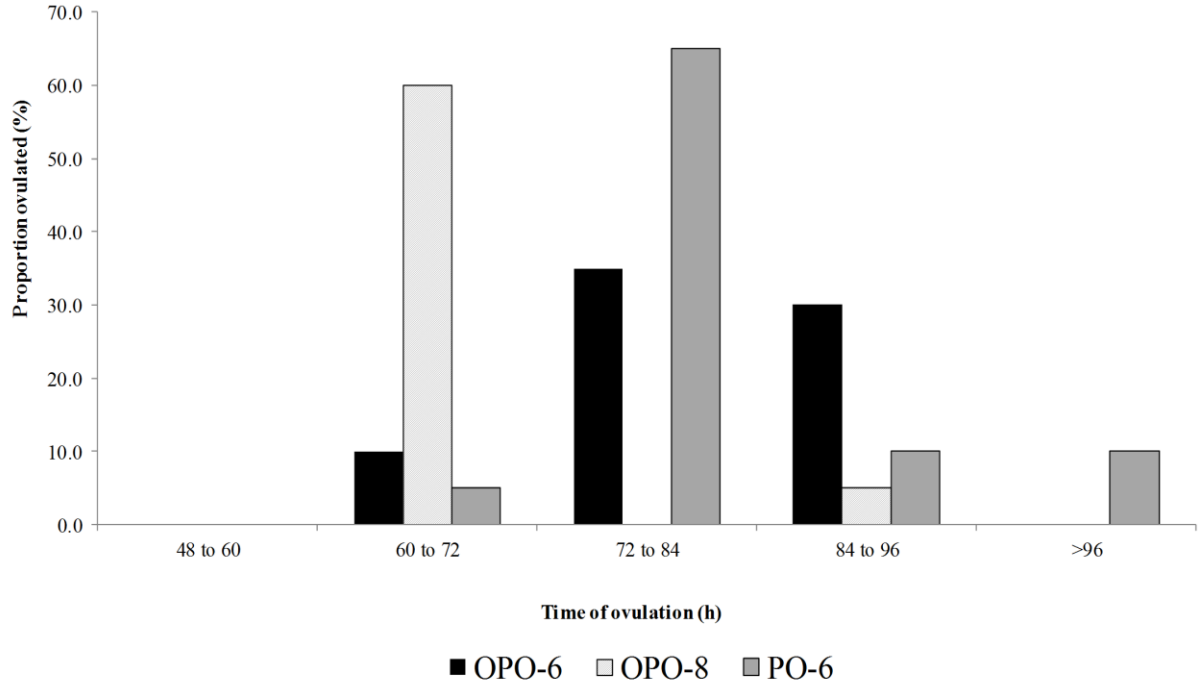


Fig. 11: Time of ovulation and the proportion of heifers that ovulated in Brahman heifers treated to synchronise ovulation with oestradiol benzoate (ODB) and intravaginal progesterone releasing device (IPRD) inserted for 8 (OPO-8) and 6 days (OPO-6) or an IPRD inserted for 6 days without ODB treatment (PO-6), and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) at removal.

4.3.4 Pregnancy rate to FTAI

The overall pregnancy rate did not significantly differ between groups (**Table 13**; *P* = 0.931). When pregnancy rate was analysed per synchronised ovulation (heifers that ovulated between 12 h prior and 24 h after FTAI), heifers in the PO-6 group had the lowest rate, although this was not significantly different from the OPO-8 and OPO-6 groups (**Table 13**). Among heifers that ovulated between 12 h prior and 24 h after FTAI, those in the OPO-6 treatment group had a 10% higher pregnancy rate than those in the OPO-8 and PO-6 treatment groups.

Table 13: Total number of heifers pregnant (at 27 to 28 days post FTAI, for OPO-6, PO-6 and OPO-8 heifers, respectively) and the proportion of pregnant heifers that had synchronously ovulated, expressed as pregnancy per ovulation and pregnancy per group, in Brahman heifers treated to synchronise ovulation with oestradiol benzoate, intravaginal progesterone releasing devices inserted for 8 (OPO-8) or 6 (OPO-6 and PO-6) days, and prostaglandin $F_{2\alpha}$.

	Synchronised Ovulation (within 12 h prior and 24 h post FTAI)			**Total Pregnant to FTAI**
Treatment Group	n	Pregnant per ovulation	Pregnant per group	-
OPO-8 (n = 20)	12 (60.0%)	6/12 (58.3%)	6/20 (30.0%)	7 (35.0%)
OPO-6 (n = 20)	15 (75.0%)	8/15 (53.3)	8/20 (40.0%)	8 (40.0%)
PO-6 (n = 20)	16 (80.0%)	6/16 (37.5%)	6/20 (30.0%)	7 (35.0%)
P – value	0.125	0.650	0.741	0.931

4.4 Study 3b

The following points summarise the major findings of Study 3b, and the following subsections provide the result details.

- The pregnancy rate to FTAI in heifers treated with the standard protocol compared to those treated with the modified protocol described above was similar (37.6 v's 40.0, respectively), with some evidence that the outcome following use of the modified protocol may in fact be significantly better than that achieved with the standard protocol.
- When all properties were included in the analysis, heifers with a CL present had a significantly higher pregnancy rate than heifers that did not have a CL present (43.9 v's 28.8, respectively.
- This study also enabled direct comparison of the outcome of natural mating of Brahman heifers with that achieved after FTAI where the standard treatment protocol was used to synchronise ovulation. The results of previous work conducted by the authors indicate that approximately 75% of Brahman heifers respond normally to the standard IPRD treatment protocol to synchronise ovulation (i.e ovulate synchronously and form a normal functional CL), and thus have a normal probability of becoming pregnant to AI. Natural mating pregnancy rates per oestrous cycle assist in understanding what is achievable to FTAI. For example, the average natural mating pregnancy rate per oestrous cycle on Property C in this study was 58.3%; if only 75% of heifers treated to synchronise ovulation synchronously ovulated then the probability of becoming pregnant to FTAI would be 43.7%. Interestingly, the actual FTAI pregnancy rate for Property C was 43.4%. Similarly, at Property B, the likelihood of becoming pregnant to FTAI was calculated to be 25.5% (34% of 75%) and the actual FTAI pregnancy rate was 27.1%. This data demonstrates that pregnancy rates to FTAI were remarkably similar to pregnancy rates per cycle to natural mating when analysed under the same conditions. This highlights the significant impact of the inherent fertility of the herd on the outcome of FTAI.

4.4.1 Animal data

The mean LW, BCS, and proportion of Brahman heifers with a CL at the commencement of oestrus synchronisation treatments on properties A, B, and C are provided in **Table 14**. Heifers at property B had a higher mean LW and BCS compared to those on properties A and C ($P < 0.001$). The proportion of heifers with a CL on Day 0 on property A (62%) was lower than on property C (71.2%; $P = 0.018$), but similar to those on property B (66.8%).

Table 14: The mean liveweight (LW), body condition score (BCS) and proportion of Brahman heifers with a corpus luteum (CL) at commencement of treatments to synchronise ovulation at Properties A, B and C.

Property	LW (kg)	BCS	CL
A	343.0 ± 1.6[b]	3.36 ± 0.01[c]	62.0[b]
B	382.6 ± 2.0[a]	3.80 ± 0.02[a]	66.8[ab]
C	337.9 ± 1.7[c]	3.42 ± 0.01[b]	71.2[a]
P-value	<0.001	<0.001	0.018

The mean LW, BCS, and proportion of heifers cycling in the NATM and FTAI groups prior to commencement of oestrus synchronisation treatments on properties B and C are provided in **Table 15**.

Table 15: The mean liveweight (LW) and body condition score (BCS), and proportion of Brahman heifers cycling that were allocated to either natural mating (NATM) or fixed-time artificial insemination with the OPO-8 protocol and exposure to bulls (FTAI) prior to commencement of study on Properties B and C.

	Property B			Property C		
Breeding strategy	NATM (n = 108)	FTAI (n = 96*)	P-value	NATM (n = 106)	FTAI (n = 196)	P-value
Proportion cycling (%)	71.3	76.0	0.525	87.7	87.8	1.000
LW** (kg)	387.6 ± 3.7	387.4 ± 3.9	0.971	323.7 ± 2.5	321.1 ± 1.8	0.410
BCS**	3.76 ± 0.03	3.79 ± 0.03	0.801	3.45 ± 0.03	3.44 ± 0.02	0.719

* Note: Only 96 heifers treated with the OPO-8 protocol were available for 2 ovarian scans and therefore were the only heifers eligible for comparison against the natural mating.
** LW and BCS recorded on first ovarian scan 24 days prior to commencement of mating.

4.4.2 Analysis of allocation procedures

Retrospective analysis of potential confounding factors was conducted and details are presented in Appendix III. At the commencement of treatments to synchronise ovulation (Day 0), heifers allocated to the OPO-8 treatment had a lower mean live-weight than the OPO-6 heifers on properties A and C (mean difference ~14 and 10 kg, respectively) but not on property B. Also, the mean BCS of the OPO-8 heifers was lower than the OPO-6 heifers (mean difference of 0.1) at property A, but not at properties B or C. The proportion of heifers with a CL at Day 0 did not differ between treatments at any property (Appendix III; Section 11.3).

The allocation of heifers to AI technicians at property A and C did not significantly differ with respect to LW, BCS or presence of a CL (Appendix III; Section 11.4). At Property A, the BCS of heifers inseminated on Day 1 of AI was significantly lower than heifers inseminated on Day 2 of AI and the stud heifers were significantly heavier in weight than the commercial heifers. At Property C, homebred heifers were significantly lower in weight, but significantly higher in BCS than the purchased heifers (Appendix III; Section 11.4).

Retrospective analysis confirmed there was no bias in sire allocation at Property A, B, or C with respect to LW (P = 0.215, P = 0.071 and P = 0.647, respectively), BCS (P = 0.320, P = 0.277 and P = 0.911, respectively), and presence of a CL (P = 0.528, P = 0.332 and P = 0.442).

4.4.3 Effects of treatment and heifer-level factors on pregnancy rates

Overall, there was no significant difference in pregnancy rates between the two synchronisation protocols (**Table 16**). However, at property A, a significant difference was observed on the second day of AI; OPO-6 heifers achieved a significantly higher pregnancy rates (52.4%) to FTAI than OPO-8 heifers (36.8%). No significant differences were found between treatments at properties B and C (**Table 16**).

When all properties were analysed together, property B was found to have a significantly lower pregnancy rate to FTAI (31.29 %), than properties A (41.38 %) and C (41.03 %) (P = 0.012).

Table 16: The effect of ovulation synchronisation treatment (OPO-8 or OPO-6)[1] on pregnancy rate to fixed-time artificial insemination (FTAI) in Brahman heifers on three different properties and the interaction of Day of artificial insemination (AI)[2]. Superscripts a,b differ significantly P<0.05.

		Ovulation synchronisation treatment[1]		
Property	Day of AI	OPO-8	OPO-6	P-Value
A	1	46/115 (39.9)[b]	42/123 (35.7)[b]	0.024
	2	39/103 (36.8)[b]	57/112 (52.4)[a]	
B	-	35/128 (26.2)	50/143 (35.4)	0.090
C	-	85/196 (43.2)	79/197 (40.3)	0.570
Total	-	205/542 (37.6)	228/575 (40.0)	0.413

[1] Ovulation synchronisation treatments: OPO-8: 1 mg oestradiol benzoate (ODB) + Intravaginal progesterone releasing device (IPRD) inserted for 8 d. 500 µg cloprostenol ($PGF_{2\alpha}$) at IPRD removal and 1 mg ODB 24 h later. Fixed-time artificial insemination (FTAI) at 54 h post IPRD removal. OPO-6: 1 mg ODB + IPRD inserted for 6 days. 500 µg at IPRD removal and 1 mg ODB 36 h later. FTAI at 72 h post IPRD removal.
[2] Day of AI: FTAI occurred over two days at Property A to enable all inseminations to occur within 4 h for each group.

The presence of a CL at the commencement of ovulation synchronisation affected pregnancy rates to FTAI. When all properties were included in the analysis, heifers with a CL present had a significantly higher pregnancy rate than heifers that did not have a CL present (43.9 v's 28.8 respectively; **Table 17**). When properties were analysed separately, at property B heifers that had a CL present had a significantly higher pregnancy rate to FTAI than heifers that did not have a CL (**Table 17**). At property A, an interaction between ovulation synchronisation treatment and presence of CL was observed; heifers receiving the OPO-6 protocol with a CL present had a higher pregnancy rate (*P* = 0.032) than heifers that did not have a CL (**Table 17**). At property C, there was a trend for heifers with a CL to have a higher pregnancy rate to FTAI than those without a CL (44.0 % and 36.0 %, respectively; *P* = 0.142).

Table 17: The effect of the presence or absence of a corpus luteum (CL)[2] on pregnancy rate to fixed-time artificial insemination (FTAI) in Brahman heifers on three different properties and the interaction of ovulation synchronisation treatment (OPO-6 or OPO-8)[2]. Superscripts a,b,c differ significantly (P<0.05) within property.

		Presence or absence of CL on day 0[1]		
Property	Ovulation synchronisation treatment[2]	CL	No CL	P-Value
A	OPO-8	61/144 (42.7)[b]	24/74 (31.4)[bc]	0.032
	OPO-6	74/137 (55.4)[a]	25/98 (24.5)[c]	
B	OPO-6 + OPO-8	67/181 (35.0)[a]	18/90 (23.1)[b]	0.049
C	OPO-6 + OPO-8	123/280 (44.0)	41/113 (36.0)	0.142
Total	OPO-6 + OPO-8-	325/742 (43.9)[a]	108/375 (28.8)[b]	<0.001

[1] Presence of a CL on the day of insertion of the intravaginal progesterone releasing device (IPRD; Day 0) diagnosed by transrectal ultrasonography.
[2]Ovulation synchronisation treatments: OPO-8: 1 mg oestradiol benzoate (ODB) + IPRD inserted for 8 d. 500 µg cloprostenol ($PGF_{2\alpha}$) at IPRD removal and 1 mg ODB 24 h later. Fixed-time artificial insemination (FTAI) at 54 h post IPRD removal. OPO-6: 1 mg ODB + IPRD inserted for 6 days. 500 µg at IPRD removal and 1 mg ODB 36 h later. FTAI at 72 h post IPRD removal.

The LW category of heifers at commencement of treatments to synchronise ovulation did not have a significant effect on the pregnancy rate to FTAI when data from all properties were analysed together. When properties were analysed separately, pregnancy rate to FTAI was significantly different between weight categories at property B, but not at properties A or C (**Table 18**). The lack of any significant relationship between LW and pregnancy rate maybe due to the confounding influence of animal age i.e as heifers become older the probability that they have reached puberty increases. Further, it is quite likely that the age of the heifers may have varied by as much as 4 - 5months.

Table 18: The effect of liveweight (LW*) on Brahman heifers at the commencement of treatments to synchronise ovulation on the pregnancy rate (PR) to fixed-time artificial insemination (FTAI) on three different properties (A, B, C). Superscripts a,b,c differ significantly (P<0.05) within property.

	Pregnancy rate to FTAI (%)			
Weight category (kg)	Property A**	Property B	Property C	Total
280- 300	40.4 (n = 42)	0.0^{d} (n = 3)	42.6 (n = 33)	35.2 (n = 78)
300 to 325	33.4 (n = 97)	54.2^{ab} (n = 11)	39.8 (n = 97)	34.3 (n = 205)
325 to 350	47.4 (n = 128)	17.7^{cd} (n = 36)	42.1 (n = 139)	39.9 (n = 303)
350 to 375	41.6 (n = 99)	22.7^{bc} (n = 59)	41.9 (n = 84)	38.2 (n = 242)
375 to 400	42.6 (n = 58)	25.4^{bc} (n = 78)	48.7 (n = 34)	39.9 (n = 170)
> 400	34.7 (n = 28)	45.6^{a} (n = 84)	22.3 (n = 6)	48.2 (n = 118)
P-value	0.354	0.005	0.875	0.374

*All weights were taken after a minimum of 12 h off feed but on water for Property A and C and off feed and water for Property B.
** One heifer had an outlying weight recorded so was excluded from this dataset.

BCS at commencement of treatments to synchronise ovulation did not significantly affect pregnancy rates to FTAI at properties A and C, but at property B heifers with a higher BCS achieved a higher pregnancy rate (*P* = 0.043) (**Fig. 12**).

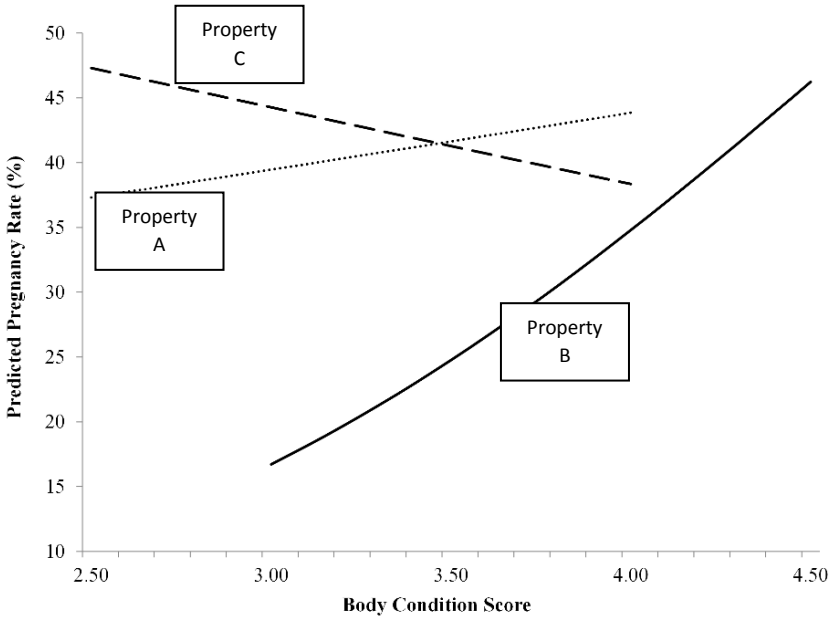


Fig. 12: The relationship between body condition score and predicted probability of becoming pregnant to fixed time artificial insemination at property A (*P* = 0.786), property B (*P* = 0.043) and property C (*P* = 0.603) in Brahman heifers treated to synchronise ovulation with intravaginal progesterone releasing devices, oestradiol benzoate and prostaglandin $F_{2\alpha}$,

The average rank of heifers for flight speed did not affect pregnancy rate to FTAI at either property A (P = 0.595) or C (P = 0.573), but did at property B (P = 0.023). The relationship of flight speed and pregnancy rate to FTAI is outlined in **Fig. 13**.

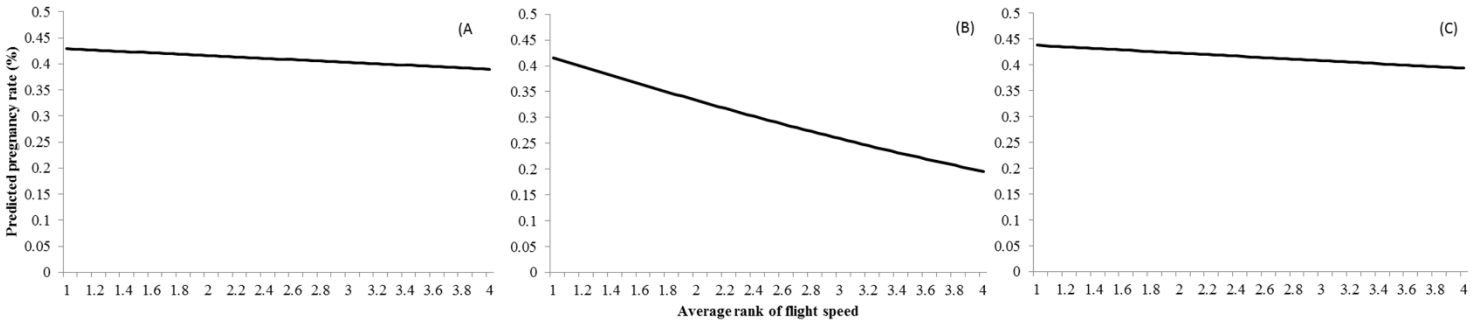


Fig. 13: The relationship average rank of flight speed in Brahman heifers (1 = fastest to 4 = slowest) and predicted probability of becoming pregnant to fixed-time artificial insemination at property A (A; *P* = 0.595), property B (B; *P* = 0.023) and property C (C; *P* = 0.573).

4.4.4 Comparisons of natural mating and FTAI

At properties B and C the NATM breeding strategy resulted in a significantly higher proportion of heifers pregnant during the first oestrous cycle of the mating period than the FTAI OPO-8 breeding strategy (Table 19). However, the proportion of heifers that were pregnant during the second oestrous cycle of the mating period and the six week in-calf rate did not differ between the breeding strategies at either property B or C (Table 19).

Table 19: The pregnancy rate (PR) per oestrous cycle of 2-year-old Brahman heifers on properties B and C that were naturally mated (NATM) or treated with the OPO-8 FTAI protocol and then exposed to bulls (FTAI).

Breeding strategy	Property B			Property C		
	NATM	FTAI + NATM	*P-value*	NATM	FTAI + NATM	*P-value*
1st Oestrous Cycle PR (%)	38.0 (41/108)	27.1 (26/96)	*0.066*	65.1 (69/106)	43.4 (85/196)	*< 0.001*
2nd Oestrous cycle PR (%)	29.9 (20/67)	30.0 (21/70)	*0.567*	51.4 (19/37)	56.8 (63/111)	*0.350*
P-value	*0.176*	*0.405*	-	*0.100*	*0.016*	-
Average natural oestrous cycle PR (%)	34.0	-	-	58.3	-	-
6 week PR	56.5 (61/108)	57.3 (55/96)	*0.510*	83.0 (88/106)	82.7 (162/196)	*0.536*

- NATM = Heifers were mated to bulls for the 1st oestrous cycle (Day 0 to 21) and 2nd oestrous cycle (Day 22 to 42) of the joining period
- FTAI + NATM = 1st FTAI on Day 0 followed natural mating to bulls between Day 14 to Day 35 after FTAI.
- 6 week PR = the proportion of females pregnant after 6 weeks of mating.

The cycling status of the heifers did not affect the pregnancy rate achieved in the first oestrous cycle for NATM or FTAI heifers at property C. However, cycling heifers at property B achieved a higher pregnancy rate in both NATM and FTAI groups (**Table 20**). At both properties, NATM had a significantly higher pregnancy rate than FTAI regardless of cycling status, except for the non-cycling heifers at Property B where the same trend was not statistically significant (**Table 20**).

Table 20: First oestrous cycle** conception rate for cycling and non-cycling 2 yo Brahman heifers on properties B and C that were naturally mated (NATM) or FTAI with the OPO-8 protocol and then exposed to bulls (FTAI).

	Property B			**Property C**		
	NATM	FTAI	*P-value*	NATM	FTAI	*P-value*
Non-cycling*	19.4 (6/31)	8.7 (2/23)	*0.245*	76.9 (10/13)	33.3 (8/24)	*0.004*
Cycling	45.5 (35/77)	32.9 (24/73)	*0.030*	63.4 (59/93)	44.8 (77/172)	*<0.001*
P-value	*0.009*	*0.017*	-	*0.265*	*0.202*	-

*Ovarian scans to examine for presence of a CL were performed 10 and 24 days prior to the commencement of mating
**1st oestrous cycle= NATM - from Day 0 to 21 and FTAI = Day 0.

4.5 Study 4

The following points summarise the major findings of Study 4, and the following subsections provide the result details.

- Optimal responses to treatments to synchronise ovulation in beef cattle usually occur when the females are cycling and only minimally exposed to stressors which may adversely affect ovulation. In heifers, the key factor is ensuring cattle have grown adequately to ensure that the majority have reached puberty prior to commencement of treatments to synchronise ovulation. Although liveweight is a reasonably good indicator of whether heifers are likely to have reached puberty, there is significant individual variation.
- This study investigated the use of biostimulation (defined as the stimulatory effects of entire males or animals treated with testosterone on female cyclic activity through genital stimulation, pheromones etc) to increase the proportion of heifers cycling prior to commencement of treatments to synchronise ovulation. Biostimulation (exposure to testosterone treated cows for 7 days approximately 26 days before FTAI) had no significant effect on proportion of heifers with a CL at time of commencement of treatments to synchronise ovulation and did not affect the percentage pregnant to FTAI.
- This study also investigated the impact of prehandling of heifers on the outcome of FTAI. Heifers were handled through the facilities that would be used for all treatments to synchronise ovulation and for AI for 3 days approximately 1 month prior to FTAI. Prehandling of heifers had no effect on percentage pregnant to AI in this herd. However, it needs to be stressed that the herd in which the study was conducted always conducts weaner training using low stress handling principles, and thus the study heifers may have been less likely to experience distress when worked through the handling facilities to complete treatments to synchronise ovulation.

4.5.1 Animal data

There was no difference between groups for mean live weight or BCS prior to or after FTAI (**Table 21**). Typically, heifers gained about 87kg over the 104 day period the FTAI

programme was conducted i.e average daily gain was 0.8kg/day. The heifers gained on average 0.5 of a BCS over this period.

Table 21: Mean live weight (LW) and standard deviation (Std Dev) and mean body condition score (BCS) of heifers 30 days before and 74 days after fixed time AI in the Biostimulation (BT), Pre handling (PH) and Control groups.

Treatment Group	Time of measurement	Mean LW (Std Dev)	Mean BCS
BT	Day -30 before FTAI (n=98)	284.7 (23.8)	3.0
	day 74 after FTAI (n=91)	371.9 (34.5)	3.5
PH	Day -30 before FTAI (n=101)	288.4 (24.5)	3.0
	Day 74 after FTAI (n=100)	374.9 (40.1)	3.5
Control	Day -30 after FTAI (n=100)	286.5 (22.9)	3.0
	Day 74 after FTAI (n=92)	374.2 (36.6)	3.5

4.5.2 Effects of biostimulation and prehandling

The proportion of heifers with a CL increased slightly (2 to 5%) between 30 days and 11 days before FTAI, but there was no difference between groups at either time point (**Table 22**). Overall, at the time of commencement of treatment to synchronise ovulation between 56 to 61% of heifers in each group had a CL.

Table 22: Proportion of heifers with a CL at 30 days and 11 days before fixed time AI for the Biostimulation (BT), Pre handling (PH) and Control groups.

Treatment Group	Time of measurement	Proportion with CL (95% CI)
BT	Day -30 before FTAI (n=98)	0.54 (0.44 - 0.64)
	Day 11 before FTAI (n=91)	0.59 (0.49 - 0.69)
PH	Day -30 before FTAI (n=100)	0.57 (0.47 - 0.66)
	Day 11 before FTAI (n=100)	0.61 (0.51 - 0.70)
Control	Day -30 after FTAI (n=100)	0.54 (0.44 - 0.63)
	Day 11 before FTAI (n=93)	0.56 (0.46 - 0.66)

The mean flight time of each group did not differ significantly and although the flight time decreased between 29 days and 1 day before FTAI in the PH group this change was not significant (**Table 23**).

Table 23: Mean flight time for heifers prior to fixed time AI in the Biostimulation (BT), Pre handling (PH) and Control groups.

Treatment	Time of measurement	Mean flight time (s) (Std Dev)
BT	Day -1 before FTAI (n=82)	1.62 (1.14)
PH	Day -1 before FTAI (n=86)	1.35 (1.15)
	Day -29 before FTAI (n=96)	1.60 (1.07)
Control	Day -1 before FTAI (n=80)	1.40 (1.07)

The mating outcomes (FTAI, Bulls, and FTAI + Bulls) were similar across all groups (**Table 24**). Overall, between 79 to 84 % of heifer became pregnant to FTAI and bulls i.e became detectably pregnant within 5 weeks of commencement of mating.

Table 24: Proportion of heifers that became pregnant to FTAI or to bulls 2-5 weeks after AI for the Biostimulation (BT), Pre handling (PH) and Control groups.

Treatment	Mating outcomes	% heifers pregnant (95% CI)
BT (n=91)	FTAI	0.47 (0.37 - 0.57)
	Bulls	0.36 (0.27 - 0.47)
	FTAI + Bulls	0.84 (0.75 - 0.90)
PH (n=99)	FTAI	0.42 (0.33 - 0.52)
	Bulls	0.36 (0.28 - 0.46)
	FTAI + Bulls	0.79 (0.70 - 0.86)
Control (n=92)	FTAI	0.39 (0.30 - 0.49)
	Bulls	0.40 (0.31 - 0.50)
	FTAI + Bulls	0.79 (0.70 - 0.86)

Multivariable analyses demonstrated that the probability of pregnancy to FTAI was not influenced by treatment group or any of the explanatory variables examined (**Table 25**).

Table 25: Multivariable logistic regression analyses: FTAI model for the Biostimulation (BT), Pre handling (PH) and Control groups.

Explanatory variable	*n* became pregnant (% of total)	Total number of heifers	Coefficient (SE)	Odds Ratio (95% Confidence Interval)	P-value
Treatment group					
BT	43 (15)	91	Reference	-	-
PH	42 (15)	99	-0.20 (0.29)	0.82 (0.46 – 1.46)[a]	0.50
Control	36 (13)	92	-0.33 (0.30)	0.72 (0.40 – 1.29)	0.27

The trend towards a difference in pregnancy rate to FTAI between the biostimulated heifers and the control heifers maybe worth investigating further by replicating the study in at least 3 different herds. The short duration of exposure to the androgenised cows was chosen on the basis of the recent findings of Tauck et al., (2010) who reported that exposure of suckled primiparous cows to bulls for 5 h daily for 9 days induced a significant increase in frequency of secretion of luteinising hormone. Although much longer periods of exposure to bulls or

androgenised cows have previously been used in prepubertal heifers it is clear from the review by Fiol and Ungerfeld (2012) that there is significant uncertainty about what is the optimum duration of exposure to bulls or androgenised cows. Further, it is likely that the magnitude of effect is influenced by whether or not at the time of exposure heifers are in the transition phase prior to reaching puberty.

Treatment group did not influence the probability of heifers becoming pregnant to the bulls. However, heifers with a CL were at least 3 times more likely to become pregnant to the bulls compared with those without a CL (**Table 26**; **Table 27**).

Table 26: Multivariable logistic regression analyses: Bull model with FTAI heifer information included for the Biostimulation (BT), Pre handling (PH) and Control groups.

Explanatory variable	*n* became pregnant (% of total)	Total number of heifers	Coefficient (SE)	Odds Ratio (95% Confidence Interval)	P-value
Treatment group					
BT	33 (15)	91	Reference	-	-
PH	36 (15)	99	-0.20 (0.29)	0.82 (0.46 – 1.46)	0.50
Control	37 (13)	92	-0.33 (0.30)	0.72 (0.40 – 1.29)	0.27
CL					
No	30 (8)	107	Reference	-	-
Yes	76 (21)	155	1.14 (0.26)	3.13 (1.88 -5.31)	<0.001

Table 27: Multivariable logistic regression analyses: Bull data only model (Heifers that became pregnant to FTAI were removed) for the Biostimulation (BT), Pre handling (PH) and Control groups.

Explanatory variable	*n* became pregnant (% of total)	Total number of heifers	Coefficient (SE)	Odds Ratio (95% Confidence Interval)	P-value
Treatment group					
BT	33 (13)	48	Reference	-	-
PH	36 (14)	57	-0.26 (0.48)	0.77 (0.29 – 1.97)	0.59
Control	37 (14)	56	-0.27 (0.49)	0.76 (0.29 – 1.97)	0.58
CL					
No	30 (11)	71	Reference	-	-
Yes	76 (29)	90	2.05 (0.39)	37.76(3.72 -17.14)	<0.001
Horn status					
0ther	15 (6)	29	Reference	-	-
H	91 (34)	106	0.97 (0.49)	2.63 (1.02 – 6.97)	0.05

4.6 Study 5

Bos indicus ancestry analysis confirmed that the heifers genotyped were “high grade Brahman”—closer to Nelore (*Bos indicus*) than to Angus (*Bos taurus*) reference populations (**Fig. 14**). However, the spread of values observed in the principal component analysis indicated that varying degrees of *Bos taurus* ancestry were present in the studied population. There were no sub-structures in the population when pregnant heifers were compared to non-pregnant heifers (**Fig. 14**, insert). In general, there were no systematic differences in the genetic background of the studied heifers that could confound the outcome of FTAI or the results of the GWAS analysis, which indicates an effective population sample and sampling method.

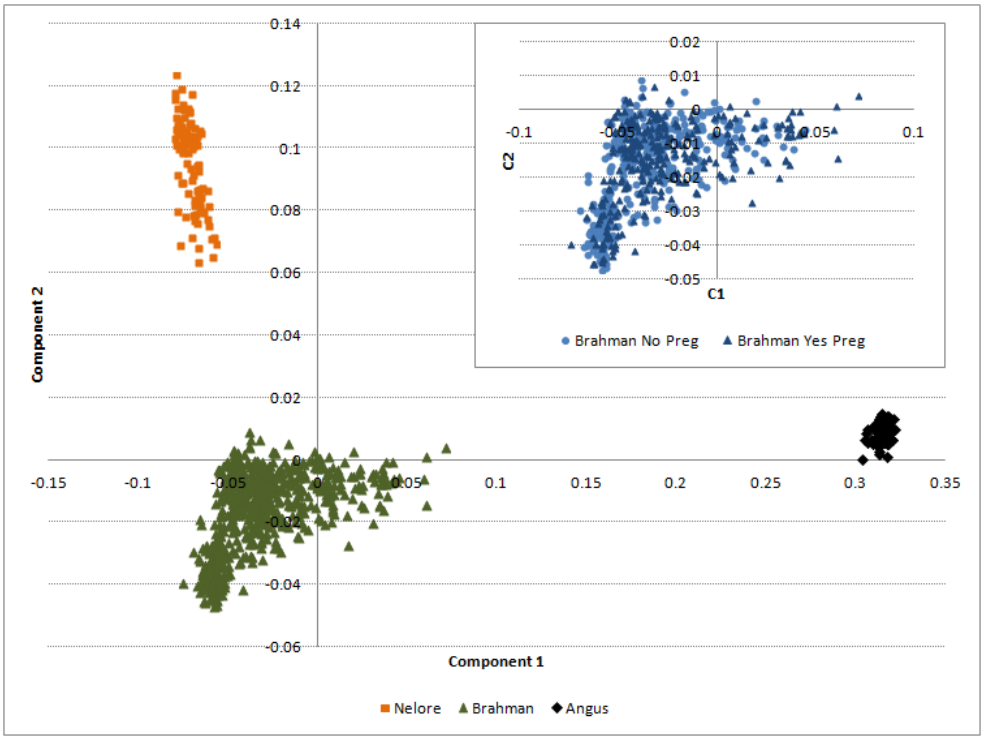


Fig. 14: Multidimensional scaling analyses. Characterization of the genetic background and identification of potential substructure among the studied Brahman population.

The heritability for PREG1 (pregnancy outcome after FTAI) was estimated at $h^2 = 0.18$. Individual records for *Bos indicus* content were significantly associated to the trait PREG1 ($P < 0.05$): on average, the genome of non-pregnant heifers showed a higher percentage of *Bos indicus* ancestry.

Bos indicus genomic content was also significantly associated with BW ($P < 0.05$): on average, heifers with a higher percentage of *Bos indicus* ancestry had lower BW.

Body weight was not significantly associated with PREG1 ($P > 0.05$).

SNP associations for PREG1 and BW are presented as Manhattan plots (**Fig. 15**). There were 101 (P <0.001, FDR = 0.53) SNPs associated with PREG1, and 117 (P <0.01, FDR = 0.46) SNPs associated with BW. These 'top' genomic regions—defined as a genomic regions harbouring SNP(s) highly associated ($P < 0.001$) to the phenotype under observation—were observed across nearly all chromosomes (**Table 28**; **Fig. 16**).

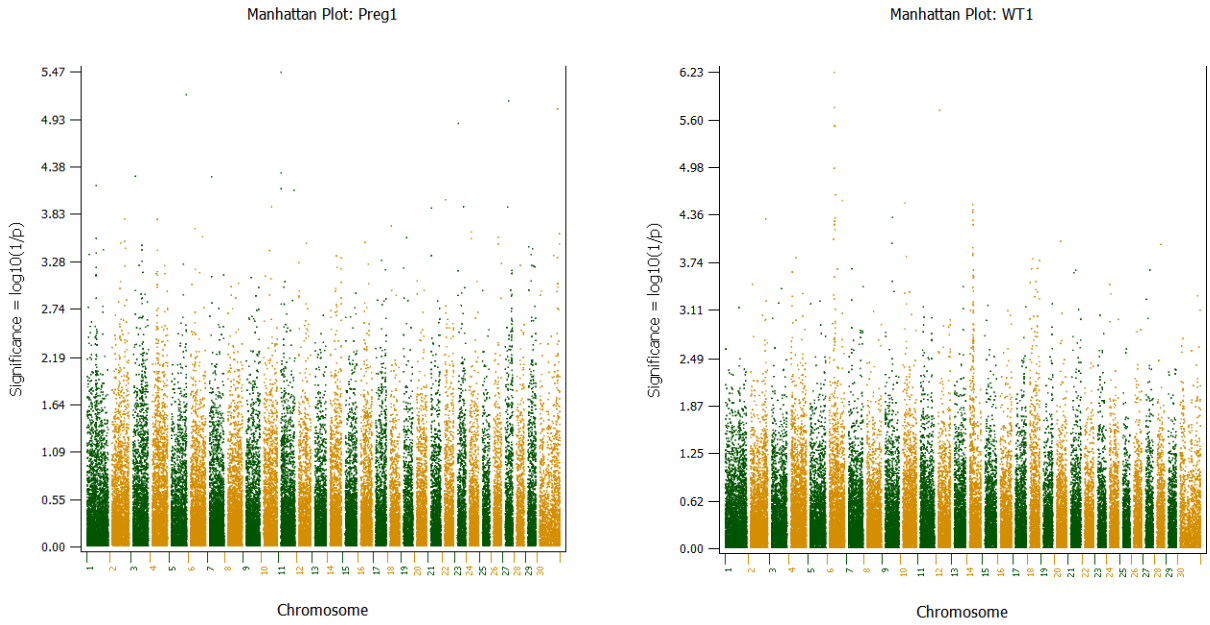


Fig. 15: Manhattan plot for pregnancy outcome after FTAI and BW.

Table 28: Top genomic regions associated ($P < 0.001$) to pregnancy outcome after FTAI (PREG1) and body weight (BW).

Genome-wide associations for PREG1						
BTA	**#SNP**	**Min *P*-value**	**SNP with min *P*-value**	**Pos**	**%Vg[1]**	**Gene**
1	9	$6.79x10^{-05}$	ARS-BFGL-NGS-31379	68,891,058	5.64	CCDC14,
2	6	$1.66x10^{-04}$	BTA-48503-no-rs	102,981,611	4.90	VWC2L
3	7	$5.35x10^{-05}$	BovineHD0300006479	20,516,548	5.54	PLEKHO1
4	8	$1.68x10^{-04}$	BTB-00890646	41,150,639	4.77	-
5	2	$6.13x10^{-06}$	ARS-BFGL-NGS-17229	118,375,557	8.41	-
6	5	$2.15x10^{-04}$	BovineHD0600009692	34,777,773	5.71	-
7	3	$5.42x10^{-05}$	ARS-BFGL-NGS-86836	14,889,952	5.84	-
8	1	$9.20x10^{-04}$	BTB-00362255	81,866,074	3.47	-
9	1	$7.91x10^{-04}$	BTB-01229331	38,412,334	3.60	-
10	4	$1.20x10^{-04}$	BovineHD1000031472	61,585,649	5.40	-
11	5	$3.35x10^{-06}$	ARS-BFGL-NGS-15287	1,417,721	8.06	ACOXL
12	2	$3.18x10^{-04}$	ARS-BFGL-BAC-14372	55,583,608	5.24	-
14	5	$4.45x10^{-04}$	Hapmap60270-ss46526106	47,141,282	4.31	MAL2
16	4	$3.11x10^{-04}$	BovineHD1600008832	31,097,118	4.06	-
17	2	$5.00x10^{-04}$	BTA-17209-no-rs	43,052,005	4.08	GLRB
18	1	$2.01x10^{-04}$	ARS-BFGL-NGS-56265	8,163,550	4.50	bta-mir-2883
19	2	$2.74x10^{-04}$	Hapmap60163-rs29015084	22,120,443	4.95	GOSR1
20	1	$8.68x10^{-04}$	BovineHD2000001473	4,594,721	2.83	ERGIC1
21	2	$1.25x10^{-04}$	ARS-BFGL-NGS-103866	5,690,539	5.55	LRRK1
22	1	$1.00x10^{-04}$	ARS-BFGL-NGS-34986	6,021,745	5.89	-
23	3	$1.32x10^{-05}$	ARS-BFGL-NGS-68679	6,124,341	6.61	MLIP
24	3	$2.36x10^{-04}$	ARS-BFGL-NGS-79951	10,966,864	4.68	-
26	3	$2.70x10^{-04}$	ARS-BFGL-NGS-21775	30,657,502	4.73	-
27	5	$7.18x10^{-06}$	ARS-BFGL-NGS-113043	20,067,064	8.09	-
28	1	$5.73x10^{-04}$	BovineHD2800005989	22,912,589	3.99	CTNNA3
29	8	$3.48x10^{-04}$	BovineHD2900001510	5,256,338	4.26	-
X	7	$8.94x10^{-06}$	BovineHD3000037489	132,052,128	8.10	-
Genome-wide associations for BW						
BTA	**#SNP**	**Min *P*-value**	**SNP with min *P*-value**	**Pos**	**%Vg[1]**	**Gene**
1	1	$7.19x10^{-04}$	BTB-01631727	100,800,828	0.63	SERPINI2
2	4	$4.94x10^{-05}$	Hapmap30526-BTA-134605	121,573,506	1.01	RNF19B
3	3	$4.05x10^{-04}$	BTA-82678-no-rs	78,265,433	0.72	IL12RB2
4	5	$1.60x10^{-04}$	BTB-00176906	39,763,972	0.83	-
5	2	$5.93x10^{-04}$	ARS-BFGL-NGS-116435	76,157,488	0.61	-
6	11	$5.95x10^{-07}$	Hapmap29925-BTC-036976	37,526,622	1.78	HERC3
7	3	$2.22x10^{-04}$	ARS-BFGL-NGS-26930	26,561,056	0.84	-
8	1	$8.07x10^{-04}$	BovineHD0800015443	51,511,606	0.71	-
9	4	$4.74x10^{-05}$	BTB-00392939	55,828,214	0.82	-
10	5	$3.06x10^{-05}$	BovineHD1000005734	17,105,679	1.07	-

11	3	$7.02x10^{-04}$	Hapmap36745-SCAFFOLD105004_39631	22,783,522	0.96	SLC8A1
12	1	$1.87x10^{-06}$	BTA-31536-no-rs	11,701,542	1.21	VWA8
13	1	$3.84x10^{-04}$	BovineHD1300018349	64,366,788	0.70	ITCH
14	8	$3.78x10^{-05}$	Hapmap46986-BTA-34282	25,307,116	1.39	SDR16C6, XKR4
15	1	$6.71x10^{-04}$	BTA-60562-no-rs	22,298,615	0.78	SIK2
16	2	$7.91x10^{-04}$	BovineHD1600014949	53,873,671	0.55	-
17	1	$7.06x10^{-04}$	ARS-BFGL-NGS-41599	73,118,011	0.58	IGLL1
18	9	$1.65x10^{-04}$	ARS-BFGL-NGS-23457	16,515,915	0.81	-
19	1	$6.41x10^{-04}$	ARS-BFGL-NGS-116261	61,478,388	0.69	-
20	2	$9.71x10^{-05}$	BovineHD2000008535	28,951,173	1.47	-
21	3	$2.33x10^{-04}$	ARS-BFGL-BAC-29153	35,921,990	0.78	-
23	1	$9.01x10^{-04}$	ARS-BFGL-NGS-5042	15,345,878	0.84	-
24	2	$3.56x10^{-04}$	ARS-BFGL-NGS-111602	2,114,444	0.91	-
27	3	$2.31x10^{-04}$	ARS-BFGL-NGS-60861	25,392,013	0.72	TMEM66
28	1	$1.07x10^{-04}$	ARS-BFGL-NGS-351	23,764,335	0.93	CTNNA3
X	2	$5.02x10^{-04}$	BovineHD3000036241	127,849,324	0.74	-

[1] Percentage of genetic variance explained.

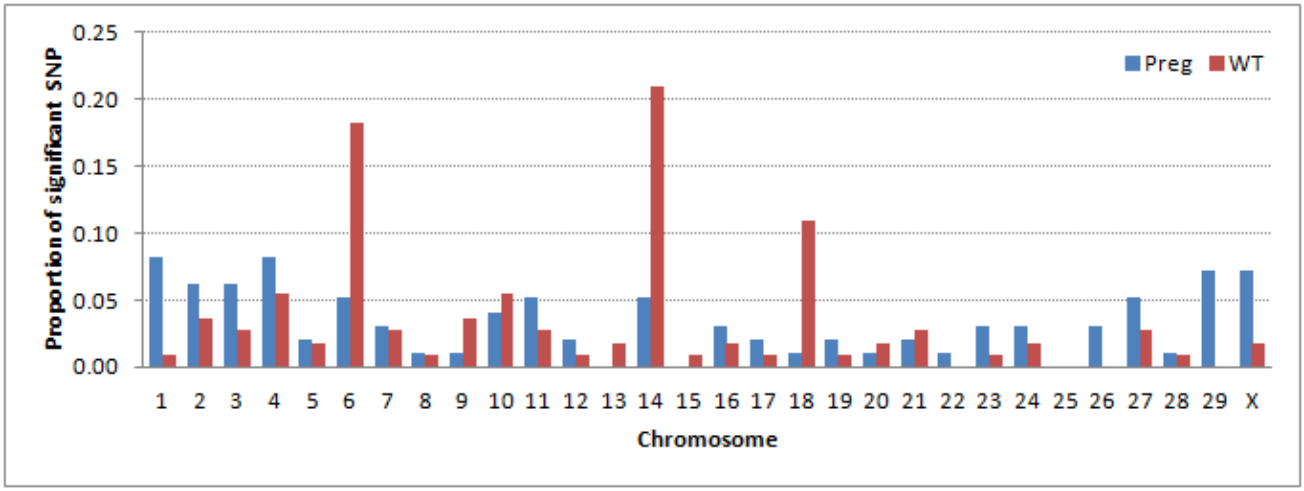


Fig. 16: Proportion of significant markers at P<0.001 on each bovine chromosome.

To further explore the influence of *Bos taurus* vs *Bos indicus* ancestries on the observed phenotypes, allele frequencies of the significant SNP and of all SNP in the study population were compared with those in the Nelore and Angus reference populations. Allele frequency differences between Nelore and Angus populations for significant SNP (PREG1, n = 88; BW n = 102) and for all SNP (n = 45,841) are represented graphically in Fig. 17.

Allele frequency differences for the significant PREG1 and BW SNP are clearly greater than the differences for all SNP.

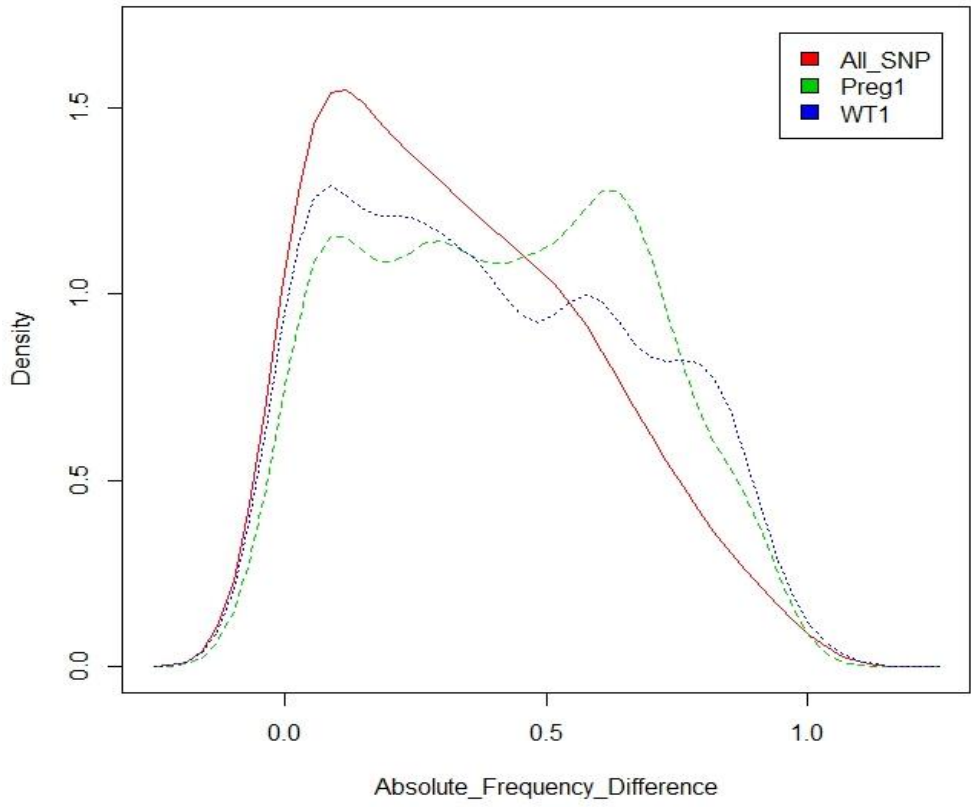


Fig. 17: Density plot of the absolute allelic frequency differences between *Bos indicus* (Nelore) and *Bos taurus* (Angus) for associated SNP (P<0.001) to pregnancy outcome after fixed time artificial insemination (Preg1) and body weight (WT1).

SNPs associated with PREG1 that point to genomic regions identified in previous studies of bovine reproduction are detailed in **Table 29**.

Table 29: Top genomic regions identified for FTAI that are also associated with age at puberty (AGECL[1]) or post-partum anoestrus interval (PPAI[1]) (P < 0.05).

BTA	SNP with min P-value	Position	Gene	*P*-Value		
Top regions PREG1				**PREG1**	**AGECL**	**PPAI**
1	ARS-BFGL-NGS-31379	68,891,058	CCDC14, U4, ROPN1	$6.79x10^{-05}$	0.007	0.488
7	ARS-BFGL-NGS-86836	14,889,952	-	$5.42x10^{-05}$	0.05	0.043
9	BTB-01229331	38,412,334	-	$7.91x10^{-04}$	0.028	0.837
Top regions BW				**BW**	**AGECL**	**PPAI**
1	BTB-01631727	100,800,828	SERPINI2	$7.19x10^{-04}$	0.475	0.023
3	BTA-82678-no-rs	78,265,433	IL12RB2	$4.05x10^{-04}$	0.595	0.02
14	Hapmap46986-BTA-34282	25,307,116	SDR16C6, XKR4	$3.78x10^{-05}$	$4.45x10^{-08}$	0.017
19	ARS-BFGL-NGS-116261	61,478,388	-	$6.41x10^{-04}$	0.018	0.945

[1] From (Hawken et al., 2012)

5 Discussion

5.1 New oestrus synchronisation protocols for FTAI in *Bos indicus* heifers

The results of the initial pilot study characterised follicular dynamics in Brahman heifers and evaluated the efficacy of GnRH as compared to ODB to successfully control the development of a new follicular wave and the emergence of a new follicle. In an ovulation synchronisation programme, the occurrence of ovulation and the time of ovulation relative to the time of AI are key determinants of the likelihood a female will conceive after FTAI. These determinants are reliant on hormonal treatments to initially control the development of a new follicular wave and the emergence of a new follicle, and then to ensure that the follicle has reached optimal maturity so that it has maximal capacity to respond to exogenous hormones for induction of a fertile ovulation (Bo et al., 1995b). Additionally, females must ovulate synchronously in an FTAI program to result in an acceptable pregnancy rate.

Currently the most common protocol used to synchronise ovulation in *Bos indicus* heifers in Australia involves insertion of an intravaginal progesterone (P4) releasing device (IPRD) for 8 days, treatment with PGF2α at the time of IPRD removal, and treatment with oestradiol benzoate (ODB) at IPRD insertion and 24 h after IPRD removal (Bo et al., 2003, Edwards et al., 2014). Recent research has demonstrated that treatment of Brahman heifers with IPRD and ODB for ovulation synchronisation suppresses secretion of FSH prior to synchronised wave emergence compared to heifers treated with only $PGF_{2\alpha}$ (Edwards et al., 2013b). An increase in secretion of FSH is responsible for the emergence of a new follicular wave and the growth of a new cohort of follicles (Adams et al., 1992, Ginther et al., 1998). Therefore, we investigated the ability of FSH treatment prior to wave emergence to enhance follicular growth and development. The FSH used in this study was formulated to ensure a slow release, as FSH typically has a relatively short half-life of less than 5 hrs. The slow release formulation has been reported to result in superovulation of cows after a single-split injection (Tríbulo et al., 2011, Tríbulo et al., 2012).

The administration of FSH four days after the commencement of IPRD+ODB (at the reported time of follicular wave emergence in *Bos indicus* cattle; (Bo et al., 2003, Edwards et al., 2013b) did not significantly improve the diameter of the developing follicle at ovulation or ovulation rate, and tended to reduce the synchrony of ovulation. Although not significant, FSH treatment was found to delay follicular wave emergence. As expected, treatment with FSH increased the number of follicles recruited at day 13, which consequently delayed the selection and dominance growth phase of the dominant follicle. Interestingly, heifers in this experiment that eventually ovulated had a mean day of FWE earlier than heifers that did not ovulate, regardless of treatment.

As the use of oestrogenic compounds in food producing animals is currently banned in Europe and New Zealand, this pilot study also investigated the use of GnRH as a replacement for ODB in oestrus synchronisation protocols for Brahman cattle. Neither the day of FWE, ovulation rate, nor the synchrony of ovulation differed significantly between ODB and GnRH protocols, although the synchrony of ovulation at 60-72 h after IPRD removal was lower in GnRH treated heifers (50%) compared to the ODB treated heifers (75%). However, heifers receiving the GnRH protocol had a significantly larger dominant follicle at the time of ovulation and at 54 hrs post IPRD removal than those receiving the ODB protocol. Based on this result, a larger field trial comparing these protocols was justified.

A large field trial, involving 1143 heifers across 4 north Queensland cattle properties found that pregnancy rates in heifers treated with ODB to synchronise oestrus (40.66%) were significantly higher (11.4%) than those treated with GnRH (29.52%). The pilot study showed that the GnRH protocol produced a significantly larger dominant follicle at ovulation, but 25% fewer heifers ovulated synchronously within 60 to 72 h after removal of the IPRD. Although follicle diameter at the time of ovulation is an important factor influencing the likelihood of pregnancy after FTAI (Sá Filho et al., 2010), the results of the current studies highlight the importance of heifers ovulating synchronously in FTAI programs to achieve acceptable pregnancy rates.

In the first pilot trial, although heifers responded poorly to the first GnRH treatment a high proportion (18/18) of those heifers that did not respond to the first GnRH treatment subsequently ovulated, suggesting that the IPRD treatment alone may be sufficient to synchronise follicular wave emergence in Brahman heifers. Colazo and Ambrose (2011) reported similar findings in dairy (Holstein) heifers (n = 64): only 31.7% of heifers ovulated to the first GnRH treatment and a larger proportion of the heifers that did not ovulate became pregnant (65.1 vs 45.0%).

A modified protocol for synchronising oestrus was also tested using ODB at the time of IPRD insertion and GnRH to induce ovulation. This protocol required a reduced number of treatments and therefore handling occasions, but resulted in a poorer ovarian response (lower ovulation rate) than the protocol that used ODB, and poorer ovulation synchrony than either protocol with only ODB or GnRH. This result did not support further validation in a field trial. However, a study in South America involving *Bos indicus* Nelore heifers found no significant difference in pregnancy rates to FTAI after inducing ovulation with GnRH as compared to ODB (Sa Filho et al. (2011). This study used a progestagin-based ear implant instead of an IPRD and also administered eCG at the time of implant removal. Exploration of such modified protocols may be worthy of further research. For example, as *Bos indicus* genotypes have been shown to be less sensitive to GnRH (gonadorelllin) (Portillo et al., 2008), they may require twice the recommended dose to induce ovulation. Also, the use of eCG at the time of IPRD removal may improve follicular growth as it has FSH and LH like activity. Such protocols would be advantageous in extensive beef operations as they require a less labour, and common industry feedback from AI programs in northern Australia identifies the time and frequency of handling as a barrier to implementing an AI program. Reducing handling would reduce labour expenses, reduce the stress on females and reduce the time females need to be held in yards and consequently the requirement for supplementary feeding.

Based on published studies and the results from Study 2, further modifications to oestrous synchronisation protocols were trialled before being implemented in another large scale trial.

The first modification trialled an increased time from IPRD removal to ODB treatment as well as a decreased duration of IPRD insertion compared with the current best practice protocol. As it has been reported that the likelihood of ovulation increases with increasing dominant follicle diameter (Gimenes et al., 2008, Simoes et al., 2012), it was hypothesised that the proportion of dominant follicles synchronously ovulating would be increased if the induced LH surge occurred when the dominant follicle was closest to maximal growth or at the static growth phase. In an attempt to achieve this, the timing of treatment with ODB to induce ovulation was increased from 24 h to 36 h after IPRD removal. Published studies support this hypothesis. Increasing the proestrus period has been associated with an increase in pregnancy rate after AI (Colazo and Ambrose, 2011), and a shortened proestrus from $PGF_{2\alpha}$

treatment to a GnRH induced LH surge (1.25 vs. 2.25 days) has been associated with shortened luteal phases after induced ovulation (Bridges et al., 2010).

As the growth of the ovulatory follicle during sub-luteal circulating P4 concentrations is associated with increased diameter of the pre-ovulatory follicle and improved CL function (Dadarwal et al., 2013), it was hypothesised that reducing the interval of IPRD insertion from 8 or 7 days to 6 days would improve dominant follicle development from FWE to the time of IPRD removal.

The first modification was also trialled without ODB treatment at the time of IPRD insertion. Treatment of *Bos indicus* heifers with P4 and ODB has been shown to adversely affect follicular development (Carvalho et al., 2008, Butler et al., 2011c) and markedly decrease FSH secretion (Edwards et al., 2013b). It was therefore hypothesised that omission of the ODB treatment at the time of IPRD insertion may further improve dominant follicle development from FWE to the time of IPRD removal.

The pilot trials demonstrated that an IPRD insertion time of 6 days coupled with a 12 h delay in ODB treatment (increased from 24 h to 36 h after IPRD removal) extended the IPO period by approximately 12 h (range 11 – 13 h for OPO-6 and PO-6 groups, respectively) compared with the current best-practice protocol for Brahman heifers (OPO-8). Assuming an LH surge occurs 18 to 20 h post ODB treatment (Rhodes et al., 1978), the duration from $PGF_{2\alpha}$ treatment to LH surge in this study would have been 44 h (1.83 days) for the OPO-8 group and 56 h (2.33 days) for the OPO-6/PO-6 groups. These estimates for the OPO-6/PO-6 groups concur with the findings of Bridges et al. (2010), who also reported a significant increase in pregnancy rate in a protocol associated with an increased IPO.

An increase in the length of the IPO period was associated with a favourable improvement in ovulation rate by 10% and 25% in heifers treated with the OPO-6 and PO-6 protocols, and an improvement in synchronised ovulation (12 h prior and 24 h after FTAI) by 15% and 20% in the OPO-6 and PO-6 protocol groups as compared to the OPO-8 protocol. The increased ovulation rate could be explained by the fact that the dominant follicle in these heifers had 12 h longer to mature and acquire LH receptors (Simoes et al., 2012), which enabled more heifers to have a dominant follicle that had reached ovulatory capacity prior to the ODB induced LH surge. Ovulatory capacity in *Bos indicus* heifers is related to the diameter of the dominant follicle at the time of the pre-ovulatory LH surge. In a study of *Bos indicus* heifers treated with LH when their dominant follicle was 7–8.4 mm, 8.5–10 mm and >10 mm, the ovulation rate was 33%, 80% and 90%, respectively (Gimenes et al., 2008). Interestingly, in the present study the proportion of OPO-6 and PO-6 heifers that had a dominant follicle of ovulatory capacity (>8.5 mm) was 10%and 15%, respectively, higher than heifers treated with the OPO-8 protocol, corresponding with the observed higher ovulation rates. Although delaying the time of ODB treatment creates a higher risk of early ovulations, only 10% and 5% of heifers in OPO-6 and PO-6 groups ovulated prior to FTAI at 72 h with normal probabilities of conceiving.

The diameter of the dominant follicle at the time of AI and ovulation has been associated with an increased likelihood of conception after FTAI of *Bos indicus* cattle (Sá Filho et al., 2010). In this study, the dominant follicle at the time of AI tended to be larger in heifers treated with the OPO-6 and PO-6 protocols as compared to heifers treated with the OPO-8 protocol. Other studies have failed to demonstrate differences in dominant follicle diameters with increasing length of proestrus (Bridges et al., 2010, Colazo and Ambrose, 2011).

Maximal ovulation synchrony and fertility occurs when the pre-ovulatory follicle has had a dominance phase of <4 d (Austin et al., 1999). In this study, all heifers treated with the OPO-

6 and OPO-8 protocols initiated a new follicular wave, ensuring all follicles had an optimum dominance phase. By contrast, 25% of heifers treated with the PO-6 protocol failed to initiate a new follicular wave after IPRD treatment. From published reports (Gimenes et al., 2008), it could be expected that the dominance phase of existing follicles in these animals would be at least 6.5 d. It is uncertain whether these ovulatory follicles may have reduced fertility due to this prolonged dominance phase. Ovulating an aged oocyte has been previously associated with declined fertility (Mihm et al., 1994, Revah and Butler, 1996), but other studies failed to demonstrate differences in fertility when the ovulatory follicle was 6.3 vs. 9.3 days in *Bos taurus* cows (Abreu et al., 2014b) and 5.3 vs. 8.8 days in *Bos taurus* heifers (Abreu et al., 2014a). Although the effect on fertility is unknown, the observed increase in the proportion of OP-6 heifers that ovulated could offset any decline in fertility associated with follicles with prolonged dominance phases. To fully understand the fertility of the ovulatory follicles that did not develop from a new follicular wave in heifers treated with the PO-6 protocol, a field trial with larger numbers of heifers would be required.

Overall, the pregnancy rate in the OPO-6 group was 5% greater than heifers in the PO-6 and OPO-8 groups, but the difference was not significant (P=0.125). When only heifers that synchronously ovulated were analysed, the PR in the OPO-6 group was 10% greater than heifers in the PO-6 and OPO-8 groups. Although the proportion of heifers conceiving per synchronised ovulation was similar in the OPO-6 and OPO-8 groups, the increased ovulation rate observed in the OPO-6 group resulted in a higher overall pregnancy rate. As heifers in the PO-6 group had the largest dominant follicle diameters at ovulation and FTAI, and the greatest overall and synchronised ovulation rate, it was expected that this group would have the highest pregnancy rate. However, those PO-6 heifers that ovulated synchronously had a 15.8 to 20.8% lower pregnancy rate than the OPO-6 and OPO-8 groups, respectively. These results, although not significant, indicate a potential reduced fertility in heifers receiving the PO-6 protocol. The apparent improvements in ovarian response observed in heifers receiving the OPO-6 protocol (increased IPO period, increased diameter of dominant follicle at ovulation, and increased synchronicity of ovulation) were promising and warranted further investigation in a larger scale trial, as these factors are considered key indicators of the likelihood of conception after FTAI (Sá Filho et al., 2010).

The larger field trial, involving 2234 heifers across 3 north Queensland cattle properties found that heifers treated with the OPO-6 protocol to synchronise oestrus had significantly higher pregnancy rates on one of three properties (52.4%) compared to the current best practice synchronisation protocol (OPO-8; 36.8%). Pregnancy rates in the other comparison groups were not significantly different. The variation in response to the OPO-6 protocol across the three properties could not readily be explained. Retrospective analysis of the allocation procedure demonstrated that there were some potential confounding bias between groups due to differences in weight and/or BCS, but these differences were accounted for in the statistical model used and thus did not explain the observed variation in response to the OPO-6 protocol.

The timing of AI treatments with the current ‘best practice’ ovulation synchronisation protocol is problematic for northern Australian beef herds as the recommended time for FTAI is 54 h after the removal of the IPRD, which frequently means that FTAI is conducted around mid-day. The majority of northern herds are bred during the summer months to maintain an optimal calving window (MLA, 2006), and thus FTAI is commonly conducted when the temperature and humidity is maximal (Bureau of Meterology, 2014). The OPO-6 FTAI protocol extends the time of FTAI from 54 h to 72 h after IPRD removal, enabling both the hormonal treatments and AI to be conducted during the coolest periods of the day. In

northern Australia this is logistically important and improves animal welfare (Petherick, 2005) and potentially reproductive performance (Burns et al., 2010).

This study has confirmed that the OPO-6 protocol may improve the pregnancy rates to FTAI compared to the current best practice OPO-8 protocol; however, whether this difference is significant is dependent on property factors which are yet to be defined.

This study also enabled direct comparison of the outcome of natural mating of Brahman heifers with that achieved after FTAI where the standard treatment protocol was used to synchronise ovulation. Natural mating pregnancy rates per oestrous cycle assist in understanding what is achievable to FTAI. Butler et al.,(2011b) have estimated that approximately 75% of Brahman heifers respond to the standard treatment to synchronise ovulation, that is, synchronously ovulate and form a normal functional CL (Butler et al., 2011c). In study 3b, the average oestrous cycle pregnancy rate on property C was 58.3%; if only 75% of heifers treated to synchronise ovulation ovulated normally then the probability of becoming pregnant to FTAI would be 43.7%. Interestingly, the actual FTAI pregnancy rate for property C was 43.4%. Similarly at property B, the likelihood of becoming pregnant to FTAI was calculated to be 25.5% (34% of 75%) and the actual FTAI pregnancy rate was 27.1%. This data demonstrates that pregnancy rates to FTAI were remarkably similar to pregnancy rates per cycle to natural mating when analysed in the same conditions. This highlights the significant impact of the inherent fertility of the herd on the outcome of FTAI.

Finally, during the course of the project there was opportunity to evaluate the outcome of FTAI of lactating Brahman cows on three commercial properties. Pregnancy rates to FTAI ranged between 48% to 55% demonstrating that where producers can access and manage lactating cows during the wet season, very acceptable pregnancy rates to FTAI can be achieved.

5.2 Biostimulation and prehandling to improve pregnancy rates to FTAI

Neither biostimulation nor prehandling prior to commencement of treatments to synchronise ovulation significantly affected the proportion of heifers that became pregnant to FTAI, or bulls, or overall for the first 5 weeks of mating. The numerically higher pregnancy rate to FTAI in the biostimulation group is of interest and may warrant further investigation. Despite the fact that the mean weight of the heifers at the commencement of the programme was only 286.5kg (compared to approximately 330kg for previous programmes), overall 43% of heifers became pregnant after treatment to synchronise ovulation with the OPO-6 protocol and FTAI.

5.3 New criteria for selection of heifers for FTAI

5.3.1 Selection based on heifer level factors

Results from study 2 and study 3b were used to investigate the use of the following heifer level factors as selection criteria for Brahman heifers that are likely to conceive to FTAI: presence of CL, presence and diameter of the dominant follicle, LW and BCS at commencement of ovulation synchronisation.

In study 2, heifers that had a CL present at the time the IPRD was inserted had a higher pregnancy rate to FTAI (but not to natural conception) than heifers that did not have a CL. The same result was found in study 3b at two of three properties (property A and B), but not at the other (property C). Other studies in Nelore heifers (Sa Filho et al., 2010) have

demonstrated that the presence of a CL at the commencement of ovulation synchronisation improved the overall pregnancy to FTAI. However, previous research conducted by this group (Edwards et al., 2012, Butler et al., 2011b) found that presence of a CL at the time an IPRD was inserted in Brahman heifers did not affect the pregnancy rate to FTAI after ovulation synchronisation. These differing results may be explained by the proportion of females that are pre-pubertal, peri-pubertal or pubertal at the time of FTAI, and the length of time from puberty to FTAI (Claro Júnior et al., 2010, Polat et al., 2009). Results from property A showed a treatment by CL effect, where OPO-6 heifers with a CL had significantly higher pregnancy rates to FTAI than OPO-6 heifers with no CL present. It is currently not known whether the progesterone dose (half the standard dose) and duration of IPRD insertion (6 days) used in the OPO-6 protocol is sufficient to induce cyclicity in pre-pubertal Brahman heifers. If future research demonstrates that the OPO-6 protocol is not sufficient to induce puberty in pre-pubertal heifers, then the presence of a CL could be used as a criterion to select heifers that have a higher probability of conceiving to FTAI.

Results from study 2 also demonstrated that the diameter of the dominant follicle at insertion of the IPRD had a significant effect on the likelihood of pregnancy. Heifers with follicle ≥ 10 mm or ≤ 5 mm were more likely to conceive to FTAI than heifers with a dominant follicle of 6 to 9 mm. This may be due to the fact that follicles ≥ 10 mm are most likely ovulatory follicles, as at that diameter in *Bos indicus* heifers granulosa cells have acquired LH receptors (Sartori and Barros, 2011). The higher proportion of heifers pregnant to FTAI in the ≤ 5 mm category could be similarly explained as representing ovulatory heifers: if a heifer is examined shortly after ovulation very few follicles > 5 mm would be present, subsequently resulting in this heifer being assigned to this category.

It is recognised that nutritional homeostasis is fundamental to achieve an optimal result to FTAI. Brahman heifers that have high circulating insulin-like growth factor 1 and high circulating glucose prior to ovulation synchronisation are more likely to respond normally to ovulation synchronisation treatment (Butler et al., 2012). Previous research has demonstrated that heifers that increase their BCS and/or LW from commencement of ovulation synchronisation to pregnancy diagnosis have a higher likelihood of conception to FTAI (Butler et al., 2011d, Butler et al., 2011b, Phillips et al., 2010). Results of the present studies, however, were equivocal. In study 2, there was no effect of LW, LW gain, BCS or BCS change on the likelihood of heifers becoming pregnant to FTAI. Even when heifers were segregated into LW categories in an attempt to identify a critical mating weight for FTAI, no significant LW effect was observed. This may be due to the fact that all heifers enrolled in the study were well grown, as most properties had experienced above average rainfall from 2010 to 2012 and particularly above average rainfall in the months preceding the study (BOM, 2012). A similar result was obtained at two of three properties involved in study 3b, except at property B where the probability of pregnancy to FTAI was affected by BCS.

Flight speed was also investigated in study 3b as a potential criteria for heifer selection. Flight speed had an effect on heifer pregnancy rates to FTAI only on Property B. Heifers that had the highest flight speed ranking had the highest pregnancy rate to FTAI compared with heifers that were slowest leaving the crush facility. It is unclear why flight speed only affected pregnancy rates to FTAI at Property B. Stock handlers at Property A and C utilise low stress stock handling and have good cattle working facilities. Although flight speed cannot be directly compared across properties, handling facilities were suboptimal and quality of livestock handling was observed to be lower at Property B compared to Properties A and C. The results from property B are not consistent with a previous study in *Bos indicus* cows that found pregnancy rates to FTAI to be negatively affected by increasing flight speed and poor

temperament as measured by crush score. As there is a strong relationship between flight speed ranking and temperament (Burrow et al., 1988), selecting for increased flight speed to improve pregnancy rates to FTAI would be undesirable due to the well-known negative effects on welfare and correlations with poor temperament.

Although the findings of this study support the recommendation that Brahman heifers should be selected on the basis of having a CL and a dominant follicle either ≤5mm or ≥10mm at commencement of treatments to synchronise ovulation, there are published results indicating that selection for these traits may not significantly affect the outcome of FTAI.

5.3.2 Selection based on genetic characteristics

The estimated heritability for pregnancy to FTAI in this study was 0.18. There is little published data available on estimates of heritability or any genetic evaluation for FTAI. The few studies that exist report low heritability estimates for AI traits. In Brangus (*Bos indicus* x *Bos taurus*) beef heifers the heritability of first service conception was estimated as $h^2 = 0.06 \pm 0.05$ (Fortes et al., 2012b). In dairy herds, the heritability of days to first breeding ranged from 0.01 to 0.09 (Goodling Jr et al., 2005), and these estimates were inflated by the use of oestrus synchronization in the animals studied. The relatively high heritability for FTAI outcome in the present study could be explained by the fact that the animals were high grade Brahmans. Higher phenotypic variation and higher heritability for reproductive traits, especially puberty, have previously been noted in *Bos indicus* influenced herds (Johnston et al., 2009, Nogueira, 2004). Also, heifers were selected for genomic analysis on the basis of presenting a CL and so all heifers analysed were post-pubertal. Nevertheless, other factors no doubt underpin, at least partially, the response of Brahman heifers to FTAI—such as age at puberty. Age at puberty, defined as the age at first CL (AGECL), was genetically correlated with post-partum anoestrus interval in 4 year old cows ($r = 0.74 \pm 0.29$) (Johnston et al., 2010). It is possible that age at puberty would also correlate with FTAI outcome in Brahman heifers, but further AGECL data would be necessary to test this hypothesis and it was not available for the commercial populations studied.

The finding that 'becoming pregnant after FTAI' is heritable supports the selection of heifers conceiving to FTAI into a property's bull breeding herd. Applying a second round of selection to these heifers when they are 1st lactation cows to identify those that have cycled within 2 months of calving and then subsequently become pregnant again to FTAI may be a strategy to increase the rate of genetic improvement for fertility in bull breeding herds.

In this study, BW was also significantly correlated to *Bos indicus* genomic content ($P < 0.05$); heifers with a higher percentage of *Bos indicus* ancestry had lower BW on average. Heifers in this study were, however, selected to have similar BW and BCS, reducing the variability in BW. Neither BW nor BCS were significant when added to the model for PREG1 ($P > 0.05$), which indicates that simple selection of heavier heifers will not be translated into better FTAI outcomes. Although all heifers included in this genomic analysis were deemed appropriate for FTAI—based on adequate BW and BCS, and evidence of a previous CL—not all became pregnant from FTAI. A better understanding of the molecular basis for FTAI failure may help to improve hormonal protocols and guide selection for better pregnancy rates.

The main objective of including a GWAS for BW in the present study was to have a "positive control". Genomic regions and SNPs associated with BW in Brahman and other cattle breeds have been previously reported. One of the most prominent regions is on chromosome 14 encompassing the *PLAG1* gene (Karim et al., 2011, Nishimura et al., 2012, Fortes et al., 2013), which has been shown to influence bovine stature (Karim et al., 2011). The gene *XKR4* maps to a genome region nearby the *PLAG1* gene, and the region around

XKR4 has been associated with a number of different phenotypes, including BW (Porto Neto et al., 2012). The fact that we were able to find predictable results for BW—identifying genomic regions of major effect despite the pressures of multiple testing on sample size—demonstrates that experimental design and methodology were appropriate, and implies that the SNP associations found for PREG1 are valid.

As expected, multiple genes of low to moderate effect were identified as influencing FTAI outcomes. The estimated effect size of significant SNP for PREG1 was much larger than for BW, but SNPs were more distributed across the genome, which is suggestive of a more complex genetic architecture. The finding that PREG1 is a polygenic, 'noiser' trait than BW is not surprising. Female reproduction can be considered a highly complex phenotype in terms of physiology and genetic inheritance, given the large number of interacting genes associated with traits such as age at puberty, post-partum anoestrus interval, or first service conception (Fortes et al., 2010, Fortes et al., 2012b, Hawken et al., 2012). The finding does, however, have relevance for genomic selection strategies, indicating that selection strategies appropriate for highly complex traits would be most appropriate for PREG1.

Some SNP associated with PREG1 pointed to genomic regions identified in previous studies of bovine reproduction. For example, 35 SNP associated with PREG1 ($P < 0.05$) were shown to be associated with puberty (AGECL, $P < 0.05$) in a previous GWAS analysis of a different population of Brahman heifers (Hawken et al., 2012). Also, 15 SNP with $P < 0.05$ for PREG1 were associated at the same level with post-partum anoestrus interval (PPAI) (Hawken et al., 2012). Further scrutiny of the "top" regions that are in common for PREG1, AGECL and PPAI might help to elucidate the underlying molecular mechanisms and lead to mutation discoveries that are important for overall Brahman heifer fertility.

The "top" region on chromosome 1 was defined by a SNP close to *CCDC14* (nearest gene). In humans, the protein coded by *CCDC14* (NP_073594) interacts with calmodulin (CaM) (Shen et al., 2005). In mice, female fertility is markedly reduced in CaMKIV-deficient mice due to impaired follicular development and ovulation (Wu et al., 2000). Ovulation is preceded by a marked increase in LH releasing hormone (LHRH), which culminates in pre-ovulatory LH surge. The expression of LHRH appears to be mediated by the calcium/calmodulin-dependent protein kinase (Wu et al., 2005). If *CCDC14* regulates calmodulin activity in cattle, then it could have an impact on LH signalling and ovulation, with consequences for how heifers respond to hormonal protocols in FTAI. Such hypotheses require further research and testing to understand the mechanisms underlying the association of the "top" region on chromosome 1.

The "top" region in chromosome 7 (SNP at 14,889,952 bp) sits within a large QTL associated with still birth in dairy cattle (Höglund et al., 2012), and encompasses an uncharacterized protein (ENSBTAG00000039809). Improved genome annotation is required to elucidate the possible link between this uncharacterized protein, PGREG1, and still birth.

The "top" region in chromosome 9 was defined by an intergenic SNP at 38,412,334 bp, which is 200 kb away from the nearest gene: *LAMA4*. The only other gene close to that SNP is a predicted tRNA (Ensembl data). Once more, improved genome annotation is required to elucidate the link between this genomic region and heifer reproductive traits. Ongoing efforts such as FAANG (http://www.faang.org/) should improve the cattle genome annotation and aid future GWAS interpretation.

5.4 Potential strategies to increase use of AI in northern Australia

At the commencement of the project, Beef Breeding Services (BBS) Rockhampton were contacted and asked to provide an estimate of the number of females that were AI'ed annually in northern Australia. Their estimate was less than 1%. We recently contacted BBS and asked the same question – they said there had been a considerable increase in usage of AI in Queensland beef cattle and estimated that the percentage being AI'ed each year had at least doubled over the past few years. One BBS AI technician said he was now annually doing at least 2,000 inseminations of beef cattle. Further, although most of north Queensland has been experiencing severe drought conditions, Dr Sophia Edwards has reported that she recently helped manage several large scale FTAI programmes, including one run in a large commercial bull breeding herd. She said 'We are consistently achieving pregnancy rates around 43% in well managed Brahman heifers and about 55% in well managed Brahman cows.' These results are very similar to what is been routinely achieved in South American beef herds (Bo pers.com.). Producers in northern Australia need to accept that these are the pregnancy rates that they are likely to achieve if they have planned and executed their AI programmes carefully, and these rates will readily generate the numbers of genetically improved males and females they need for their herds.

There is an urgent need for development of producer/staff training packages on how to plan and conduct AI programmes to generate replacement bulls in particular. Producers/managers need to understand what is realistically achievable when best practice protocols are used. They need to understand what are the major factors affecting AI outcome, particularly the critical impact of the inherent fertility of their herd.

A critical area of concern is the limited number of experienced AI technicians/veterinarians in northern Australia. This project has demonstrated that standard beef cattle handling facilities can be readily modified to enable two technicians to work simultaneously. This enables 400-600 females to be AI'ed per day. However, inexperienced technicians will not be able to AI this many in a day and due to fatigue and lack of attention to detail will achieve significantly lower pregnancy rates than where experienced technicians carry out the AI.

Further, strategies need to be developed to significantly reduce the cost per straw of semen to at least levels similar to those in the dairy industry. These need to be developed in conjunction with existing artificial breeding centres in northern Australia as well as with individual or groups of producers who wish to purchase genetically elite bulls and then collect semen for use in AI programmes. It is critically important that before semen is collected from bulls managed on property that they undergo testing to ensure they are free of recognised infectious diseases (bovine pestivirus, *Campylobacter fetus venerealis, Tritrichomonas foetus*) and heritable congenital abnormalities e.g Pompe's disease.

During the course of the project the authors explored the use of either chilled or ambient temperature stored semen for use in FTAI programmes. This approach is commonly used in the dairy industry in New Zealand and Ireland and the authors feel that the approach could be adapted and applied in northern Australia, which could significantly reduce the cost of semen per straw and facilitate more widespread use of AI in herds. Further research needs to be done to develop semen diluents and semen extension protocols which enable AI of cattle with ambient stored semen over periods of perhaps up to a week.

6 Conclusions/recommendations

This project has demonstrated that there are effective treatment protocols for synchronisation of ovulation in Brahman heifers. These protocols are likely to be at least as efficacious in other tropically adapted genotypes. Currently, treatment of heifers with these protocols results in approximately 75% of females ovulating synchronously. If the herd is fertile i.e the probability of conception per oestrous cycle during mating is 60%, then use of FTAI will typically result in 40 to 45% of Brahman heifers becoming pregnant. Further modifications to the synchronisation protocols may result in modest increases to the percentage pregnant after FTAI, but the authors conclude that the current protocols yield satisfactory results and producers need to be encouraged to more widely adopt the technology as part of their genetic improvement programme. Further, by using an over-mating strategy it is likely that producers can generate sufficient pregnant heifers for their herd within a 4-6 week mating period, which affords significant nutritional and weaning management advantages.

The findings from this project that becoming pregnant to FTAI is likely to be a heritable trait in cycling Brahman heifers may offer further opportunity for selection for improved fertility. However, the authors very strongly feel that the potential real opportunity for improved selection for fertility in herds using FTAI in their heifers is where they then identify those pregnant heifers which subsequently calve and recommence cycling within 2 months of calving. Heifers that become pregnant to FTAI will calve over a 2 week period (approximately). Producers could then organise to have the ovaries examined using ultrasonography 50 to 60 days after the last calf was born. Those 1st lactation cows that have a CL could then be again treated to synchronise ovulation, undergo FTAI and the bulls resulting from these mating are likely to carry the genetics for enhanced lifetime productivity, as demonstrated in Beef CRC III.

7 Key Messages

- To achieve a satisfactory pregnancy rate either using AI after oestrous detection or FTAI requires careful planning and attention to detail. Planning must start at the time heifer calves are weaned to ensure they grow adequately through to time of first calving. A good rule of thumb for Brahman heifers is that they must weigh 70% of mature body weight for the majority of them to have reached puberty (Fordyce pers. com). However, it is very important to recognise that age at puberty is influenced by both genetic and nutritional factors.
- It is critical that the timing of AI programmes ensure females calve at the time of the year when they are most likely to become pregnant within 4 months after calving i.e between October and March depending on typical timing of the onset of the wet season.
- Producers need to discuss with their veterinary advisor the risks of infectious disease outbreaks during the AI programme and during gestation. The two infectious disease of major concern in northern Australia are bovine viral diarrhoea virus (BVDV) and vibriosis (campylobacteriosis).
- Cycling heifers are more likely to conceive to FTAI – select heifers that have a CL at the time of first hormonal treatment to synchronise ovulation. Experienced veterinarians and technicians can readily scan 400 to 600 heifers per day.
- Treatments to synchronise ovulation and FTAI do NOT improve fertility. There is evidence that 20 to 25% of treated heifers (especially 100% *Bos indicus* genotypes)

do not respond normally to the hormonal treatments and thus are very unlikely to become pregnant to FTAI.

- Pregnancy rates to FTAI in heifers are significantly higher in herds in which the pregnancy rate in the first 3weeks of natural mating of heifers is high (i.e ≥ 60%), and where pregnancy rates to natural mating are low pregnancy rates to FTAI are more likely to be lower than expected.
- In well managed Brahman herds that have been selected for fertility, pregnancy rates to FTAI of 40 to 43% can be consistently achieved, even under severe drought conditions.
- Sires to be used in bull breeding herds should be selected on the basis that their EBV for scrotal circumference is in the top 10% for the breed and they are producing ejaculates with at least 70% morphologically normal sperm.
- Semen to be used in any AI programme should be assessed prior to use to verify that it is of satisfactory quality i.e immediately after thawing at least 70% of sperm are morphologically normal and 60% of sperm have an intact acrosome and 25% of sperm are progressively motile at a moderate speed (A Barth Society of Theriogenology). Storage and handling of sperm is critical; the level of liquid nitrogen in AI tanks should be checked once a week and when searching for particular straws of semen the bucket containing the semen should not be lifted above the frost line.
- Bulls to be used for natural mating after the AI programme should undergo a breeding soundness examination and only bulls that are physically sound and are producing ejaculates with at least 30% progressively motile sperm and at least 70% morphologically normal sperm used. Selected bulls should be vaccinated against any infectious diseases known to cause death or illness e.g botulism, tick fever and bovine ephemeral fever, or venereal disease.
- If producers are planning to AI more than 50 heifers in a day, they should strongly consider employing someone who has the experience of AI 200+ per day. Completion of an AI course provides an excellent introduction to how to conduct an AI programme but does not prepare people for inseminating large number of females in a single day.
- Producers who are considering conducting FTAI on 200+ heifers in a single day should consider installing a second crush and race in the yards they intend to use to ensure all heifers undergo FTAI within the correct time period after treatment to induce ovulation. Further, producers should ensure there is effective shade over the handling facility and in the holding paddocks adjacent to the yards.
- ODB is superior to GnRH in current IPRD based ovulation synchronisation protocols. If there is evidence of any potential change in usage of ODB in beef cattle further work needs to be done to identify a more effective alternate treatment protocol.
- Pregnancy diagnosis and foetal aging should always be conducted after FTAI to identify those heifers that became pregnant to FTAI. Further works is needed to determine whether the trait ‘becoming pregnant to FTAI’ is positively associated with other key fertility traits such as age at puberty and interval from calving to first ovulation.

8 Bibliography

ABRAMSON, J. H. 2004. WINPEPI (PEPI-for-Windows): computer programs for epidemiologists. Epidemiologic Perspectives & Innovations 2004, 1:6.

ABREU, F. M., CRUPPE, L. H., MAQUIVAR, M., UTT, M. D., MADSEN, C. A., VASCONCELOS, J. L. M., MUSSARD, M. L., DAY, M. L. & GEARY, T. W. 2014a. Effect of follicle age on conception rate in beef heifers. *Journal of Animal Science,* 92**,** 1022-1028.

ABREU, F. M., GEARY, T. W., CRUPPE, L. H., MADSEN, C. A., JINKS, E. M., POHLER, K. G., VASCONCELOS, J. L. M. & DAY, M. L. 2014b. The effect of follicle age on pregnancy rate in beef cows. *Journal of Animal Science,* 92**,** 1015-1021.

ABRI. 2013. *Understanding the EBVs, selection indicies and accuracy* [Online]. Brahman BREEDPLAN, Agricultural Business Research Institute. Available: http://abri.une.edu.au/online/pages/understanding_ebvs_abba.htm [Accessed 8/2/2013 2013].

ADAMS, G. P. Control of ovarian follicular wave dynamics in mature and prepubertal cattle for synchronization & superstimilation. XX World Buiatrics Congress, 1998 Sydney 6-10 July. World Association for Buiatrics, 595-605.

ADAMS, G. P., EVANS, A. C. O. & RAWLINGS, N. C. 1994. Follicular waves and circulating gonadotrophins in 8-month-old prepubertal heifers. *J Reprod Fertil,* 100**,** 27-33.

ADAMS, G. P., MATTERI, R. L., KASTELIC, J. P., KO, J. C. H. & GINTHER, O. J. 1992. Association between surges of follicle-stimulating hormone and the emergence of follicular waves in heifers. *Journal of Reproduction and Fertility,* 94**,** 177-188.

AGUER, D. 1991. The management of oestrus in *Bos indicus* cattle. *Proceedings of the Annual Conference of the Australian Association of Cattle Vets.*

ALEXANDER, D. H., NOVEMBRE, J. & LANGE, K. 2009. Fast model-based estimation of ancestry in unrelated individuals. *Genome Research,* 19**,** 1655-1664.

AUSTIN, E. J., MIHM, M., RYAN, M. P., WILLIAMS, D. H. & ROCHE, J. F. 1999. Effect of duration of dominance of the ovulatory follicle on onset of estrus and fertility in heifers. *Journal of Animal Science,* 77**,** 2219-26.

BALLA, E., PINCINATO, D., MARANA, D., PERES, L. C., BORGES, L. F. K. & BO, G. A. Effect of duration of treatment with progesterone vaginal devices on pregnancy rates in cross-bred zebu cattle artificially inseminated at a fixed time. International Embryo Transfer Society, 2006 Orlando, Florida, USA. Reproduction Fertility and Development, 112-113.

BARTH, A. D. 1993. Evaluation of frozen bovine semen by the veterinary practitioner. *Theriogenology handbook B9 (6/93).* Nashville: Society for theriogenology.

BARWICK, S. A., WOLCOTT, M. L., JOHNSTON, D. J., BURROW, H. M. & SULLIVAN, M. T. 2009. Genetics of steer daily and residual feed intake in two tropical beef genotypes, and relationships among intake, body composition, growth and other post-weaning measures. *Animal Production Science,* 49**,** 351-366.

BO, G. A., ADAMS, G. P., CACCIA, M., MARTINEZ, M., PIERSON, R. A. & MAPLETOFT, R. J. 1995a. Ovarian follcular wave emergence after treatment with progesterone and estradiol in cattle. *Animal Reproduction Science,* 39**,** 193-204.

BO, G. A., ADAMS, G. P., PIERSON, R. A. & MAPLETOFT, R. J. 1995b. Exogenous control of follicular wave emergence in cattle. *Theriogenology,* 43**,** 31-40.

BO, G. A., BARUSELLI, P. S. & MARTINEZ, M. F. 2003. Pattern and manipulation of follicular development in *Bos indicus* cattle. *Animal Reproduction Science,* 78**,** 307-326.

BOLORMAA, S., PRYCE, J. E., KEMPER, K., SAVIN, K., HAYES, B. J., BARENDSE, W., ZHANG, Y., REICH, C. M., MASON, B. A., BUNCH, R. J., HARRISON, B. E., REVERTER, A., HERD, R. M., TIER, B., GRASER, H. U. & GODDARD, M. E. 2013. Accuracy of prediction of genomic breeding values for residual feed intake and

carcass and meat quality traits in Bos taurus, Bos indicus, and composite beef cattle. *Journal of Animal Science,* 91**,** 3088-104.

BOM. 2012. *Climate Data Online* [Online]. Australian Government Bureau of Meterology. Available: http://www.bom.gov.au/climate/data/ [Accessed 11/07/2012 2012].

BRIDGES, G. A., HELSER, L. A., GRUM, D. E., MUSSARD, M. L., GASSER, C. L. & DAY, M. L. 2008. Decreasing the interval between GnRH and $PGF_{2\alpha}$ from 7 to 5 days and lengthening proestrus increases timed-AI pregnancy rates in beef cows. *Theriogenology,* 69**,** 843-851.

BRIDGES, G. A., MUSSARD, M. L., BURKE, C. R. & DAY, M. L. 2010. Influence of the length of proestrus on fertility and endocrine function in female cattle. *Animal Reproduction Science,* 117**,** 208-215.

BROWNING, B. L. & BROWNING, S. R. 2011. A Fast, Powerful Method for Detecting Identity by Descent. *American Journal of Human Genetics,* 88**,** 173-182.

BUREAU OF METEROLOGY. 2014. *Climate and past weather* [Online]. Australian Government. Available: http://www.bom.gov.au/climate/ [Accessed 18/03/2014.

BURNS, B. M., FORDYCE, G. & HOLROYD, R. G. 2010. A review of factors that impact on the capacity of beef cattle females to conceive, maintain a pregnancy and wean a calf - Implications for reproductive efficiency in northern Australia. *Animal Reproduction Science,* 122**,** 1-22.

BURROW, H. M., GRIFFITH, G. R., BARWICK, S. A. & HOLMES, W. E. 2003. Where to from Brahmans in the northern Australian herd? Maintaining the economic benefit of earlier infusions of *Bos indicus*. *Association for the Advancement of Animal Breeding and Genetics.,* 15**,** 294-297.

BURROW, H. M., SEIFERT, G. W. & CORBET, N. J. 1988. A new technique for measuring temperament in cattle. *Proceedings of the Australian Society of Animal Production,* 17**,** 154-7.

BURROW, H.M., 2006. " Links between the genetics of beef quality and components of herd profitability in northern Australia." Final MLA Report. B.NBP.301.

BUTLER, S. A. A., AITKINSON, P. C., BOE-HANSEN, G., BURNS, B., DAWSON, K., BO, G. A. & MCGOWAN, M. R. 2011a. 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,* 76**,** 1416-1423.

BUTLER, S. A. A., ATKINSON, P. C., BOE-HANSEN, G., BURNS, B. M., DAWSON, K., BO, G. A. & MCGOWAN, M. R. 2011b. 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,* 76**,** 1416 - 1423.

BUTLER, S. A. A., PHILLIPS, N., BOE-HANSEN, G., BO, G. A., BURNS, B., DAWSON, K. & MCGOWAN, M. R. 2011c. Ovarian responses in *Bos indicus* heifers treated to synchronise ovulation with intravaginal progesterone releasing devices, oestradiol benzoate, prostaglandin $F_{2\alpha}$, and eCG. *Animal Reproduction Science,* 129**,** 118-126.

BUTLER, S. A. A., PHILLIPS, N. J., BOE-HANSEN, G. B., BO, G. A., BURNS, B. M., DAWSON, K. & MCGOWAN, M. R. 2011d. Animal-level factors affecting ovarian function in Bos indicus heifers treated to synchronise ovulation with intravaginal progesterone releasing devices and oestradiol benzoate. *Reproduction in Domestic Animals*.

BUTLER, S. A. A., PHILLIPS, N. J., BOE-HANSEN, G. B., BO, G. A., BURNS, B. M., DAWSON, K. & MCGOWAN, M. R. 2012. Animal-level factors affecting ovarian function in *Bos indicus* heifers treated to synchronise ovulation with intravaginal progesterone releasing devices and oestradiol benzoate. *Reproduction in Domestic Animals,* 47**,** 463-471.

CARVALHO, J. B. P., CARVALHO, N. A. T., REIS, E. L., NICHI, M., SOUZA, A. H. & BARUSELLI, P. S. 2008. Effect of early luteolysis in progesterone-based timed AI

protocols in *Bos indicus*, *Bos indicus* x *Bos taurus*, and *Bos taurus* heifers. *Theriogenology,* 69**,** 167-175.

CAVALIERI, J., COLEMAN, C., RODRIGUES, H., MACMILLAN, K. L. & FITZPATRICK, L. A. 2002. The effect of timing of administration of oestradiol benzoate on characteristics of oestrus, timing of ovulation and fertility in Bos indicus heifers synchronised with a progesterone releasing intravaginal insert. *Australian Veterinary Journal,* 80**,** 217-223.

CHENOWETH, P. J. 1994. Aspects of reproduction in female *Bos indicus* cattle: a review. *Australian Veterinary Journal,* 71**,** 422-426.

CHESTA, P., CUTAIA, L. & BO, G. A. Effecto del tratamiento con DIB por 7 u 8 dias en los porcentajes de preñez en vaquillonas cruza cebu inseminadas a tiempo fijo. Vº Simposio Internacional de Reprodución Animal, 2003 Huerta Grande, Córdoba, 27 al 29 de junio de 2003. Abstr. 387.

CLARO JÚNIOR, I., SÁ FILHO, O. G., PERES, R. F. G., AONO, F. H. S., DAY, M. L. & VASCONCELOS, J. L. M. 2010. Reproductive performance of prepubertal *Bos indicus* heifers after progesterone-based treatments. *Theriogenology,* 74**,** 903-911.

COLAZO, M. G. & AMBROSE, D. J. 2011. Neither duration of progesterone insert nor initial GnRH treatment affected pregnancy per timed-insemination in dairy heifers subjected to a Co-synch protocol. *Theriogenology,* 76**,** 578-588.

DADARWAL, D., MAPLETOFT, R. J., ADAMS, G. P., PFEIFER, L. F. M., CREELMAN, C. & SINGH, J. 2013. Effect of progesterone concentration and duration of proestrus on fertility in beef cattle after fixed-time artificial insemination. *Theriogenology,* 79**,** 859-866.

DOBSON, H. & SMITH, R. F. 2000. What is stress, and how does it affect reproduction? *Animal Reproduction Science,* 60-61**,** 743-752.

EDWARDS, S. A. A., ATKINSON, P. C., SATAKE, N., BOE-HANSEN, G. B. & MCGOWAN, M. R. 2014. Ovarian dynamics in response to two modified intravaginal progesterone releasing device and oestradiol benzoate based ovulation synchronisation protocols designed for use in Brahman heifers. *Animal Reproduction Science,* 148**,** 18-25.

EDWARDS, S. A. A., BO, G. A., DAWSON, K., ATKINSON, P. C. & MCGOWAN, M. R. 2015. Comparison of the pregnancy rates and costs per calf born after fixed time AI of *Bos indicus* heifers following synchronization of ovulation, or AI following estrus detection before and after a single prostaglandin $F_{2\alpha}$ treatment. *Theriogenology,* 83**,** 114-120.

EDWARDS, S. A. A., BO, G. A., DAWSON, K. & MCGOWAN, M. R. Comparison of the proportion of *Bos indicus* heifers pregnant following synchronisation of ovulation and fixed-time AI or oestrous detection before and after a single prostaglandin $F_{2\alpha}$ treatment. 17th International Congress on Animal Reproduction (ICAR), 2012 Vancouver, July 2012.

EDWARDS, S. A. A., BURNS, B. M., ALLEN, J. & MCGOWAN, M. R. Potential economic return from use of fixed-time artificial insemination as part of a genetic improvement programme. Proceedings of the 20th conference of the association for the advancement of animal breeding and genetics (AAABG). , 2013a 20 - 23 October 2013, Napier, New Zealand. Association for the advancement of animal breeding and genetics 90.

EDWARDS, S. A. A., PHILLIPS, N. J., BOE-HANSEN, G. B., BO, G. A., BURNS, B. M., DAWSON, K. & MCGOWAN, M. R. 2013b. Follicle stimulating hormone secretion and dominant follicle growth during treatment of *Bos indicus* heifers with intra-vaginal progesterone releasing devices, oestradiol benzoate, equine chorionic gonadotrophin and prostaglandin $F_{2\alpha}$. *Animal Reproduction Science,* 137**,** 129-136.

ENTWISTLE, K. W. & FORDYCE, G. 2003a. Clinical history and physical evaluation - assessment and standards. *Evaluating and reporting bull fertility.* Indooroopilly, Queensland, Australia: Australian Association of Cattle Veterinarians.

ENTWISTLE, K. W. & FORDYCE, G. 2003b. *Evaluating and reporting bull fertility,* Indooroopilly, Queensland, Australia, Australian Association of Cattle Veterinarians.

FIOL, C., QUINTANS, G. & UNGERFELD, R. 2010. Response to biostimulation in peri-puberal beef heifers: influence of male-female proximity and heifer's initial body weight. *Theriogenology,* 74**,** 569-575.

FIOL, C. & UNGERFIELD, R. 2012. Biostimulation in cattle: Stimulation pathways and mechanisms of response. *Tropical and Subtropical Agroecosystems,* 15**,** S29-S45.

FORDYCE, G., BURNS, B. M. & HOLROYD, R. 2005. Minimising pregnancy failure and calf loss. Final Report, Project NBP.336. Sydney: Meat and Livestock Australia.

FORDYCE, G., DODT, R. M. & WYTHES, J. R. 1988. Cattle temperaments in extensive beef herds in northern Queensland. 1. Factors affecting temperament. *Australian Journal of Experimental Agriculture,* 28**,** 683-687.

FORTES, M. R. S., KEMPER, K., SASAZAKI, S., REVERTER, A., PRYCE, J. E., BARENDSE, W., BUNCH, R., MCCULLOCH, R., HARRISON, B., BOLORMAA, S., ZHANG, Y. D., HAWKEN, R. J., GODDARD, M. E. & LEHNERT, S. A. 2013. Evidence for pleiotropism and recent selection in the PLAG1 region in Australian Beef cattle. *Animal Genetics,* 44**,** 636-647.

FORTES, M. R. S., LEHNERT, S. A., BOLORMAA, S., REICH, C., FORDYCE, G., CORBET, N. J., WHAN, V., HAWKEN, R. J. & REVERTER, A. 2012a. Finding genes for economically important traits: Brahman cattle puberty. *Animal Production Science,* 52**,** 143-150.

FORTES, M. R. S., REVERTER, A., ZHANG, Y. D., COLLIS, E., NAGARAJ, S. H., JONSSON, N. N., PRAYAGA, K. C., BARRIS, W. & HAWKEN, R. J. 2010. Association weight matrix for the genetic dissection of puberty in beef cattle. *Proceedings of the National Academy of Sciences of the United States of America,* 107**,** 13642-13647.

FORTES, M. R. S., SNELLING, W. M., REVERTER, A., NAGARAJ, S. H., LEHNERT, S. A., HAWKEN, R. J., DEATLEY, K. L., PETERS, S. O., SILVER, G. A., RINCON, G., MEDRANO, J. F., ISLAS-TREJO, A. & THOMAS, M. G. 2012b. Gene network analyses of first service conception in Brangus heifers: Use of genome and trait associations, hypothalamic-transcriptome information, and transcription factors. *Journal of Animal Science,* 90**,** 2894-2906.

FORTES, M. R. S., SUHAIMI, A. H. M. S., PORTO-NETO, L. R., MCWILLIAM, S. M., FLATSCHER-BADER, T., MOORE, S. S. M., D'OCCHIO, M. J., MEIRA, C., THOMAS, M. G., SNELLING, W. M., REVERTER, A. & LEHNERT, S. A. 2014. Post-partum anoestrus in tropical beef cattle: A systems approach combining gene expression and genome-wide association results. *Livestock Science,* 166**,** 158-166.

GASSER, C. L., BEHLKE, E. J., GRUM, D. E. & DAY, M. L. 2006. Effect of timing of feeding a high-concentrate diet on growth and attainment of puberty in early-weaned heifers. *J Anim Sci,* 84**,** 3118-22.

GENSTAT 2013. GenStat for Windows, Release 14.1. Oxford: VSN International Ltd.

GIBBS, R. A., TAYLOR, J. F., VAN TASSELL, C. P., BARENDSE, W., EVERSOLE, K. A., GILL, C. A., GREEN, R. D., HAMERNIK, D. L., KAPPES, S. M., LIEN, S., MATUKUMALLI, L. K., MCEWAN, J. C., NAZARETH, L. V., SCHNABEL, R. D., WEINSTOCK, G. M., WHEELER, D. A., AJMONE-MARSAN, P., BOETTCHER, P. J., CAETANO, A. R., GARCIA, J. F., HANOTTE, O., MARIANI, P., SKOW, L. C., SONSTEGARD, T. S., WILLIAMS, J. L., DIALLO, B., HAILEMARIAM, L., MARTINEZ, M. L., MORRIS, C. A., SILVA, L. O., SPELMAN, R. J., MULATU, W., ZHAO, K., ABBEY, C. A., AGABA, M., ARAUJO, F. R., BUNCH, R. J., BURTON, J., GORNI, C., OLIVIER, H., HARRISON, B. E., LUFF, B., MACHADO, M. A., MWAKAYA, J., PLASTOW, G., SIM, W., SMITH, T., THOMAS, M. B., VALENTINI, A., WILLIAMS, P., WOMACK, J., WOOLLIAMS, J. A., LIU, Y., QIN, X., WORLEY, K. C., GAO, C., JIANG, H., MOORE, S. S., REN, Y., SONG, X. Z., BUSTAMANTE, C. D., HERNANDEZ, R. D., MUZNY, D. M., PATIL, S., SAN LUCAS, A., FU, Q., KENT, M. P., VEGA, R., MATUKUMALLI, A., MCWILLIAM, S., SCLEP, G., BRYC, K., CHOI, J., GAO, H., GREFENSTETTE, J. J., MURDOCH, B., STELLA, A., VILLA-ANGULO, R., WRIGHT, M., AERTS, J., JANN, O., NEGRINI, R., GODDARD, M. E., HAYES, B.

J., BRADLEY, D. G., BARBOSA DA SILVA, M., LAU, L. P., LIU, G. E., LYNN, D. J., PANZITTA, F. & DODDS, K. G. 2009. Genome-wide survey of SNP variation uncovers the genetic structure of cattle breeds. *Science,* 324**,** 528-32.

GIMENES, L. U., SA, M. F., CARVALHO, N. A. T., TORRES, J. R. S., SOUZA, A. H., MADUREIRA, E. H., TRINCA, L. A., SARTORELLI, E. S., BARROS, C. M., CARVALHO, J. B. P., MAPLETOFT, R. J. & BARUSELLI, P. S. 2008. Follicle deviation and ovulatory capacity in Bos indicus heifers. *Theriogenology,* 69**,** 852-858.

GINTHER, O. J., BERGFELT, D. R., KULICK, L. J. & KOT, K. 1998. Pulsatility of systemic FSH and LH concentrations during follicular-wave development in cattle. *Theriogenology,* 50**,** 507-519.

GINTHER, O. J., KASTELIC, J. P. & KNOPF, L. 1989a. Composition and characteristics of follicular waves during the bovine estrous cycle. *Animal Reproduction Science,* 20**,** 187-200.

GINTHER, O. J., KNOPF, L. & KASTELIC, J. P. 1989b. Ovarian follicular dynamics in heifers during early-pregnancy. *Biology of Reproduction,* 41**,** 247-254.

GINTHER, O. J., KNOPF, L. & KASTELIC, J. P. 1989c. Temporal associations among ovarian events in cattle during oestrous cycles with two and three follicular waves. *Journal of Reproduction and Fertility,* 87**,** 223-230.

GOODLING JR, R. C., SHOOK, G. E., WEIGEL, K. A. & ZWALD, N. R. 2005. The Effect of Synchronization on Genetic Parameters of Reproductive Traits in Dairy Cattle. *Journal of Dairy Science,* 88**,** 2217-2225.

HAWKEN, R. J., ZHANG, Y. D., FORTES, M. R. S., COLLIS, E., BARRIS, W. C., CORBET, N. J., WILLIAMS, P. J., FORDYCE, G., HOLROYD, R. G., WALKLEY, J. R. W., BARENDSE, W., JOHNSTON, D. J., PRAYAGA, K. C., TIER, B., REVERTER, A. & LEHNERT, S. A. 2012. Genome-wide association studies of female reproduction in tropically adapted beef cattle. *Journal of Animal Science,* 90**,** 1398-1410.

HÖGLUND, J. K., GULDBRANDTSEN, B., LUND, M. S. & SAHANA, G. 2012. Analyzes of genome-wide association follow-up study for calving traits in dairy cattle. *BMC genetics* [Online], 13. Available: http://europepmc.org/abstract/MED/22888914

http://www.pubmedcentral.nih.gov/picrender.fcgi?tool=EBI&pubmedid=22888914&action=stream&blobtype=pdf

http://www.pubmedcentral.nih.gov/articlerender.fcgi?tool=EBI&pubmedid=22888914

http://dx.doi.org/10.1186/1471-2156-13-71

http://europepmc.org/articles/PMC3465222

http://europepmc.org/articles/PMC3465222?pdf=render [Accessed 2012].

JEPHCOTT, S. & NORMAN, S. 2004. Interpreting results of pregnancy diagnosis in extensive beef herds. *Pregnancy diagnosis in cattle.* Eight Mile Plains, Queensland, Australia: Australian Association of Cattle Veterinarians.

JOHNSTON, D. J., BARWICK, S. A., CORBET, N. J., FORDYCE, G., HOLROYD, R. G., WILLIAMS, P. J. & BURROW, H. M. 2009. Genetics of heifer puberty in two tropical beef genotypes in northern Australia and associations with heifer- and steer-production traits. *Animal Production Science,* 49**,** 399-412.

JOHNSTON, D. J., BARWICK, S. A., FORDYCE, G. & HOLROYD, R. G. 2010. Understanding the Genetics of Lactation Anoestrus in Brahman Beef Cattle to Enhance Genetic Evaluation of Female Reproductive Traits. *9th WCGALP.* Leipzig, Germany.

KARIM, L., TAKEDA, H., LIN, L., DRUET, T., ARIAS, J. A. C., BAURAIN, D., CAMBISANO, N., DAVIS, S. R., FARNIR, F., GRISART, B., HARRIS, B. L., KEEHAN, M. D., LITTLEJOHN, M. D., SPELMAN, R. J., GEORGES, M. & COPPIETERS, W. 2011. Variants modulating the expression of a chromosome domain encompassing PLAG1 influence bovine stature. *Nature Genetics,* 43**,** 405-+.

KASTELIC, J. P., PIERSON, R. A. & GINTHER, O. J. 1990. Ultrasonic morphology of corpora lutea and central luteal cavities during the estrous cycle and early pregnancy in heifers. *Theriogenology,* 34**,** 487-498.

LANE, E. A., AUSTIN, E. J. & CROWE, M. A. 2008. Oestrous synchronisation in cattle - Current options following the EU regulations restricting use of oestrogenic compounds in food-producing animals: A review. *Animal Reproduction Science,* 109**,** 1-16.

MARIASEGARAM, M., HARRISON, B. E., BOLTON, J. A., TIER, B., HENSHALL, J. M., BARENDSE, W. & PRAYAGA, K. C. 2012. Fine-mapping the POLL locus in Brahman cattle yields the diagnostic marker CSAFG29. *Animal Genetics,* 43**,** 683-688.

MATUKUMALLI, L. K., LAWLEY, C. T., SCHNABEL, R. D., TAYLOR, J. F., ALLAN, M. F., HEATON, M. P., O'CONNELL, J., MOORE, S. S., SMITH, T. P., SONSTEGARD, T. S. & VAN TASSELL, C. P. 2009. Development and characterization of a high density SNP genotyping assay for cattle. *PLoS One,* 4**,** e5350.

MCCOSKER, T., MCLEAN, D. & HOLMES, P. 2010. MLA final report B.NBP.0518: Northern beef situation analysis 2009. North Sydney, NSW: Meat and Livestock Australia Ltd.

MCLAREN, W., PRITCHARD, B., RIOS, D., CHEN, Y. A., FLICEK, P. & CUNNINGHAM, F. 2010. Deriving the consequences of genomic variants with the Ensembl API and SNP Effect Predictor. *Bioinformatics,* 26**,** 2069-2070.

MIHM, M., BAGUISI, A., BOLAND, M. P. & ROCHE, J. F. 1994. Association between the duration of dominance of the ovulatory follicle and pregnancy rate in beef heifers. *Journal of Reproduction and Fertility,* 102**,** 123-130.

MLA 2006. Managing the breeder herd - Practical steps to breeding livestock in northern Australia. Meat and Livestock Australia Limited.

NISHIMURA, S., WATANABE, T., MIZOSHITA, K., TATSUDA, K., FUJITA, T., WATANABE, N., SUGIMOTO, Y. & TAKASUGA, A. 2012. Genome-wide association study identified three major QTL for carcass weight including the PLAG1-CHCHD7 QTN for stature in Japanese Black cattle. *BMC Genetics,* 13.

NOGUEIRA, G. P. 2004. Puberty in South American *Bos indicus* (Zebu) cattle. *Animal Reproduction Science,* 82-83**,** 361-372.

PEREZ-ENCISO, M. & MISZTAL, I. 2011. Qxpak.5: Old mixed model solutions for new genomics problems. *BMC Bioinformatics,* 12**,** 202.

PETHERICK, J. C. 2005. Animal welfare issues associated with extensive livestock production: The northern Australian beef cattle industry. *Applied Animal Behaviour Science,* 92**,** 211-234.

PHILLIPS, N. J., FORDYCE, G., BURNS, B., WILLIAMS, P., MAYER, D., BO, G. A. & MCGOWAN, M. R. 2010. Does the amount of progesterone in intravaginal implants used to synchronise oestrus affect the reproductive performance of Brahman heifers artificially inseminated at a fixed time. *Reproduction in Domestic Animals,* 45**,** e392-e397.

PHILLIPS, N.J., HOCKEY,C.,BOOTHBY, D., NORMAN, S.T.& MCGOWAN M.R. 2006: Comparison of the reproductive performance of tropically adapted heifers treated with three different protocols for synchronization of estrus. 7th International Ruminant Reproduction Symposium. Te Papa, Wellington, New Zealand

POLAT, B., COLAK, A., KAYA, M. & UCAR, O. 2009. Stimulation of delayed puberty in heifers by using a PRID regime. *Rev Med Vet,* 160**,** 149-153.

PORTILLO, G. E., BRIDGES, G. A., DE ARAUJO, J. W., SHAW, M. K. V., SCHRICK, F. N., THATCHER, W. W. & YELICH, J. V. 2008. Response to GnRH on day 6 of the estrous cycle is diminished as the percentage of *Bos indicus* breeding increases in Angus, Brangus, and Brahman x Angus heifers. *Animal Reproduction Science,* 103**,** 38-51.

PORTO-NETO, L. R., REVERTER, A., PRAYAGA, K. C., CHAN, E. K., JOHNSTON, D. J., HAWKEN, R. J., FORDYCE, G., GARCIA, J. F., SONSTEGARD, T. S., BOLORMAA, S., GODDARD, M. E., BURROW, H. M., HENSHALL, J. M., LEHNERT, S. A. &

BARENDSE, W. 2014. The genetic architecture of climatic adaptation of tropical cattle. *PLoS One,* 9**,** e113284.

PORTO-NETO, L. R., SONSTEGARD, T. S., LIU, G. E., BICKHART, D. M., DA SILVA, M. V., MACHADO, M. A., UTSUNOMIYA, Y. T., GARCIA, J. F., GONDRO, C. & VAN TASSELL, C. P. 2013. Genomic divergence of zebu and taurine cattle identified through high-density SNP genotyping. *BMC Genomics,* 14**,** 876.

PORTO NETO, L. R., BUNCH, R. J., HARRISON, B. E. & BARENDSE, W. 2012. Variation in the XKR4 gene was significantly associated with subcutaneous rump fat thickness in indicine and composite cattle. *Animal Genetics,* 43**,** 785-9.

R DEVELOPMENT CORE TEAM 2014. R: A language and environment for statistical computing. . Vienna, Austria: R Foundation for Statistical Computing.

REVAH, I. & BUTLER, W. R. 1996. Prolonged dominance of follicles and reduced viability of bovine oocytes. *Journal of Reproduction and Fertility,* 106**,** 39-47.

RHODES, R. C., RANDEL, R. D. & HARMS, P. G. 1978. Reproductive studies of Brahman cattle. 4. Luteinizing hormone levels in ovariectomized Brahman, Brahman-x Hereford and Hereford cows following a 20 mg dose of estradiol-17β. *Theriogenology,* 10**,** 429-437.

SA FILHO, M. F., BALDRIGHI, J. M., SALES, J. N. S., CREPALDI, G. A., CARVALHO, J. B. P., BO, G. A. & BARUSELLI, P. S. 2011. Induction of ovarian follicular wave emergence and ovulation in progestin-based timed artificial insemination protocols for Bos indicus cattle. *Animal Reproduction Science,* 129**,** 132-139.

SÁ FILHO, M. F., CRESPILHO, A. M., SANTOS, J. E. P., PERRY, G. A. & BARUSELLI, P. S. 2010. 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. *Animal Reproduction Science,* 120**,** 23-30.

SA FILHO, M. F., TORRES, J. R. S., PENTEADO, L., GIMENES, L. U., FERREIRA, R. M., AYRES, H., PAULA, L., SALES, J. N. S. & BARUSELLI, P. S. 2010. Equine chorionic gonadotropin improves the efficacy of a progestin-based fixed-time artificial insemination protocol in Nelore (*Bos indicus*) heifers. *Animal Reproduction Science,* 118**,** 182-187.

SA FILHO, O. G., MENEGHETTI, M., PERES, R. F. G., LAMB, G. C. & VASCONCELOS, J. L. M. 2009. Fixed-time artificial insemination with estradiol and progesterone for *Bos indicus* cows II: Strategies and factors affecting fertility. *Theriogenology,* 72**,** 210-218.

SARTORI, R. & BARROS, C. M. 2011. Reproductive cycles in *Bos indicus* cattle. *Animal Reproduction Science,* 124**,** 244-250.

SARTORI, R., BASTOS, M. R., BARUSELLI, P. S., GIMENES, L. U., ERENO, R. L. & BARROS, C. M. 2010. Physiological differences and implications to reproductive management of Bos taurus and Bos indicus cattle in a tropical environment. *Soc Reprod Fertil Suppl,* 67**,** 357-75.

SHEN, X., VALENCIA, C. A., SZOSTAK, J. W., DONG, B. & LIU, R. 2005. Scanning the human proteome for calmodulin-binding proteins. *Proc Natl Acad Sci U S A,* 102**,** 5969-74.

SIMOES, R. A. L., SATRAPA, R. A., ROSA, F. S., PIAGENTINI, M., CASTILHO, A. C. S., ERENO, R. L., TRINCA, L. A., NOGUEIRA, M. F. G., BURATINI, J., JR. & BARROS, C. M. 2012. Ovulation rate and its relationship with follicle diameter and gene expression of the LH receptor (LHR) in Nelore cows. *Theriogenology,* 77**,** 139-147.

STATACORP 2007. Stata Statistical Software: Release 10. College Station, Texas, USA: StataCorp.

TAUCK, S.A., OLSEN, J.R., WILKINSON, J.R.C., WEDLAKE, R.J., DAVIS, K.C., BERARDINELLI, J.G. 2010a. Characteristics of temporal patterns of cortisol and luteinizing hormone in primiparous, postpartum, anovular, suckled, beef cows exposed acutely to bulls. *Reproductive Biology & Endochrinology*. 8, 89.

TRÍBULO, A., ROGAN, D., TRIBULO, H., TRIBULO, R., ALASINO, R. V., BELTRAMO, D., BIANCO, I., MAPLETOFT, R. J. & BÓ, G. A. 2011. Superstimulation of ovarian

follicular development in beef cattle with a single intramuscular injection of Folltropin-V. *Animal Reproduction Science,* 129**,** 7-13.

TRÍBULO, A., ROGAN, D., TRÍBULO, H., TRÍBULO, R., MAPLETOFT, R. J. & BÓ, G. A. 2012. Superovulation of beef cattle with a split-single intramuscular administration of Folltropin-V in two concentrations of hyaluronan. *Theriogenology,* 77**,** 1679-1685.

VERONESI, M. C., GABAI, G., BATTOCCHIO, M., MOLLO, A., SOLDANO, F., BONO, G. & CAIROLI, F. 2002. Ultrasonographic appearance of tissue is a better indicator of CL function than CL diameter measurement in dairy cows. *Theriogenology,* 58**,** 61-68.

WANG, S. W., DVORKIN, D. & DA, Y. 2012. SNPEVG: a graphical tool for GWAS graphing with mouse clicks. *Bmc Bioinformatics,* 13.

WILTBANK, M. C. & PURSLEY, J. R. 2014. The cow as an induced ovulator: Timed AI after synchronization of ovulation. *Theriogenology,* 81**,** 170-185.

WOLFE, D. F. 1986. Surgical procedures of the reproductive system of the bull. *In:* MORROW, D. A. (ed.) *Current therapy in theriogenology.* 2nd Edition ed.: W. B. Saunders Co, Philadelphia.

WU, J. Y., GONZALEZ-ROBAYNA, I. J., RICHARDS, J. S. & MEANS, A. R. 2000. Female fertility is reduced in mice lacking Ca2+/calmodulin-dependent protein kinase IV. *Endocrinology,* 141**,** 4777-83.

WU, T. J., MANI, S. K., GLUCKSMAN, M. J. & ROBERTS, J. L. 2005. Stimulation of luteinizing hormone-releasing hormone (LHRH) gene expression in GT1-7 cells by its metabolite, LHRH-(1-5). *Endocrinology,* 146**,** 280-6.

ZAR, J. H. 1984. *Biostatistical analysis,* The University of Michigan, Prentice-Hall.

ZIMIN, A. V., DELCHER, A. L., FLOREA, L., KELLEY, D. R., SCHATZ, M. C., PUIU, D., HANRAHAN, F., PERTEA, G., VAN TASSELL, C. P., SONSTEGARD, T. S., MARCAIS, G., ROBERTS, M., SUBRAMANIAN, P., YORKE, J. A. & SALZBERG, S. L. 2009. A whole-genome assembly of the domestic cow, Bos taurus. *Genome Biol,* 10**,** R42.

9 Appendix I: Potential economic return from use of fixed-time artificial insemination as part of a genetic improvement programme

Summary

To investigate the potential return on investment of implementing a genetic improvement program in a self-replacing commercial Brahman breeding herd, three different selection and breeding strategies were evaluated: 1) Natural mating with no genetic improvement (NATM-G), 2) Natural mating with genetic improvement (NATM+G), and 3) Fixed-time AI (FTAI) with genetic improvement (FTAI+G). In each scenario, the Jap Ox Index was used to quantify genetic gain and improvements were made using a Brahman sire with a top 10% Jap Ox Index ($45). A sire was selected from the progeny generated in Year 1. This sire was then used in Year 3 for natural mating in a multiplier herd. A partial budget was used to calculate the cost per calf weaned. The costs per calf weaned in Year 1 were calculated to be $46.83, $371.42 and $173.76 for NATM-G, NATM+G and FTAI+G, respectively. The Jap Ox Index for the progeny was calculated to be $20.00, $32.50 and $32.50 for NATM-G, NATM+G and FTAI+G, respectively. However, when progeny from Year 1 were used in Year 3 for breeding, the costs per calf weaned in Year 3 were calculated to be $46.83, $10.27 and $4.35 for NATM-G, NATM+G and FTAI+G, respectively. In Year 3, Total Genetic Profit was calculated to be $0, $237.25 and $1275.00 for NATM-G, NATM+G and FTAI+G, respectively. This model supports the return on investment in genetic improvement in Brahman cattle in northern Australia, and demonstrates the value of FTAI in both disseminating improved genetics and improving rate of genetic gain.

Introduction

A range of local and global factors are impacting on the Australian beef industry contributing to an average return on assets of only 0.3 to 2.0%. Poor reproductive performance in extensively managed tropically adapted herds (McCosker *et al*. 2010) is one factor contributing to this poor financial performance. Genetic improvement to increase herd productivity with a strong emphasis on reproduction has the ability to improve the financial performance of northern breeder herds. The findings from recent molecular and quantitative genetic research enable selection of superior tropical breed sires for a range of trait, such as age of puberty, postpartum re-conception interval and lifetime productivity (Fortes *et al.* 2012; Johnston *et al.* 2009). The large genetic variation in reproduction traits observed in the Brahman genotype provides substantial opportunity for improvement through genetic selection (Johnston *et al.* 2009). Artificial insemination (AI) provides a practical method of increasing the dissemination of superior genetics in commercial and seed-stock bull breeding herds. The use of AI in northern Australia is currently estimated to be less than 1% of the breeder herd and traditionally considered difficult to implement in extensively managed herds. A strategy to increase the dissemination of superior genetics in northern beef herds is use of fixed-time AI (FTAI) as it is often associated with lower labor inputs, and enables insemination of large numbers of females and production of more calves than typical oestrus detection programs (Edwards *et al.* 2012). The objective of this study was to compare the potential return on investment of implementing three different selection and breeding strategies 1) Natural mating with no genetic improvement (NATM-G), 2) Natural mating with genetic improvement (NATM+G), and 3) FTAI with genetic improvement (FTAI+G), in a self-replacing commercial Brahman breeding herd

Materials and methods

The Brahman Jap Ox index was used to quantify genetic merit of sires (ABRI 2013) used in three different selection and breeding strategies: Strategy 1—NATM using breed average sires with no genetic improvement (NATM-G), Strategy 2—NATM with genetic improvement using a purchased top 10% Jap Ox sire (NATM+G), and Strategy 3—FTAI with genetic improvement using a top 10% Jap Ox sire (FTAI+G). In each strategy, bulls were produced by NATM or FTAI in Year 1 from the bull breeding herd and used in Year 3 in the multiplier herd. Assumptions for purchase of sire and frozen semen, pregnancy rate to FTAI and overall weaning rate, and costs of FTAI in a 200 cow breeding herd are presented in Table 1.

The cows mated in each strategy were all assumed to have a breed average Jap Ox Index ($20). Genetic gain was calculated for each strategy using the following equations: *[(Sire Jap Ox Index) – ($20)]/2 = Calf Genetic Improvement.* In Year 3, when bulls produced from the Year 1 mating are used in the multiplier herd, the genetic gain is calculated as described above.

Table 1. List of assumptions and costs associated with NATM or FTAI

Item	***Parameters and costs***	***Source***
Breed average Brahman sire	Purchase price: $5,000	
Top 10% Jap Ox Brahman sire	Purchase price: $40,000; Semen Price: $50	
Station labour (@ $200/day)	FTAI: 5 personnel x 3 days = 15 units = $3000 NATM: 2 personnel x 1 days = 2 units = $400	
FTAI costs	Drugs to synchronise ovulation: $3524 AI technician: $1500	
Expected sire working life	4 years	(Smith *et al.* 2011)
Weaning rate (% cows joined)	71 %	(Schatz and Hearnden 2008)
Pregnancy rate to FTAI	35 %	(Edwards *et al.* 2012)
Bull:Cow ratio (NATM)	5 bulls for 200 cows (2.5%)	(Smith *et al.* 2011)

Results and discussion

The costs per calf born in Years 1 and 3 of each strategy are presented in Table 2. In the genetic improvement strategies, more genetically superior progeny were produced using FTAI than NATM (63 vs. 28, respectively). In the NATM+G scenario, as the purchase price of a natural mating sire is relatively high, only one sire was used, and thus the number of cows which could be mated to this sire was only 40 (using a 2.5% mating ratio). This strategy limits the production of genetically superior calves compared to that achieved using FTAI, where all cows in the bull breeding herd were AI once, resulting in a higher total number of genetically superior calves being produced. As a result, in both Years 1 and 3 the cost per genetically superior calf born was lower for the FTAI strategy compared to the NATM-G strategy.

Table 2. Cost per calf generated from NATM-G, NATM+G and FTAI+G strategies.

		Strategy		
Year 1	*Calculation*	*NATM-G*	*NATM+G*	*FTAI+G*
Bull breeding herd (n)	(A)	200	40[a]	200
FTAI costs[c]	(B)	-	-	$ 15,024.00
Cost per sire *(Table 1)*	(C)	$ 5,000.00	$ 40,000.00	$ 5,000.00
Sires (n) *(Table 1)*	(D)	5	1	5
Total sire expenses	C*D = (E)	$ 25,000	$ 40,000.00	$ 25,000
Labour costs	(F)	$ 400.00	$ 400.00	$ 3,400.00
Mating costs for Yr 1[d]	[B+(E/4)] + F = (G)	$ 6,650.00	$10,400.00	$ 6,650.00
Progeny by high genetic merit bull[e]	NATM: (A*0.71) = (H) FTAI: (A*0.35) = (H)	-	28 calves	63 calves
Progeny by average genetic merit bulls	NATM: (A*0.71) = (I) FTAI: (A*0.71)-H = (I)	142 calves	-	79 calves
Cost per calf	G/(H+I) = (K)	$ 46.83	$ 371.42	$173.76
Year 3		*Natural mating using sires generated in Yr 1*		
Bull breeding herd (n)	(L)	200	80	200
Cost per sire	NATM-G: New Sires = (M) NATM+G, FTAI+G: K = (M)	$ 5,000.00	$ 371.42	$173.76
Sires (n) *(Table 1)*[b]	(N)	5	2	5
Total Sire expenses	N*M= (O)	$ 25,000	$ 742.84	$ 868.80
Labour costs	(P)	$ 400.00	$ 400.00	$ 400.00
Mating costs for Yr 3	(O/4) + P = (Q)	$ 6,650.00	$ 585.71	$ 617.20
Progeny from mating	L*0.71 = (R)	142 calves	57 calves	142 calves
Total cost per calf	Q/R = (S)	**$ 46.83**	**$ 10.27**	**$ 4.35**

[a]Due to the relatively high purchase price it is assumed that only 1 purchased sire was used to breed replacement bulls.

[b] A selection intensity of 16% was applied to sires generated from Year 1. Therefore, only 2 sires were retained to join 80 cows in the NATM+G strategy, however, 5 sires were available to join the entire bull breeding herd in the FTAI+G strategy.

[c] Insemination expenses include: Drugs to synchronise ovulation and, AI technician and semen costs.

[d] Mating costs include: Sire expenses and labour costs for mustering and yard handling associated with the mating strategy.

[e] Genetically improved progeny include: Number of calves born from genetic improvement mating. Weaning rate and pregnancy rates to FTAI are as per Table 1.

The lack of adoption of artificial breeding technologies in the northern beef industry could be due to a perceived high cost per calf born. As FTAI+G can generate more high genetic merit calves than

natural mating, the total costs of genetic improvement are spread across a greater number of progeny, resulting in a lower cost per calf born than NATM+G. This model assumes that price of a natural mating sire is correlated with its genetic merit and in turn is correlated with price of semen from this sire. Some assumptions that have not been included in the model are: 1) Genetically improved male progeny not retained for use in the herd may be sold for a higher price than average genetic merit progeny, 2) As a high selection pressure is applied to male progeny (only 16% of available progeny selected) the retained sires should have a higher actual Jap Ox index than calculated in the model, 3) Transport and other associated expenses of purchase of a high genetic merit natural mating sire have not been included, and 4) An increased proportion of females conceiving earlier in the mating period in FTAI may improve weaner values (Spitzer 1986). Total Genetic Profit was calculated to be $ 0, $ 237.25 and $ 1275.00 for NATM-G, NATM+G and FTAI+G, respectively (Table 3). In this comparison the FTAI+G strategy improved the genetic profit of the calves 5.4 times more than the NATM+G strategy. This is explained by the FTAI+G strategy producing 85 more calves by high genetic merit sires multiplying the effects of the genetic improvement strategy.

Table 3. Genetic profit from NATM-G, NATM+G and FTAI+G strategies.

		Strategy		
Year 1	*Calculation*	*NATM-G*	*NATM+G*	*FTAI+G*
Bull breeding herd (n)	(A)	200	40[a]	200
Jap Ox Index of sires	(B)	$ 20	$ 45	$ 45
Average Jap Ox Index of cows	(C)	$ 20	$ 20	$ 20
Genetic gain per calf born	(B-C)/2 = (D)	$ 0	$ 12.50	$ 12.50
Progeny by genetically superior sire(n)	(E)	0	28 calves	63 calves
Progeny by average genetic merit sire (n)	(F)	142 calves	-	79 calves
Total genetic gain	E*D = (G)	$ 0.00	$ 350.00	$ 787.50
Jap Ox Index of progeny	(H)	$ 20.00	$ 32.50	$ 32.50
Year 3		*Natural mating using sires generated in Yr 1*		
Bull breeding herd(n)	(I)	200	80	200
Jap Ox Index of sire	= (H)	$ 20.00	$ 32.50	$ 32.50
Progeny from mating	I*0.71 = (J)	142 calves	57 calves	142 calves
Genetic gain over average females	(H-C)/2 = (K)	$ 0	$ 6.25	$ 6.25
Genetic gain – replacement females Yr 1[c]	D*(E*0.5)=(L)	$ 0	$ 175.00	$ 400.00
Progeny from mating	(M)	140 calves	56 calves	140 calves
Total genetic gain of progeny Yr 3	M*K = (N)	$ 0	$ 62.25	$ 875.00
Total Genetic Profit	L + N = (O)	**$ 0**	**$ 237.25**	**$ 1275.00**

[a]Due to the relatively high purchase price it is assumed that only 1 purchased sire will be used to breed replacement bulls.

[b] A selection intensity of 16% is applied to sires generated from Year 1. Therefore only 2 sires are retained to join 80 cows in the NATM+G Strategy, however, 5 sires are available to join to the entire bull breeding herd in the FTAI+G strategy.

[c] Assume all cows from Year 1 are retained and bred in Year 3. Assume 50% of the calves born in Year 1 are female.

Conclusion

This study supports the return on investment in genetic improvement in Brahman cattle in northern Australia and demonstrates the value of FTAI in both disseminating improved genetics and improving rate of genetic gain.

References

ABRI. (2013). *http://abri.une.edu.au/online/pages/understanding_ebvs_abba.htm* Accessed 8/2/2013 2013.

Edwards, S. A. A., G. A. Bo, K. Dawson, and M. R. McGowan. (2012). In: 'Proceedings 17th Interational Congress on Animal Reproduction (ICAR), Vancouver, July 2012

Fortes, M. R. S. et al. (2012). *Anim. Prod. Sci.* **52**: 143.

Johnston, D. J. et al. (2009). *Anim. Prod. Sci*. **49**: 399.

McCosker, T., D. McLean, and P. Holmes. (2010). Meat and Livestock Australia Ltd., North Sydney, NSW.

Schatz, T. J., and M. N. Hearnden. (2008). *Aust. J. Exp. Agr.* **48**: 940.

Smith, D., McCosker, K., Butler, S. Fordyce, G., Burns, B., Jephcott, S. and McGowan, M. (2011) *Proc. North. Beef. Res. Updat. Conf*. 122

Spitzer, J. C. (1986). In: Current therapy in theriogenology: Diagnosis, treatment and prevention of reproductive diseases in small and large animals. p 320-341. editor D. A. Morrow, W.B. Saunders Company, Philadelphia.

10 Appendix II: Extension

10.1 Publications

S.A.A. Edwards, G.A. Bo, K.A. Chandra, P.C. Atkinson, M.R. McGowan. Comparison of the pregnancy rates and costs per calf born after fixed-time artificial insemination or artificial insemination after estrus detection in Bos indicus heifers. *Theriogenology* 2015, **83** (1) 114-120.

S.A.A. Edwards, P.C. Atkinson, N. Satake, G. Boe-Hanson, M.R. McGowan. Ovarian dynamics in response to two modified intravaginal progesterone releasing device and oestradiol benzoate based ovulation synchronisation protocols designed for use in Brahman heifers. *Animal Reproduction Science* 2014, **148**, 18-25.

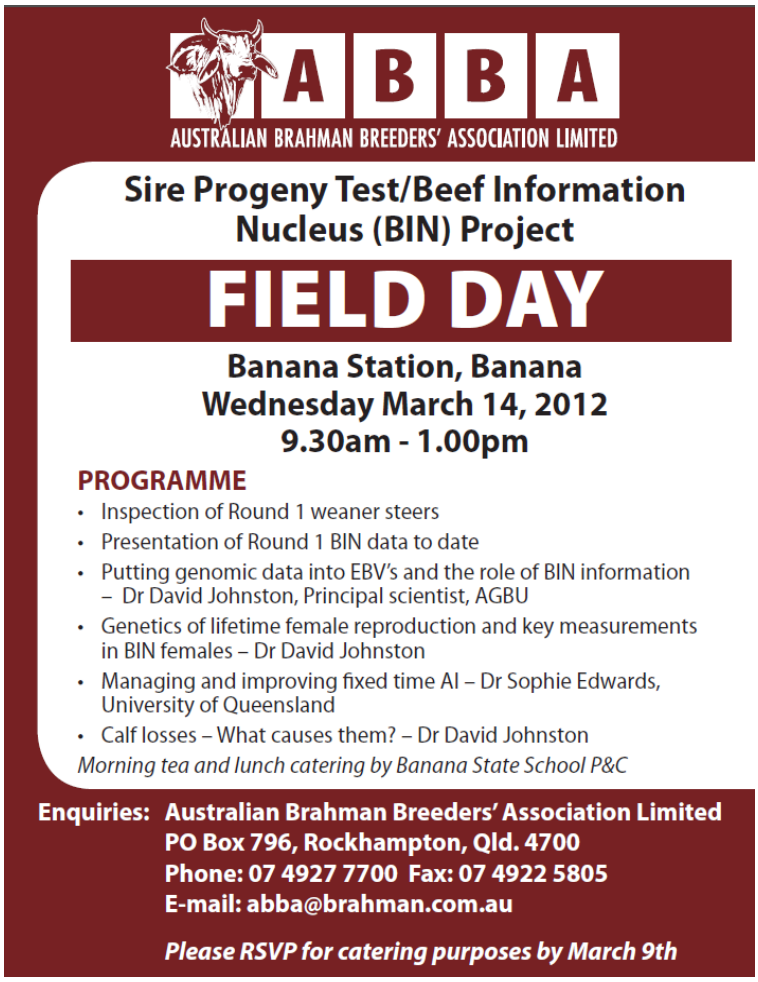
S.A.A. Edwards, N.J. Phillips, G.B. Boe-Hansen, G.A. Bo, B.M. Burns, K. Dawson, M.R. McGowan. Follicle stimulating hormone secretion and dominant follicle growth during treatment of Bos indicus heifers with intra-vaginal progesterone releasing devices, oestradiol benzoate, equine chorionic gonadotrophin and prostaglandin F2α. *Animal Reproduction Science*, 2013, 137: 3–4, 129-136

S.A.A. Edwards, G B Boe-Hansen, N Satake, K Chandra and M R McGowan. 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. *Animal Reproduction Science,* 2014 **submitted***.*

Sophia Edwards, Gabriel Bo, Kerri Dawson, Michael McGowan. Comparison of the proportion of *Bos indicus* heifers pregnant following synchronisation of ovulation and fixed-time AI or oestrous detection before and after a single prostaglandin $F_{2\alpha}$ treatment.*Abstract accepted to the 17th International Congress on Animal Reproduction (ICAR), Vancouver July 2012.*

S.A.A. Edwards, G. Boe-Hansen, B. Burns, G.A. Bo, and M.R. McGowan. Fixed-time artificial insemination in Bos indicus heifers in northern Australia - Recent research. Proceedings of the Australian Cattle Veterinarians 2013 Conference (Darwin) 2013. 25th - 28th June, 134.

10.2 Presentations



10.3 Media releases

Cloncurry AI program builds on Brahman base

CLONCURRY Brahman breeders Dan and Sue Lynch recently teamed up with Queensland's leading artificial insemination (AI) experts to put best practice AI protocols to the test.

The Lynch family run 8000 Brahman and Brahman-Romagnola cross cattle on their 20,000ha property, Tara, 80km north of Cloncurry.

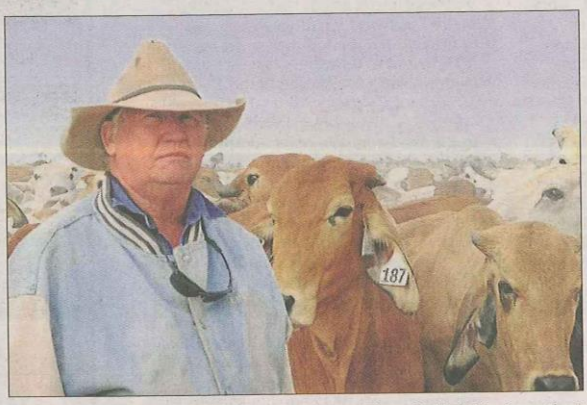
They wanted to use fixed-time AI (FTAI) to investigate the use of new genetics in their Brahman herd.

The Lynchs worked with Dr Sophia Butler from The University of Queensland, Dr Brian Burns from Queensland Alliance for Agriculture and Food Innovation and Ian Braithwaite, a renowned veterinarian from Mt Isa, to carry out the FTAI program using current best practice protocols in January and February this year.

Mr Lynch said undertaking an FTAI program was the easiest way to trial several breeds of cattle over his own cows.

"This was the first time we had done AI so it was a massive learning curve for us," he said.

"We have been crossbreeding in our Brahman based herd for many years and wanted to investigate the use of some new breeds over our Brahman cows."



Cloncurry cattleman Dan Lynch used Fixed Time AI (FTAI) to investigate the use of new genetics in his Brahman herd.

The program involved inseminated 669 Brahman cows that were divided into four groups of around 165 head. The mixed aged cows were inseminated with semen from Angus, Belmont Red, Belmont Red Senepol and Brahman bulls.

As consistent with the Lynch's normal joining requirements, the cows all had a body score of 3.5 or greater and had all delivered a calf within a 12-month calving interval.

All cows were individually tagged so that accurate records could be kept on what semen was used with each cow.

They were selected at random from the herd in January and ovary scanned in early February to remove any with reproductive abnormalities and ensure that cows submitted to the AI program had a sufficient proportion cycling.

The AI program began in earnest 10 days later after a series of treatments to synchronise ovulation.

A Cue-Mate device was inserted, with an injection of oestradiol benzoate and prostaglandin. Eight days later the Cue-Mate device was removed and cows were injected with Pregnecol (equine chorionic gonadotrophin) and prostaglandin. After 24 hours, the cows received another injection of oestradiol benzoate before insemination, 30 hours later.

The mobs were then held in holding paddocks for14 days before being joined to cover bulls. This lapse of 14 days ensured the technicians could accurately determine how many of the cows fell pregnant through the FTAI process.

The mob was pregnancy tested in June when it was determined that 57pc were pregnant as a result of the AI program.

"Because we had never done FTAI before we didn't know what a good result was, but we had been told that anywhere between 40 and 50 percent was reasonable," Mr Lynch said.

Mr Lynch said there were several logistical challenges to overcome to ensure best practice was followed but he said none were insurmountable.

"It was definitely necessary to divide the cows into mobs of 200 because you can't handle 669 cows in a day," he said.

"It was also important that cows were inseminated within a four-hour window on the day of AI, without rushing cattle through and elevating stress levels of both cattle and technicians.

"Because we were dealing with cows we had calves to pull off as well, which made handling more difficult and time consuming.

"We used all low-stress stock handling techniques and I think that played a major role in ensuring the cows were as quiet and calm as possible.

"We also ensured the cows had fresh green feed when they were in the holding paddocks so they came into the yards full and content."

Mr Lynch is now planning to AI 600 Brahman heifers in February using FTAI.

"That will probably be a bit easier because we won't have to handle calves as well," he said.

"We will use the FTAI program again and are looking forward to seeing what results we get this time."

Queensland Country Life, 22 September 2011

Queensland Country Life, 22 September 2011

Fixed time AI picks up in Northern Australia

By **PENELOPE ARTHUR**

ARTIFICIAL insemination (AI) is widely recognised as the most cost-effective way to accelerate genetic improvement in beef herds around the world.

But the adoption of fixed time AI (FTAI) has been lagging in northern Australia, largely due to lower than expected pregnancy rates in tropical beef herds.

Dr Sophia Butler and Professor Michael McGowan from the School of Veterinary Science at the University of Queensland's Gatton Campus have been researching AI programs in northern Australia for the past four years and have recently developed a series of best practice recommendations for producers.

Dr Butler believes the adoption of FTAI in northern herds would dramatically increase the rate of genetic gain in northern beef herds.

She said semen from a genetically superior bull had the potential to produce hundreds of calves annually using FTAI.

"This technology would be particularly beneficial for producers looking to breed their own bulls," she said.

Ms Butler said the use of FTAI could also increase the value of weaners by producing a more compact calving period.

"Currently we can achieve a pregnancy rate of 65pc using FTAI and mop-up sires, so 65pc of the calves would be born at the beginning of the season and therefore weigh heavier at weaning," she said.

"The heifers also have more time to fall pregnant the next year as a first lactation female which is one of the largest restrictions on fertility in the north."

"FTAI could set them up for life in terms of being able to achieve a 12-month calving interval."

What is current best practice for FTAI?

Dr Butler said producers looking to undertake an AI program, first need to consider a whole management approach.

"This includes heifer selection and heifer management through to selecting what AI protocols they wish to use," she said.

"In general there are two approaches to AI, fixed time AI (FTAI) or AI on detected heat."

"FTAI involves synchronising ovulation in females so that all females are inseminated at a predetermined time while detected heat AI involves monitoring groups of heifers two to three times daily for 45 minutes to record the time of standing heat."

"In northern Australia, FTAI is often the better option because accurately detecting heat in large groups, and particularly Bos Indicus genotypes, can be difficult and lead to a reduced number of calves born to AI."

"Producers also need to ask themselves what they hope to achieve out of their AI programs and what condition their breeders are in."

"Most AI programs should be planned well ahead so that adequate nutrition and vaccinations are completed prior to the time of breeding so that their investment in AI is maximised."

Getting the mob prepared

Once the producer has settled on an AI method, they can start preparing for the start of the AI program.

Females should be selected and managed separately to other females at least three months prior to the start of the AI program to ensure they are of adequate body condition or cycling status.

Dr Butler said all females should also be individually identified with either an electronic NLIS tags or property ear tags so accurate breeding records can be maintained.

"There are also a number of viral and venereal infections that can significantly reduce pregnancy and calving rates," she said.

"Producers should consider vaccinating well in advance of the AI programme against pestivirus (BVDV), 3-day sickness (ephemeral fever) and possibly vibrioisis where bull control is difficult."

Management of females for AI programs

Dr Butler said best practice protocols differed slightly for cows and heifers.

She said the task of preparing heifers for AI needed to start at weaning, when the heifers should be yard weaned for a minimum of two weeks to familiarise them with humans and cattle handling facilities.

Turn to page 126

KEY POINTS

- **Best practice protocols for FTAI now available for northern producers.**
- **FTAI recognised as the fastest way to accelerate genetic gains in the north.**
- **Producers can improve profits by up to 45pc by using AI.**
- **New research under way to build on current best practice protocols to help boost FTAI rates in northern Australia.**

Fixed time AI picks up in Northern Australia

From 125

Nutrition is also a vital part of preparing heifers for AI and has been consistently shown to be a key determinant in the likelihood that heifers will be pregnant to AI.

Dr Butler said that depending on breed, heifers needed to achieve liveweight gains of 0.6 to 0.75 kg/day from weaning through to calving to enable heifers to be bred as yearlings.

"However, due to poorer nutritional conditions typical of many parts of northern Australia, heifer growth rates can be much lower and heifers are typically bred as two-year-olds in these conditions," she said.

"It is generally recommended that Bos Indicus heifers and their cross-breeds reach weights of >330kg before being included in an AI program.

"The provision of supplementary feed may be necessary in many instances to ensure the majority of heifers are cycling when the AI program is due to start."

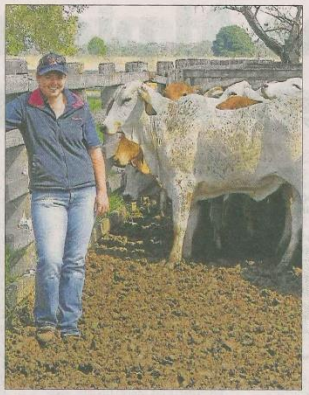
Cows should not be included in a program until 60 days or more after calving to ensure completion of uterine involution and return to cyclicity.

"Breeding earlier than this should not have an adverse effect on subsequent breeding performance but a reduced conception rate can be expected," Dr Butler said.

"There is also good evidence to suggest that 48-hour calf removal is beneficial in inducing the LH surge in postpartum cows.

"We often recommend that heifers and cows are pregnancy tested prior to the start of the AI program so that all females that are pregnant or have abnormalities can be excluded.

"Where bull control is difficult we often find many females presenting for AI that are already pregnant."



LEFT: Dr Sophia Butler conducts research on Brahman heifers at the Belmont Research Station last month.

"We hope to get some results by the middle of next year after pregnancy testing so that the best practice protocol can be used for the 2012-13 breeding season."

"At the same time an ovarian examination may be performed to determined the proportion of animals cycling and this will help determine the protocols you may wish to use"

Dr Butler said that groups of heifers or cows where a proportion of females were anovulatory, the use of ovulation synchronisation protocols for FTAI could be useful to induce cyclicity.

"Ovulation synchronisation using intravaginal progesterone releasing devices (IPRD's) can improve conception rates of Bos Indicus cows and heifers in tropical environments as it can 'kick-start' females to cycle therefore increasing the liklehood of pregnancy earlier in the breeding season," she said.

What's new in research?

Dr Butler has also recently embarked on a new three-year research project that seeks to build upon current best practice protocols and improve the success rate of FTAI in northern herds.

Funded by Meat & Livestock Australia (MLA), Dr Butler said the first study in the project aimed to examine two slightly different methods of FTAI to determine how effective they would be when implemented in northern Australia.

"Our current best practice protocol for FTAI requires the females to be handled about four times," she said.

"We want to test a protocol that will remove one of those handlings.

"The second FTAI method we are testing is one which doesn't use Oestradiol Benzoate (ODB), which is the hormone used to synchronise females for AI.

"ODB is already banned in New Zealand and Europe and has even been voluntarily banned by Australian dairy farmers because of a perceived issue of hormones in food producing animals.

"We wanted to test an FTAI method that doesn't use ODB so that, in the event that this product is banned from use in Australia, we have some protocols for FTAI in Bos Indicus cattle that doesn't require ODB."

The two protocols were tested in the concept stage at the Belmont Research Station in September and researchers hope to test the methods over four cattle stations across northern Australia later this year.

"We hope to get some results by the middle of next year after pregnancy testing so that the best practice protocol can be used for the 2012-13 breeding season," Dr Butler said.

North Queensland Register, November 17 2011

Northern fixed time AI focus for researcher

A HARSH climate, erratic weather and unreliable food stocks make Northern Australia a volatile place to breed cattle. But one researcher says timed artificial reproduction can turn things around.

Dr Sophia Butler from the University of Queensland thinks times are changing and there is a move in the north from cattle harvesting to cattle breeding, opening a platform for fixed time artificial insemination (FTAI).

She said the rate of natural breeding was not meeting demand, and AI was the only way to fast-track genetic gain and glean the beef industry's full potential.

AI offers two significant benefits to the north, according to Dr Butler's research.

She said it paved access to sires with particular traits which couldn't be readily purchased, and offered another market option through crossbreeding with British or European genetics.

The idea is to control and synchronise programs to produce earlier, heavier calves, more compact calving and weaning schedules, and ultimately genetically-superior cattle.

Dr Butler explained a tighter calving interval meant more efficient weaning and more time for the cow to re-conceive during the mating period.

This would also help the problem in the north of post-partum anoestrous where cows won't slip back into the calving cycle for some time because of a lag from calf and lactation.

Boosting profitability is a big focal point of AI.

Dr Butler's figures showed ovulation synchronisation produced more calves earlier in the breeding season than natural reproduction, equating to an estimated profit of $54 per cow.

It operated on generating relatively cheap genetically-superior sires rather than buying them, when they may not be available.

Dr Butler said this outweighed the expense involved in the AI process in the long term.

"It is investing in the profitability of your herd and I believe economically it does have benefits."

It was particularly targeted towards the nucleus and multiplier bull breeding herds.

Dr Butler explained the nucleus was the peak FTAI gene flow, made up of an estimated 126,000-plus females and 50,000 bulls.

She believed it could pick up in these groups, despite estimating less than 1 percent of northern Australia's breeding herd was currently AI.

"I definitely want to see it reach there, and I certainly believe it would become routine in the nucleus and multiplier bull breeding herds."

Based on tropical conditions, Dr Butler's research suggested AI be invested in when cattle were gaining weight, such as the start of the wet season.

"Otherwise, results are not optimised. The likelihood you're going to attract a good result is higher during these times," she said.– *Story: ELIZA ROGERS.*

North Queensland Register, February 23 2012

PRODUCTIVE AGRICULTURE – BEEF

Sophia Butler has done extensive research into artificial fixed-time insemination.

AI opening up new opportunities

By ELIZA ROGERS

A QUEENSLAND veterinarian and researcher is expanding her work in timed artificial reproduction out in the field.

Sophia Butler, from the University of Queensland, presented her work at an expo in the Gulf last year, claiming fixed-time artificial insemination (FTAI) could negate northern Australia's volatile breeding conditions.

She said a harsh climate, erratic weather and unreliable food stocks make the region a difficult breeding ground, but a move from cattle harvesting to cattle breeding opened a new platform.

Dr Butler said the rate of natural breeding was not meeting demand, and AI was the only way to fast-track genetic gain and glean the beef industry's full potential.

AI offers two significant benefits to the north, according to her research.

She said it paved access to sires with particular traits which cannot be readily purchased, and offered another market option through cross-breeding with British or European genetics.

The idea is to control and synchronise programs to produce earlier, heavier calves, more compact calving and weaning schedules, and ultimately, genetically superior cattle.

Dr Butler explained a tighter calving interval meant more efficient weaning and more time for the cow to re-conceive during the mating period.

This would also help the problem in the north of post-partum anoestrus where cows won't slip back into the calving cycle for some time because of a lag from calf and lactation.

Boosting profitability is a big focal point of AI.

Dr Butler's figures show ovulation synchronisation produces more calves earlier in the breeding season than natural reproduction, equating to an estimated profit of $54/cow.

It operates on generating relatively cheap, genetically superior sires rather than buying them, when they may not be available.

Dr Butler said this outweighed the expense involved in the AI process in the long term.

"It is investing in the profitability of your herd, and I believe economically it does have benefits," she said.

It's particularly targeted towards the nucleus and multiplier bull-breeding herds.

Dr Butler explained the nucleus is the peak FTAI gene flow, made up of an estimated more than 126,000 females and 50,000 bulls.

She believes it can pick up in these groups, despite estimating less than 1 per-cent of northern Australia's breeding herd is currently AI.

"I definitely want to see it reach there, and I certainly believe it would become routine in the nucleus and multiplier bull-breeding herds," she said.

Based on tropical conditions, Dr Butler's research suggests AI be invested in when cattle are gaining weight, such as at the start of the wet season.

"Otherwise, results are not optimised," Dr Butler said.

"The likelihood you're going to attract a good result is higher during these times."

10.4 Capacity building

Support and advice for beef producers wishing to conduct AI programs:

During the 2011/2012 breeding season we have been involved in providing support and assistance to a number of commercial producers interested in conducting AI programs. In particular, advice has been given to Annie Donoghue at Barranga Station, QLD to assist in the AI programmes that have been run in conjunction with the Brahman BIN project. This programme enrolled well prepared, majority cycling, Brahman heifers, in an oestrus detection (n = 405) and FTAI (n = 261) program. There was bias in selection of heifer in these groups, due to the FTAI heifers being selected after not being observed in oestrus. The programme resulted in a 90.1% submission rate and 56% conception rate (CR) to oestrus detected AI and a 43.7% pregnancy rate (PR) to FTAI.

Advice for the NT Department of Resources:

We have been providing support and advice to the NT to assist in the development of the PDS site for the polled gene marker. This PDS heavily relies on the use of AI to be able to deliver the appropriate genetics. Females have been synchronised and inseminated and the pregnancy diagnosis is pending.

11 Appendix III: Statistical analysis of allocation procedures

11.1 Study 2: Heifer allocation to treatment group, AI group and technician

Retrospective analysis of allocation procedures for assigning Brahman heifers on Properties A, B, C and D to AI group (Day 1, 2 or 3), treatment group (OPO or GPG), and AI technician (1 to 7). Mean ± SEM of liveweight (LW) and body condition score (BCS) and proportion of heifers with a CL.

		Group	*n*	*LW (Kg)*	*BCS (1-5)**	*Presence of CL (%)*
Property A	***AI Group***	1	197	381.1 ± 2.7	3.24 ± 0.03	78.41
		2	211	369.5 ± 2.4	3.28 ± 0.02	77.51
		P-value	-	*<0.001*	*0.157*	*0.837*
	Treatment	OPO	207	378.1 ± 2.5	3.25 ± 0.02	83.06
		GPG	201	372.5 ± 2.4	3.27 ± 0.02	72.65
		P-value	-	*0.065*	*0.522*	*0.01*
	Technician	4	100	378.3 ± 3.4	3.25 ± 0.03	76.01
		5	114	374.8 ± 3.0	3.25 ± 0.03	82.33
		6	62	373.5 ± 4.5	3.30 ± 0.04	85.91
		7	132	374.6 ± 3.0	3.24 ± 0.03	71.86
		P-value	-	*0.767*	*0.516*	*0.072*
Property B	***Treatment***	OPO	36	355.8 ± 5.2	3.5	91.87
		GPG	37	363.7 ± 5.9	3.5	83.61
		P-value	-	0.274	-	0.294
	Technician	1	38	352.1 ± 4.9	3.5	85.13
		2	26	358.3 ± 5.9	3.5	91.93
		3	9	368.9 ± 10.1	3.5	86.17
		P-value	-	*0.318*	-	*0.701*
Property C	***AI Group***	1	198	354.1 ± 2.9	3.67 ± 0.02	75.66
		2	197	342.8 ± 3.3	3.69 ± 0.03	78.33
		3	55	347.5 ± 5.1	3.71 ± 0.04	74.02
		P-value	-	*0.005*	*0.511*	*0.732*
	Treatment	OPO	224	349.8 ± 3.2	3.69 ± 0.03	75.94
		GPG	226	346.5 ± 3.3	3.69 ± 0.03	77.32
		P-value	-	*0.290*	*0.799*	*0.728*
	Technician†	1	197	348.7 ± 2.6	3.70 ± 0.02	76.21
		2	230	350.5 ± 2.5	3.71 ± 0.02	77.13
		3	22	345.3 ± 7.1	3.67 ± 0.06	75.20
		P-value	-	*0.703*	*0.764*	*0.963*
Property D	***AI Group***	1	108	387.1 ± 7.5	3.56 ± 0.04	81.79
		2	105	387.7 ± 7.7	3.49 ± 0.04	89.86
		P-value	-	*0.927*	*0.017*	*0.097*
	Treatment	OPO	107	387.3 ± 7.5	3.53 ± 0.04	85.11
		GPG	106	387.5 ± 7.5	3.51 ± 0.04	86.43
		P-value		*0.979*	*0.324*	*0.773*
	Technician§	1	198	385.5 ± 4.9	3.50 ± 0.03	85.84
		3	13	389.3 ± 11.6	3.54 ± 0.06	84.60
		P-value	-	*0.727*	*0.507*	*0.893*

** All heifers on Property B had a BCS of 3.5.*

† One heifer did not have a technician recorded.
§ Two heifers did not have a technician recorded.

11.2 Study 2: Sire allocation

Retrospective analysis of allocation procedure of Brahman heifers to AI sires 1 to 61. Mean ± SEM of liveweight (LW) and body condition score (BCS) and proportion of heifers with a CL. Superscripts indicate significant differences (P < 0.05).

Sire	*n*	*LW (Kg)*	*BCS (1-5)**	*Presence of CL (%)*
Property A				
5	6	375.6 ± 11.9	3.33 ± 0.11	66.65
17	20	377.0 ± 6.6	3.31 ± 0.06	85.38
18	6	365.4 ± 12.1	3.14 ± 0.11	73.14
19	17	373.0 ± 7.1	3.27 ± 0.07	81.36
20	2	381.3 ± 20.6	3.29 ± 0.19	99.97
21	6	361.1 ± 12.0	3.26 ± 0.11	99.97
22	4	364.4 ± 14.6	3.17 ± 0.13	46.85
23	5	348.0 ± 13.3	3.30 ± 0.12	99.96
24	16	382.6 ± 7.4	3.26 ± 0.07	77.91
25	7	383.2 ± 11.1	3.23 ± 0.10	99.96
26	17	381.6 ± 7.1	3.33 ± 0.06	94.66
27	8	379.8 ± 10.3	3.31 ± 0.09	60.46
28	20	368.0 ± 6.6	3.29 ± 0.06	86.63
29	6	375.0 ± 11.9	3.43 ± 0.11	66.33
31	25	389.2 ± 5.9	3.30 ± 0.05	80.19
32	19	371.5 ± 6.7	3.19 ± 0.06	64.99
33	7	380.9 ± 11.1	3.30 ± 0.10	99.96
34	7	379.9 ± 11.9	3.29 ± 0.10	66.37
35	11	372.9 ± 8.8	3.14 ± 0.08	81.76
36	17	372.6 ± 7.1	3.18 ± 0.06	71.62
37	15	382.4 ± 7.6	3.28 ± 0.07	72.51
38	2	375.7 ± 20.7	3.28 ± 0.19	99.96
39	5	379.9 ± 13.1	3.27 ± 0.12	99.98
40	14	371.7 ± 8.0	3.22 ± 0.07	75.59
41	42	370.2 ± 4.5	3.27 ± 0.04	79.28
42	20	375.6 ± 6.6	3.25 ± 0.06	70.16
43	18	382.9 ± 7.0	3.25 ± 0.06	86.37
44	20	374.5 ± 6.6	3.27 ± 0.06	75.60
45	16	383.6 ± 7.3	3.20 ± 0.07	61.86
46	10	375.6 ± 9.2	3.19 ± 0.08	71.70
47	20	378.7 ± 6.5	3.23 ± 0.06	67.09
P-value		0.820	0.985	0.296
Property B				
15	38	355.8 ± 5.3	3.5	89.63
16	35	363.7 ± 5.7	3.5	85.56
P-value		0.276	-	0.600

Sire	*n*	*LW (Kg)*	*BCS (1-5)**	*Presence of CL (%)*
Property C				
1	22	350.8 ± 7.6	3.67 ± 0.06	64.30^{bc}
2	20	353.1 ± 7.7	3.66 ± 0.07	50.48^{c}
3	21	344.8 ± 7.4	3.70 ± 0.06	90.79^{ab}
4	20	343.5 ± 7.7	3.71 ± 0.07	70.58^{bc}
5	47	346.8 ± 5.3	3.68 ± 0.05	75.42^{abc}
6	46	353.9 ± 5.2	3.69 ± 0.04	69.56^{bc}
7	46	352.7 ± 5.2	3.68 ± 0.04	84.77^{ab}
8	46	353.0 ± 5.3	3.76 ± 0.04	74.03^{bc}
9	47	345.5 ± 5.1	3.70 ± 0.04	74.59^{bc}
10	45	344.0 ± 5.3	3.66 ±0.04	91.03^{a}
11	47	340.9 ± 5.3	3.64 ± 0.04	80.39^{ab}
12	43	348.8 ± 5.2	3.73 ± 0.04	77.00^{ab}
P-value		0.676	0.823	0.035
Property D†				
7	38	396.5 ± 7.5	3.53 ± 0.04	87.27
20	3	371.3 ± 22.4	3.57 ± 0.12	99.98
27	4	388.2 ± 19.5	3.55 ± 0.10	99.98
29	2	379.4 ± 26.9	3.52 ± 0.14	99.99
30	3	349.9 ± 22.2	3.19 ± 0.12	63.94
48	13	399.4 ± 11.0	3.59 ± 0.06	92.59
49	3	361.6 ± 22.4	3.57 ± 0.12	99.98
50	11	393.7 ± 12.6	3.64 ± 0.07	86.38
51	27	396.1 ± 8.8	3.49 ± 0.05	90.05
52	8	402.3 ± 14.3	3.64 ± 0.07	62.91
53	2	377.9 ± 26.9	3.52 ± 0.14	0.00
54	6	390.1 ± 16.2	3.54 ± 0.08	60.72
55	7	373.5 ± 14.7	3.50 ± 0.08	99.99
56	1	396.7 ± 37.7	3.54 ± 0.20	99.99
57	54	396.1 ± 7.1	3.54 ± 0.04	89.50
58	12	427.0 ± 12.1	3.47 ± 0.06	83.97
59	1	363.1 ± 37.9	3.50 ± 0.20	99.99
60	1	392.7 ± 37.7	3.54 ± 0.20	99.99
61	15	404.9 ± 10.7	3.52 ± 0.06	73.04
P-value		0.236	0.377	0.207

** All heifers on Property B had a BCS of 3.5.*

† One heifer did not have a technician recorded.

11.3 Study 3b: Treatment group allocation

Retrospective analysis of allocation procedure of Brahman heifers on Farm A, B and C to treatment (OPO-8 or OPO-6) with respect to mean ± SEM of liveweight (LW), body condition score (BCS) and proportion of heifers with a corpus luteum (CL).

		Group	n	Weight (Kg)	BCS (1-5)[1]	CL (%)[2]
Property A	Treatment	OPO-8	218[3]	335.2 ± 3.4	3.30 ± 0.03	66.81
		OPO-6	235	349.2 ± 3.4	3.40 ± 0.03	58.16
		P-value	-	<0.001	<0.001	0.055
Property A	Treatment	OPO-8	128[3]	382.1 ± 4.4	3.76 ± 0.04	64.50
		OPO-6	143	376.4 ± 4.1	3.78 ± 0.04	68.86
		P-value	-	0.222	0.559	0.448
Property C	Treatment	OPO-8	196	328.7 ± 2.0	3.45 ± 0.02	70.16
		OPO-6	197	339.4 ± 2.0	3.42 ± 0.02	72.25
		P-value	-	<0.001	0.288	0.644
All Properties	Treatment	OPO-8	542	350.4 ± 1.5	3.51 ± 0.01	65.1
		OPO-6	575	358.6 ± 1.4	3.55 ± 0.01	67.8
		P-value	-	<0.001	0.038	0.331

[1] Body condition score measured on a 1 to 5 scale (1 = poor, and 5 = fat)
[2] Proportion of heifers that had a CL present as determined by transrectal ultrasonography.

11.4 Study 3b: Allocation of technician, day of AI, management group and heifer origin for properties A and C

Retrospective analysis of allocation procedure of Brahman heifers on Farm A, B and C to treatment (OPO-8 or OPO-6) with respect to mean ± SEM of liveweight (LW), body condition score (BCS) and proportion of heifers with a corpus luteum (CL).

		Group	n	Weight (Kg)	BCS (1-5)[1]	CL (%)[2]
Property A	Day of AI	1	238	340.4 ± 3.3	3.28 ± 0.03	63.31
		2	215	344.1 ± 3.6	3.42 ± 0.03	61.22
		P-value	-	0.250	<0.001	0.663
	Management group	Stud	160	361.6 ± 5.1	3.31 ± 0.04	57.38
		Commercial	293	322.9 ±7.5	3.39 ± 0.06	65.02
		P-value		<0.001	0.346	0.650
	Technician	1	344	344.5 ± 2.7	3.36 ± 0.02	62.00
		2	70	343.9 ± 4.4	3.38 ± 0.04	63.07
		3	39	338.3 ± 5.8	3.30 ± 0.05	63.75
		P-value	-	0.564	0.339	0.968
	Heifer origin	Homebred	343	325.6 ± 2.4	3.48 ± 0.03	66.02
		Purchased	156	342.5 ± 1.6	3.40 ± 0.02	73.59
		P-value	-	<0.001	0.014	0.130
	Technician	4	229	333.9 ± 1.8	3.43 ± 0.02	70.26
		5	164	334.2 ± 2.2	3.44 ± 0.02	72.52
		P-value	-	0.928	0.715	0.622

[1] Body condition score measured on a 1 to 5 scale (1 = poor, and 5 = fat)
[2] Proportion of heifers that had a CL present as determined by transrectal ultrasonography.