Comparison of Fixed-time Artificial Insemination and Natural Mating on Pregnancy rates in Mpwapwa Breed Cattle

***K.T. Kabuni*[1]*, D.F. Masao*[1]*, R. Laven*[2] *and T.J. Parkinson*[2]**

[1]*Department of Cattle Research, Tanzania Livestock Research Institute (TALIRI), P.O.Box 202, Mpwapwa, Tanzania;*

[2] *School of Veterinary Science, Massey University, Private bag 11 222, Palmerstone North 4442, New Zealand*

E-mail: *kabuni.kabuni@gmail.com*

Article timeline

Submitted: 26-10-2021, Revised: 28-12-2021, Accepted: 09-02-2022, Published:12-02-2022

Tanzania Veterinary Journal Vol. 36 (2) 2021 ***https://dx.doi.org/10.4314/tvj.v36i2.3***

Comparison of Fixed-time Artificial Insemination and Natural Mating on Pregnancy rates in Mpwapwa Breed Cattle

K.T. Kabuni[1], D.F. Masao[1], R. Laven[2] and T.J. Parkinson[2]

[1]Department of Cattle Research, Tanzania Livestock Research Institute (TALIRI), P.O.Box 202, Mpwapwa, Tanzania; [2] School of Veterinary Science, Massey University, Private bag 11 222, Palmerstone North 4442, New Zealand

E-mail: *kabuni.kabuni@gmail.com*

SUMMARY

The goal of this study was to assess if a designed prostaglandin (PGF2α)-protocol when used against natural mating (NM) in well-managed Mpwapwa cattle would improve pregnancy rates during the breeding season. Of the PGF2α oestrus synchronization protocols, a standard 14-day PGF2α protocol accompanied with fixed-time artificial insemination (FTAI) was tested against NM. Totals of 39 and 49 cows became pregnant in the FTAI and NM groups respectively following completion of the study of 100 cows for each group. Statistically, the difference between the two groups was not significant (P=0.21), even though there was a lower risk of pregnancy in the FTAI group compared to the NM group (unadjusted relative risk: 0.8, 95%CI: 0.58-1.09). The results from this study suggest that PGF2α–based synchronization protocol and FTAI, especially when combined with NM in Mpwapwa breed cattle, can yield better reproductive performance and enhance greater genetic improvement compared to being naturally serviced. Few cows (only 10/100 cows) displayed behavioural oestrus signs after receiving the first PGF2α injection. The poor oestrus response might be due to either ineffective detection of oestrus or cows lacked a responsive corpus luteum (CL) at the time of synchronisation. Therefore, this might reflect a large number of cows to have been in anoestrus. Further investigation on the reproductive status and performance of Mpwapwa cows is needed to identify the effects of anoestrus.

Keywords: Mpwapwa, Breed, Cattle, PGF2α, Synchronization, Protocols, Insemination, Fixed-time

INTRODUCTION

Currently in the Tanzanian context, there are only few published findings from the use of assisted reproductive technologies (ARTs), which might perhaps suggest that the outcomes of adopting and utilizing ARTs into the Tanzanian beef cattle industry have not been as good as hoped. If so, simple, adoptable and utilizable ARTs programmes need to be developed and advocated for use into the Tanzanian beef cattle farming.

However, the cheapest and simplest but yet still effective protocols for synchronizing cows are the regimens based on prostaglandin F2α (PGF2α) (Lehman and Lauderdale, 2015). PGF2α is known for its role of inducing luteolysis and thereby permitting a new ovulation (Flint and Sheldrick, 1983). Consequently, exogenous PGF2α has been widely used to regulate oestrus in cattle (Odde, 1990). The control of oestrus by PGF2α has two significant constraints. Firstly, presence of a functional corpus luteum is crucial for prostaglandin to synchronize oestrus. Hence, for anoestrus cows or cyclic cows not in luteal phase, application of prostaglandin is ineffective. Secondary, follicular development is not affected by PGF2α (Lehman and Lauderdale, 2015), so induction of oestrus/ovulation by prostaglandin becomes subjective to the stage of the follicular wave.

Cows with large dominant follicles tend to show oestrus between 1-2 days after being treated with prostaglandin, whereas, 3-4 days is required for cows will small, subdominant follicles (de Rensis and Peters, 1999; Stevenson et al., 2000). Based on these two concerns, the development of PGF2α-based oestrus synchronization protocols involving two PGF2α injections was made necessary (Stevenson et al., 2000). In these protocols, the first PGF2α injection is administered to cows followed by insemination of any cows that display oestrus within 1-3 days. Cows that do not display oestrus are then given a second PGF2α injection 11-14 days after the first PGF2α, injection, followed by insemination using either double FTAI at 72 hours or at observed oestrus (Stevenson et al., 2000). Thus, PGF2α-based protocols, being simple and inexpensive means of synchronizing oestrus, can easily fit for utilization into the Tanzanian beef industry as a strategy of improving beef cattle breeding using AI which is the common applicable ART in the Tanzanian cattle breeding system.

This study aimed to assess if a standard 14-day PGF2α-based protocol when used against natural mating in well-managed Mpwapwa cattle would improve pregnancy rates during the breeding season.

MATERIALS AND METHODS

Selection of animals

Prior to the start of the study, written approval for all animal related study was obtained from TALIRI. All animals came from the TALIR Mpwapwa research herd.

Twenty mature Mpwapwa breed bulls aged ≥3 years with no record of reproductive disorder underwent a breeding soundness evaluation (Barth, 2018) which included the measurement of scrotal circumference using a flexible tape measure. Mating ability in each bull which passed the breeding soundness evaluation was then assessed based on the following criteria: - i) ability to move, see, smell and eat; and ii) absence of injuries of the joints, muscles, prepuce and penis. The four bulls with the largest scrotal circumference (all ≥30 cm) were then selected from the bulls which passed the mating ability test. Similarly, vasectomise teaser bulls were selected basing on ability to detect cows on oestrus in a herd.

Three hundred and eighty Mpwapwa breed cows were pregnancy tested, weighed and body condition scored on a 1-5 scale (Nicholson and Sayers, 1987). Non-pregnant cows were screened for brucellosis using Rose Bengal rapid slide agglutination test. The 200 heaviest (all > 159 kg) non-pregnant, brucellosis negative Mpwapwa cows were then selected for the study.

Animal health management and pre-breeding nutrition

Once selected cows and bulls had access to unrestricted grazing and mineral block supplementation (1 kg/animal per month) (Royal Ilac, Kayseri, Turkey) for a period of four weeks. All selected animals were treated for intestinal parasites using either 0.5 mL/kg of a levamisole/oxyclozanide combination (Nilfarm, Farmers Centre-Tanzania) (lactating cows only) or 50 µg/kg ivermectin (Ivermectin, Anglian Nutrition Products-UK) (dry cows and bulls). All selected cattle were also dipped once per week using amitraz (Amitraz 12.5% w/v, Sinochem Ningbo, Ningbo City, China) for the control of ecto-parasites. At the end of this supplementation period, all cattle were weighed and body condition scored.

Allocation to treatment

The 200 selected cows were ordered based on age, parity and ear tag number. They were then divided into four mating groups of 50 cows based on that order, with the first cow going to mating group 1, the second cow going to mating group 2 and so on. Each group of 50 was then divided into two groups of 25 again based on that order, with the first group being allocated to natural mating (NM) and the second group to fixed time artificial insemination (FTAI).

Timing of mating

Mating start was staggered for the four groups (Supplementary Table 1). On day 0 for each mating group, the NM and FTAI groups were separated into different paddocks and mating commenced. Each NM mating group was allocated one of the four selected Mpwapwa bulls. This bull was kept with the group from day 0 to day 60. On day 0 in all the four FTAI treatment groups, each cow received 500 µg of cloprostenol (Estroplan, Parnell Australia), with behavioural oestrus observed using one vasectomised teaser bull per group. Cows seen in oestrus (i.e. stand to be mounted, mount others and mucus discharge) were marked with coloured crayons and inseminated using the purchased semen based on the ante meridian (AM)/post meridian (PM) rule (Trimberger, 1948). The frozen semen came from two tested Mpwapwa breed bulls kept at the Tanzania National Artificial Insemination Centre (NAIC). Prior to purchase, a post-thaw motility of >60% had been confirmed for samples from both bulls. Before insemination, semen straws were thawed in a water bath maintained at 35°C to 36.2°C for 2 minutes.

Cows that were not seen in oestrus within 120 hours of the first cloprostenol injection were retreated on Day 14, followed by FTAI at 72 hours after the second cloprostenol injection. At the end of the breeding, which lasted for two months, pregnancy diagnosis (PD) was undertaken using transrectal ultrasonography (Chison Medical Imaging Co, Jiangsu, China) in all the 4 groups.

Data analysis

Data were analysed using SPSS version 23 (IBM, Armonk, USA). Evaluation of the effect of pre-breeding treatment on BCS and comparison of BCS across treatment groups were undertaken using Chi-square analysis. The effect of treatment on relative risk (RR) of pregnancy during the breeding season was then estimated with 95% confidence intervals (CI) (95%CI) Gardner and Altman (1990). Logistic regression was then used to analyse the effect of factors on the odds of pregnancy. Initially univariable models were run with pregnancy as the outcome variable and cow age, cow parity, days from calving to start of study, body weight at selection, body weight at start of breeding season, BCS at selection, BCS at start of breeding season, mating group and treatment (FTAI vs NM) as the predictor variables.

All factors where p was <0.25 in the univariable model were then tested for strong correlation (Spearman's correlation >0.7). If a factor was not correlated with other factors, it was put forward for inclusion in the multivariable model. Where correlation was present, the most biologically plausible factor was chosen for the multivariable model. Treatment was forced in the model and all interactions between the selected variables and treatment group were also included. The final model was then selected using a backwards elimination process, starting with the variables/interactions which had the highest p-value and continuing until all remaining factors (excluding treatment) had a p-value of <0.05.

RESULTS

The BCS results for the two examinations are summarised in Supplementary Table 2. All cattle were between BCS 3 and 4 at both time points. Supplementation significantly improved BCS and body weight (p<0.001) (Table 1).

Table 1. Mean (range) for the variables used in the univariable analysis, divided by treatment group and breeding group.

Breeding group	Treatment	Age (years)	Parity	Time since calving* (days)	Body weight b4S (kg)	Body weight b4b (kg)	BCS b4S	BCS b4b
1	FTAI	8.4 (4-12)	4.3 (1-8)	37 (12-74)	228.3 (182-296)	245.4 (200-308)	3.4 (3-4)	3.8 (3-4)
	NM	8.6 (4-14)	4.6 (1-10)	66 (35-104)	221.2 (167-315)	242.4 (200-308)	3.4 (3-4)	3.7 (3-4)
2	FTAI	6.6 (4-14)	2.8 (1-10)	58 (10-112)	240.7 (200-282)	252.2 (214-287)	3.6 (3-4)	3.8 (3-4)
	NM	6.4 (4-9)	2.8 (1-5)	63 (4-108)	236.9 (204-273)	250.4 (216-287)	3.6 (3-4)	3.7 (3-4)
3	FTAI	7.7 (4-13)	3.8 (1-9)	96 (32-504)	215.2 (159-285)	238.6 (177-304)	3.2 (3-4)	3.7 (3-4)
	NM	7.5 (4-13)	3.5 (1-9)	92 (34-124)	217.6 (175-275)	242.8 (191-298)	3.3 (3-4)	3.7 (3-4)
4	FTAI	7.3 (4-13)	3.3 (0-10)	123 (32-805)	221.8 (174-308)	246.6 (190-336)	3.4 (3-4)	3.8 (3-4)
	NM	6.8 (4-13)	1.5 (1-9)	145 (32-1177)	232.3 (175-312)	250.7 (186-330)	3.5 (3-4)	3.7 (3-4)

Range for the respective parameter is shown in brackets; FTAI: PGF2α-treated group; NM: naturally-mated group; *time from calving to start of mating for breeding group; b4S: before start of supplementation regime; b4b: before start of breeding; BCS: body condition score.

Table 2. Oestrus behavioural signs for cows treated with PGF2α in the FTAI treatment groups

Breeding group	Stand to be mounted	Mount others	Mucus discharge
1	3	3	3
2	6	6	6
3	1	1	1
4	0	0	0

Table 3. Number of pregnancy in cows across groups and treatment methods.

Breeding group	Treatment groups	No. of pregnant cows
1	NM	10
	FTAI	11
2	NM	14
	FTAI	10
3	NM	14
	FTAI	12
4	NM	11
	FTAI	6
Total	NM	49
	FTAI	39

FTAI: Insemination based on signs of oestrus after the first PGF2α plus the second PGF2α at fixed time insemination, NM: naturally-mated, 50 animals in each breeding groups (1-4), with 25 per treatment in the respective breeding groups.

The data used in the univariable analysis is summarised for each treatment group, separated by breeding group, in Table 1. There were no differences observed between treatment groups in any of the variables ($p \geq 0.25$). The oestrus behaviour observed in the treated cattle are summarised in Table 2. There were significant differences between groups in the proportion of cattle which showed behavioural signs ($\chi2=25.2$, $p<0.001$). The results of the pregnancy test are summarised across groups and treatment methods in Table 3. The unadjusted RR of pregnancy of the FTAI group against that of the NM group with 95% CI was 0.8; 95% -CI 0.58-1.09.

The results of the univariable logistic regression are summarised in Table 4. Of the factors tested only three, days from last calving, breeding method and Bodyweight prior to supplementation regime (i.e. one month prior to start of breeding season) had any association with the odds of pregnancy (all $P<0.25$). These three factors (as well as their interactions with treatment group) were then included in the multivariable logistic regression. In the final model, only treatment group remained. In this final model, the odds ratio for pregnancy in cattle treated with PGF2α-based synchronisation programme, compare to those which were naturally mated for the breeding season was 0.73 (95%CI: 0.4-1.24).

Table 4. Results of univariable logistic regression analysis

Explanatory variable		Odds ratio (95%CI)	P-value
Age		1.009 (0.912 – 1.116)	0.87
Parity (categorical) Reference: parity 5+	Parity 0-1	0.89 (0.38 – 2.1)	0.72
	Parity 2-4	1.19 (0.61 – 2.3)	
Days from last calving to start of study		0.998 (0.995 – 1.001)	0.18
Breeding method		0.696 (0.397 – 1.221)	0.21
Body weight one month prior to start of breeding season		1.006 (0.996 – 1.015)	0.25
Body weight at start of breeding season		1.005 (0.995 – 1.015)	0.30
BCS one month prior to start of breeding season		0.967 (0.549 – 1.700)	0.91
BCS one month at start of breeding season		1.371 (0.728 – 2.582)	0.33
Group No. Reference: Group 1	Group 2:	1.4 (0.63 – 3.1)	0.35
	Group 3:	1.5 (0.68 – 3.3)	
	Group 4:	0.78 (0.34 – 1.7)	
Change in weight pre-breeding		0.996 (0.973 – 1.019)	0.73
Change in BCS		0.707 (0.381 – 1.310)	0.27

DISCUSSION

This study aimed to assess if a designed standard 14-day PGF2α -protocol when used against natural mating in well-managed Mpwapwa cattle would improve pregnancy rates over 12-week period during the breeding season. 39/100 and 49/100 cows were found pregnant in the FTAI and NM groups respectively following completion of the study. Statistically, the difference between the two groups was not significant (P=0.21), even though there was a lower risk of pregnancy in the FTAI group compared to the NM group (unadjusted relative risk: 0.8, 95%CI: 0.58-1.09).

Nonetheless, cows in the FTAI group were inseminated only once, while cows in the NM group were potentially served by bulls several times within the breeding season. This study however involved fewer numbers of cows and a single breeding season (Table 3), nonetheless, the FTAI treatment group produced similar results as reported from other studies conducted on *Bos. indicus* cattle. For example, Bó *et al.* (2005) reported that a FTAI programme was conducted in Argentina started from 2002/03 to 2004/05 breeding seasons in a semi-arid climate similar to this study using heifers, dry and suckled beef cows of Zebu derived and Bonsmara. At the end of the FTAI programme, 49.1% (1053/2144), 47.9% (189/394) and 41.7% (951/2278) pregnancy rates were recorded for the heifers, dry and suckled beef cows respectively.

Regardless of these results, Cutaia *et al.* (2003) pointed out that, the importance of FTAI programmes against NM on pregnancy rates in a managed beef herd is that it can facilitate a large number of cows to become pregnant earlier during the breeding season. The lower pregnancy rates obtained from these studies using FTAI programmes suggest that either ineffective ways of detecting oestrus or lack of a responsive corpus luteum (CL) at the time of synchronization and low BCS could be the reasons for this.

Furthermore, the use of the PGF2α oestrus synchronisation protocol increased utilisation of artificial insemination (AI), which is rare under the Tanzanian cattle breeding system in which natural mating is the dominant breeding method. The results from this study suggest that PGF2α–based synchronization protocol and FTAI, especially when combined with NM in Mpwapwa breed cattle, can yield better reproductive performance and enhance greater genetic improvement compared to being naturally bred.

Few cows (only 10/100 cows) were observed to display behavioural oestrus signs after receiving the first PGF2α injection. The cause for the poor response was not directly determined in the present study, although there are some clear pointers in the literature. For example, as reported by Hafs *et al*., (1975), only 23/33 suckling beef cattle showed oestrus after an injection of cloprostenol, while Landivar *et al*., (1984) reported that 46% of Zebu cattle with a palpable CL showed oestrus after a first injection of PGF2α (equivalent to an overall rate of ~25% if all cattle had been treated irrespective of CL status). On the other hand, Galina and Arthur (1990) reported that between *Bos indicus* and *Bos taurus* cattle when kept under the same conditions, the response to oestrus using PGF2α protocols is 30% less in *Bos indicus* cattle compared to *Bos taurus* cattle.

However, they suggested that the reasons for the wide variations in the response of oestrus and pregnancy rates are multifactorial caused by factors such as anoestrus, low plane of nutrition, type of breed of cattle and season. In this study, it might be the detection of oestrus was ineffective after synchronization, or a high proportion of cows did not have a responsive corpus luteum (CL) at the time of synchronization. Since the function of PGF2α in oestrus synchronisation depends on the presence of a responsive CL, it is ineffective when used to synchronize anoestrus cows, even when utilized in a double injection protocol. Likewise, if the cows were suffering from anovulatory anoestrus it would have reduced the response to the second injection of PGF2α as well (even if the cow started cycling between the two injections, thus reducing the conception rate to the second AI).

As a result, this might represent a large number of cows being in anoestrus. There is a need to investigate the reproductive status of Mpwapwa breed cows at the beginning of the mating season in order to identify how anoestrus affect the reproductive performance of Mpwapwa breed cows.

Therefore, if this is the case, treatment of anoestrus cows using progesterone-based protocols, although more expensive, would be likely to be more beneficial (McDougall, 2010). Cows with a CL may be treatable with a PGF2α injection, but identification of CL requires skilled veterinarians or technicians and may not be feasible in most situations. Furthermore, if anoestrus is common, treatment of cows with a CL with a progesterone-based programme be more effective (McDougall, 2010) as well as more feasible.

Nutrition is among the vital factor influencing fertility in cattle (Robinson *et al.,* 2006). Based on this fact, Morris *et al.,* (2006) and Selk *et al.,* (1988) reported that, it is essential to assess BCS prior to start of the breeding season, as there is a positive relationship between BCS and pregnancy rates in cattle. Similarly, during the breeding season as pointed out by DeRouen *et al.,* (1994) reproductive success is more likely to be achieved in cows with higher BCS. Thus, a one-month nutrition and preventive medicine treatment programme seemed necessary for the selected cattle in order to improve their BCS prior to the beginning of the mating season. Before the start of the programme, the proportions of cows were either 56.5% or 43.5% for BCS 3 and 4 respectively. BCS 3 was considered unsatisfactory at the start of the mating season, as such, of the 200 selected cows over 50% were in poor condition at the end of the dry season. The proportion of cows at BCS 4 increased to 72% (Supplementary Table 2) following the completion of the pre-treatment month. Nonetheless, no significant difference in pregnancy rate between BCS 3 and 4 was found, despite the lower relative risk (RR) of pregnancy in cows at BCS 4 compared to cows with BCS 3 (unadjusted RR: 0.6, 95%CI: 0.46-0.69). This result is contrary to published data, which strongly recommend BCS as a significant factor of fertility (Rae *et al*., 1993). For example, in a study conducted by Meneghetti *et al.,* (2009) it was reported that, Nellore cows which had 2.5 BCS produced lower pregnancy per AI compared to cows which had 3.5 BCS.

Nevertheless, as the difference in pregnancy rate found in the current study was not significant, introduction of programmes for checking BCS into Tanzanian farms is crucial so that cows in poor condition can be detected and monitored before the start of the mating season. Moreover, it is important to note that, regardless of the lack of the difference in pregnancy rate between BCS 3 and 4, cows were well fed a month prior to the beginning of the breeding season. Whether this would have been the case if the animals' plane of nutrition had not been improved would be a matter of further investigation.

Except for the last batch, cows body weight were improved significantly during the pre-breeding programme (Table 1); at the beginning of the mating season cows had a bodyweight >200 kg. Although there are no published data for the support of the breeding weight used on Mpwapwa cattle, 180 kg is the minimum recommended breeding weight used at TALIRI Mpwapwa farm. To better establish ideal breeding weight for Mpwapwa cattle, further research is needed to assess if there is any benefit of weighing in relation to evaluation of BCS.

Of the cows in the NM group, 49/100 were confirmed pregnant at the completion of the study, despite having a relatively intensive pre-breeding programme and the use of tested bulls. Regardless of obtaining lower percentage (39/100) in the FTAI group, cows in this group were only once inseminated and became pregnant earlier at the start of the mating season, contrary to the NM (i.e. control) group, which had longer time to be serviced to become pregnant. Moreover, 39 cows became pregnant AI in FTAI (treatment), which means they produced significantly better calves of higher genetic merit compared to calves produced in the NM group. The results from this study have demonstrated that a simple 14-day PGF2α protocol can achieve pregnancy rates to a single insemination which is as good as those obtained from natural mating through the season, while at the same time encouraging utilization of AI.

Therefore, one of the conclusions that can be drawn related to the fertility of Mpwapwa breed cattle, based on obtained low pregnancy rates in the NM group and the low proportion of treated cows displaying oestrus, strongly indicate that there are underlying issues affecting the cyclicity of the Mpwapwa cattle used in this study. Thus, further research should focus on the follicular dynamics of Mpwapwa cattle at the beginning of the mating season, mainly to identify if the issues are either at the herd level or at individual cow. More importantly, such research should assess the optimal synchronization protocol for utilization in Mpwapwa breed cattle, which can either cooperate or replace the PGF2α protocol.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the support on research costs received from the New Zealand Ministry of Foreign Affairs and Trade (MFAT) and the Tanzania Livestock Research Institute (TALIRI) for the permission to conduct this research. We also acknowledge the support provided by the Tanzania National Artificial Insemination Centre (NAIC) for the supply of semen straws and ultrasonography machine, and the Department of Veterinary Surgery and Theriogenology at Sokoine University of Agriculture (SUA) for their support and assistance. Finally, we acknowledge publication assistance provided by the Massey University from New Zealand, Tanzania Livestock Research Institute (TALIRI) and the Commission of Science and Technology (COSTECH) from Tanzania.

CONFLICT OF INTEREST

Authors do not have any conflict of interest.

REFERENCES

Barth AD. Review: The use of bull breeding soundness evaluation to identify sub fertile and infertile bulls. *Animal,* 12: 158 – 164, 2018.

Bó GA, Cutaia L, Chesta P, Balla F, Pincinato D, Peres L, Marafia D, Aviles M, Menchaca A, Veneranda G & Baruselli PS2005 Implementacion de programas de inseminación artificial en rodeos de cria de argentina. *Proc VI Simposio Internacional de Reproducción Animal,* pp: 97-128, 2005.

Chawala AR, Banos G, Komwihangilo DM, Peters A, Chagunda MGG. Phenotypic and genetic parameters for selected production and reproduction traits of Mpwapwa cattle in low-input production systems. *S Afr J Anim Sci,* 43: 307-319, 2017.

Cutaia L, Veneranda G, Tribulo R, Baruselli PS& Bó GA 2003b Programas de Inseminackin Artificial a Tiempo Fijo en Rodeos de Cria: Factores que lo Afectan y Resultados Prod uctivos. *Proc V Simposio Internacional de Reproducción Animal,* pp: 119-132, 2003.

De Rensis F, Peters AR. The control of follicular dynamics by PGF2α, GnRH, hCG and oestrus synchronization in cattle. *Reprod Dom Anim,* 34: 49-59, 1999.

DeRouen SM, Franke DE, Morrison DG, Wyatt WE, Coombs DF, White TW, Humes PE, Greene BB. Prepartum body condition and weight influences on reproductive performance of first-calf beef cows. *J Animal Sci,* 72: 1119- 1125, 1994.

Flint APF, Sheldrick EL. Evidence for a systemic role for ovarian oxytocin in luteal regression in sheep. *J Reprod Fert,* 67: 215-225, 1983.

Galina CS and Arthur GH. Review on cattle reproduction in the tropics. Part 4. Oestrus cycles. *Animal Breeding Abstracts,* 58: 697-707, 1990.

Gardner MJ, Altman DG. Confidence--and clinical importance--in research findings. *Br J Psychiatry,* 156: 472-474, 1990.

Hafs HD, Manns JG, Drew B. Onset of oestrus and fertility of dairy heifers and suckled beef cows treated with prostaglandin F2α. *Anim Prod,* 21: 13-20, 1975.

Landivar C, Galina CS, Duchateau A, Novaro-Fierro R. Fertility trial in Zebu cattle after a natural or controlled estrus with prostaglandin F2 alpha, comparing natural mating with artificial insemination. *Theriogenology,* 23: 421-429, 1984.

Lehman F, Lauderdale JW, Use of technology in controlling estrus in cattle. *In: Bovine Reproduction ed Hopper RM John Wiley & Sons Chichester UK,* pp: 655-661, 2015.

McDougall S. Effects of treatment of anoestrous dairy cows with gonadotropin-releasing hormone, prostaglandin, and progesterone. *J Dairy Sci,* 9: 1944-1959, 2010.

Meneghetti M, Sa Filho OG, Peres RF, Lamb GC, Vasconcelos JL. Fixed-time artificial insemination with estradiol and progesterone for Bos indicus cows I: basis for development of protocols. *Theriogenology,* 72: 179-189, 2009.

Morris ST, Morel PCH, Kenyon PR. The effect of individual live weight and condition of beef cows on their reproductive performance and birth and weaning weights of calves. *NZ Vet J,* 54: 96-100, 2006.

Nicholson MJ, Sayers AR. Repeatability, reproductibility and sequential use of condition scoring of *Bos indicus* cattle. *Trop Anim Hlth Prod,* 19: 127-135, 1987.

Odde KG. A review of synchronization of oestrus in postpartum cattle. *J Anim Sci*, 68: 817-830, 1990.

Rae DO, Kunkle WE, Chenoweth PJ, Sand RS, Tran T. Relationship of parity and body condition score to pregnancy rates in Florida beef cattle. *Theriogenology,* 39: 1143-1152, 1993.

Robinson JJ, Ashworth CJ, Rooke JA, Mitchell LM, McEvoy TG. Nutrition and fertility in ruminant livestock. *Anim Feed Sci Technol,* 126: 259-276, 2006.

Selk GE, Wettemann RP, Lusby KS, Oltjen JW, Mobley SL, Rasby RJ, Garmendia. JC. Relationship among weight change, body condition and reproductive performance of range beef cows. *J Animal Sci*, 66: 3153-3159, 1988.

Stevenson JS, Thompson KE, Forbes WL, Lamb GC, Grieger DM, Corah LR. Synchronizing estrus and (or) ovulation in beef cows after combinations of GnRH, norgestomet, and prostaglandin F2α with or without timed insemination. *J Anim Sci*, 78: 1747-1758, 2000.

Trimberger GW. Breeding efficiency in dairy cattle from artificial insemination at various intervals before and after ovulation. *Res Bull Nebraska Agric Expt Sta,* 153: 1-26, 1948.

Supplementary Table 1. Timing of key events for each mating group.

	Treatment groups	Day 0	Oestrus detection	2nd PG injection	Joining of treatment groups
Group 1	FTAI	16/03/16	19-21/03/16	29/03/16	16/05/16
	NM		N/A	N/A	
Group 2	FTAI	30/03/16	02-04/04/16	12/04/16	30/05/16
	NM		N/A	N/A	
Group 3	FTAI	13/04/16	16-18/04/16	26/04/16	13/06/16
	NM		N/A	N/A	
Group 4	FTAI	27/04/16	30/04-02/05/16	10/05/16	27/06/16
	NM		N/A	N/A	

FTAI: fixed time artificial insemination; NM: natural mating. All cows were pregnancy tested two months post FTAI and NM. Cows in FTAI group were treated with 500 µg of cloprostenol on Day 0 and then 14 days later if oestrus not observed. N/A, not applicable.

Supplementary Table 2. BCS results for the study cows one month prior to and at the start of the breeding season

BCS	Examination prior to start of supplementation (n)	Examination at start of breeding season (n)
3	113	56
4	87	144

Body condition score (BCS)