Research Article

Pregnancy, Parturition and Resumption of Ovarian Cyclicity in Beef Cows

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Abstract

Communal farmers find it very difficult to control animal breeding hence fixed Time Artificial Insemination (TAI) is a promising technique. The objective of this study was to determine the effects of bull age and breed on pregnancy rate, and the post-partum interval in extensively managed communal beef cows. A total of 577 female animals with a body condition score between 2.5 and 3, from three breeds; Brahman (189), Nkone (18) and Mashona (370) were used. Fixed time artificial insemination using the ovsynch protocol was done using semen from four different bull breeds (Mashona, Tuli, Brahman and Boran) aged between 3 to 6 years. The percentage of cows identified pregnant were analysed using generalized linear mixed model PROC GLIMIXX of SAS. The effects of cow parity on PPI were evaluated using the general linear model of SAS. Conception rates were 53.2; 57.3 and 75% for Brahman, Mashona and Nguni cows respectively. Animals in their third parity had the highest conception rate. Young bulls less than 3 years of age had the least pregnancy rate while Mashona bulls had the highest pregnancy rate. There was a breed by parity interaction on PPA interval (P < 0.05). It can be concluded that PGF2 α and Estradiol Benzoate (EB) could be successfully used to induce and synchronize ovulation in cattle undergoing TAI, sire age significantly influenced conception rate as well as that the post-partum interval is dependent on breed and parity.

Keywords: Conception; Indigenous breeds; Postpartum anoestrus; Small scale beef production

Introduction

Beef cattle serve a unique role in converting low quality forage to high-quality protein for human consumption [17]. In most smallholder beef enterprises, cowherds are characterised by uncontrolled breeding, low plane of nutrition and rampant calving. In sub-Saharan Africa performance of this sector is affected by various factors including breed, geographical location, season of calving, suckling status, age, parity, and body condition score [55]. In such production systems profitability is rarely talked about , however farmers realise that it hinges on producing as many calves as possible per cow per lifetime [47]. Furthermore, the businesses is predominantly part-time where small herds of animals are frequently run on fragmented land with limited access to handling facilities. Under such conditions productivity is a term not applicable, not to mention the use of advanced technologies like AI. Nonetheless the demand for beef is increasing and technologies that can improve the quantity and quality of beef are germane.

In this regard herd fertility becomes critical, and it has been promoted that fertility of the herd, among other factors, underpins the profitability of a beef enterprise [36]. At least in the cow, fertility is measured by conception rate/pregnancy rate, and calving rate. The use of oestrous synchronisation has been promoted as probably the only applicable reproductive biotechnology to facilitate AI in beef cattle [1,6]. Unfortunately, due to the extensive nature of the productive system, it is no secret that, AI has strong barriers to implementation at farm level.

In this study, pregnancy rate and Post-Partum Anoestrus (PPA) were used as a measure of fertility. In order to achieve a calf every year, cows should conceive between 75 to 85 days following parturition. More often than not, smallholder beef animals exceed this period. The reasons could be related to nutritional inadequacies [17,55] that leads to low LH pulse frequency [17], seasonal variations [2], suckling and maternal bond inhibiting ovulation [17]. It has been observed that beef cows exhibit early resumption of follicular growth within 7 to 10 days postpartum [47], however the time to ovulation is limited to more than 80 days probably due to lack of ovulation stimulus and not lack of follicle growth. Resumption of ovulation and oestrous cycles are key requirements to facilitate rebreeding of postpartum cows. It has been reported that beef cows have a prolonged interval to resumption of ovulation compared to dairy [17] and this is because of suckling and maternal bond-inhibiting ovulation. [18] Reported that following an uncomplicated calving, approximately 30 days are required for completion of uterine involution in beef cows. In addition resumption of normal ovarian cyclicity and oestrus depends on the recovery of the hypothalamic-anterior pituitary- ovarian axis, in particular the attainment of a GnRH/LH pulse frequency of 4 to 5 pulses per 10 h period. This can only happen in commercialised entities with expect gadgets to monitor these hormonal levels and is generally not practical in smallholder farming systems.

Furthermore, beef cows in good Body Condition (BC) have been shown to have a mean of 3.2 ± 0.2 dominant follicles (~30 days) to first ovulation [2,17]. If these animals are in poor BC the number of follicles drops and period to first ovulation increase to ~70 to 100 days

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[2]. The lack of ovulation and dominant follicles during this period has been associated with infrequent LH pulses, suckling, maternal-offspring bonding and low Body Condition Score (BCS) [17]. The key to optimizing the resumption of ovulation in beef cows relates to appropriate pre-calving nutrition and management, this ensures that cows calve down in optimal body condition ranging between 2.75 to 3.0 [2,47]. Conception and subsequent pregnancy rate in beef cows following the initiation of postpartum ovarian cyclicity solely is a function of bull fertility in small holder naturally serviced herds [18]. To this end bull age and breed are critical in the evaluation of beef cows' fertility status. As reported by [26,47-49,52,55], bull age and breed significantly affect pregnancy rates in beef cattle production. Therefore the objective of this study was to determine the effects of bull age and breed on pregnancy rate, and the post-partum interval in extensively managed communal beef cows.

Materials and Methods

Study Site

The study was carried out in Mwenezi District which is located in Agro-ecological Region V (21° 25 $^{\circ}0"S,$ 30 $^{\circ}44'$ 0" E) on the South East Lowveld of Zimbabwe. Mwenezi District lies at an altitude of 558masl. The district is drought prone, characterized by low and erratic rainfall of about 400mm/ year [29] recording maximum temperatures of 34°C in October and an average minimum temperature of 5°C occurring in July [15]. This area falls in a transition zone between broken granite country to the north and the flat, fertile mixed bushlands of the south east lowveld. There are many domed hills and castle kopjes interspersed with gently sloping areas and thickly wooded river valleys. Acacia, Combretum and Mopane trees provide livestock food, together with perennial forbes and shrubs which supply nutritious green shoots [29]. Sand veld sites are characterised by species low in digestible crude protein, while clay veld areas are typified by high quality fodder grasses. Grasses include Themedatriandra, Eragrostis spp, Cenchrusciliaris. Urochloa spp, and some Panicum maximum, and these are mostly palatable and of high value as grazing. Heteropogoncontortus, Themedatriandra, Eragrostissuperba and Digitaria spp), and where soils are deep a good sward of Panicum maximum and Urochloapullulans and other good grazing grasses can be found. (Figure 1) shows the study area.

Study Animals and Selection Criteria

A total of 567 female animals from three breeds; Brahman (189), Nkone (8) and Mashona (370) were selected. Heifers and cows from first to sixth parity with a body condition score ranging from 2.5 to 4 were selected. These animals were derived from 8 wards in which farmers on a willing basis were required to provide them with feed and drink. A central insemination holding paddock in each ward was erected, which lasted for two weeks, with the help of Agritex and Veterinary officers. Two paravets from each ward among the local farmers were identified and trained with the help of Agritex and Veterinary officers. They were responsible for mobilizing farmers to join the program, general health of the cows. Insemination was done by two trained technicians from Matopos Research Institute.

Cow Breeding

Fixed time artificial insemination using the ovsynch protocol that uses Estradiol benzoate (estrogens), Prostaglandins $F2\alpha$

(PGF2 α) analogue and Controlled Internal Drug Release (CIDR), was doneusing semen from four different breeds (Mashona, Tuli, Brahman and Boran) aged between 3 to 6 years, with an unbalanced semen breed allocation design following farmer preferences. A shot of Estradiol Benzoate was administered to the animals at 2ml/ animal intramuscular on day 0 and a CIDR loop inserted. The inserted CIDR was in form of an intravaginal insert containing 1.9g of progesterone. On Day 7 the CIDR loop was removed and the animals received a PGF2 α (Estrumate) injection at 2ml/animal and the animals confined. A second shot of Estradiol Benzoate was administered intramuscular on Day 8 at 1ml/animal. Timed artificial insemination was performed on Day 10. Farmers provided feed and water to their animals during the confinement period as this was one of the preselection requirements. Animals were let go on Day 11.

Data Collection

Insemination records that included insemination date, semen breed and age, dam predominant breed, post calving period (months), and parity were recorded. Pregnancy diagnosis through rectal palpation was done 90 days following the fixed time artificial insemination with the pregnancy statuses being recorded.

Confirmation of Pregnancy

To confirm pregnancy all animals were subjected to pregnancy diagnosis after 90 days post breeding as described by Arthur [3]. Conception Rates (CR) were estimated from the proportion of pregnancies confirmed by rectal palpation among the total number of cows and heifers bred using the formulae prescribed by Banerjee [5].

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Conception Rate = \frac{Number of cows or heifers pregnant}{Number of cows or heifers exposed to the bull} x 100
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Post-Partuminterval

Determination of period post-partum was through farmer oral reports which were confirmed through direct observation by paravets. Animals were to show clinical heat signs after parturition and this period represented the first re-breeding interval. Whenever an animal was presumed/ observed to be in oestrus, milk samples were collected for progesterone evaluation to confirm ovarian activity. Rapid on-farm progesterone test kits (Bovites; Firme R. Turmel, Inc., Beloeil, Quebec, Canada) based on the principle of Enzyme Immunoassay (EIA) were used. Color development (dark blue color) was regarded as confirmation of ovarian activity [40]. Milk progesterone concentration was determined using a solid phase radioimmunoassay (Coat-a count, Diagnostic Products Corporation, Los Angeles, Calif). The intra inter-assay coefficient of variation was 9.4. Cows that had plasma progesterone concentrations of at least 1 ng/ml were considered to have ovulated [11].

Experimental Design

Data from a total of 567 animals was evaluated in the current study, in which different bull breeds (Boran, Brahman, Mashona and Tuli) of different age groups (< 3 years; 3- 5 years; > 5 years) at a bull: cow ratio of 4% were used across different cows breeds (Brahman= 189, Mashona=370 and Nguni=18) of different parities (Heifers =174, 1st calvers = 124, 2nd calvers = 123, P3=80, P4=76) in a completely randomised design to determine the effects of bull breed and age on pregnancy rate as well as the post-partum interval of different cow parities from different breeds.



Statistical Analysis

Descriptive statistics and mixed ANOVA was performed to estimate the effect of breed, age of sire, breed of dam and parity on conception. The percentage of cows identified pregnant were analysed using generalized linear mixed model PROC GLIMIXX SAS 9.4 (SAS Institute Inc., Cary, NC) to estimate the effects of sire breed, sire age, dam breed, parity and their interaction to predict the likelihood that any of the given factors have on pregnancy rate of cattle from a communal production system. Data was subjected to general linear model of SAS 9.4 2009 to evaluate the effects of cow parity on PPI using the following model:

 $Y_{iik} = \mu + Pi + Bj + PBij + e_{iik}$

Where μ = overall mean common to all observations

 P_i = effect of parity (i = heifers, 1st calvers, 2nd calvers, P3, P4)

B_i = effect of breed (j = Brahman, Mashona, Nguni)

 $PB_{ii} = is$ the interaction of treatments and diameter

 $e_{iik} = is$ the error term

Means were separated using the Tukeys' Studentized Range Test method at P < 0.05.

Results

An overview of the data for different bull semen used to breed

cows are shown in (Table 1).

A greater proportion of cows were inseminated by semen from bulls aged between 3 - 5 years for all breeds, with variations for Boran and Brahman bulls where older or younger bulls were used respectively. Breeding cows were grouped according to parity and the summary results are presented in Table 2. There were fewer Nguni cows within the population.

A greater proportion of the animals were from the Mashona breed followed by Brahman and lastly Nguni, while there were more heifers, first and second calvers than were mature cows in parities three and four. The effect of cow breed on conception was evaluated and the results are presented in Table 3. Conception was highest in Nguni cows compared to Mashona and Brahman. Overall conception rate was 56%.

The highest pregnancy rate was recorded for animals in their third parity. Although a greater proportion of the breeding animals were heifers, their pregnancy rate was mediocre. In total 43.8% of all the breeding females were not pregnant.

Pregnancy was related to the sire breed, Mashona bulls recorded the highest pregnancy rate followed by Boran, Brahman and lastly Tuli bulls. Older bulls showed the highest pregnancy rate followed by middle aged bulls and lastly young bulls.

PPA

The PPA for all breeds was quite high except for Nguni cattle (P

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| Variable | | Bull breed | | | | |
|----------|-------------|------------|------------|------------|-----------|-----|
| | | Boran | Brahman | Mashona | Tuli | |
| Bull age | < 3 years | 15 (21.1) | 22 (30.9) | 15(21.1) | 19 (26.7) | 71 |
| | 3 – 5 years | 83(26.3) | 80(25.4) | 64 (21.3) | 88 (27.9) | 315 |
| | 5 years | 66 (36.5) | 44 (24.3) | 35 (19.3) | 36 (19.9) | 181 |
| Total | | 164 (28.9) | 146 (25.7) | 114 (20.1) | 143(25.2) | 567 |

Table 1: The number of cows inseminated with semen from different bullsin Mwenezi in the 2019/20 farming season.

Table 2: Breed vs parity of cows inseminated in Mwenezi District.

| Parity | | | | | | | | |
|-----------|---------|------------|-------------------------|-------------------------|-----------|------------|-------|--|
| | | Heifers | 1 st calvers | 2 nd calvers | P3 | P4 | Total | |
| Cow breed | Brahman | 58 (30.7) | 39 (20.6) | 38 (19.6) | 28 (14.8) | 27(14.3) | 189 | |
| | Mashona | 111 (30.0) | 79 (21.4) | 85 (23.0) | 46 (12.4) | 49 (13.20) | 370 | |
| | Nguni | 6 (28.6) | 6 (28.6) | 0 | 6 (57.1) | 0 | 18 | |
| Total | | 170 (30.0) | 120 (21.2) | 123 (21.7) | 78 (13.8) | 76 (13.4) | 567 | |

Table 3: Conception rate in different cow breeds of Mwenezi.

| | | | Total | | | |
|-----------------|----------|------------|------------|-----------|-------|--|
| | | Brahman | Mashona | Nguni | TOLAI | |
| Dragnant status | Empty | 90 (46.8) | 158 (42.7) | 4 (25.0) | 249 | |
| Pregnant status | Pregnant | 100 (53.2) | 212 (57.3) | 14 (75.0) | 318 | |
| Total | | 190 (33.5) | 370 (65.1) | 18 (1.4) | 567 | |

⁰figures in parenthesis are percent.

Table 4: Conception rate in different parity of cow.

| Parity | | | | | | | | |
|---|----------|-----------|-----------|------------|-----------|-----------|------------|--|
| Heifers 1 st calvers 2 nd calvers P3 P4 Total | | | | | | | Total | |
| Pregnancy status | Empty | 79 (46.2) | 50 (41.7) | 51 (41.5) | 27 (34.6) | 42 (55.3) | 249(43.9) | |
| | Pregnant | 92 (53.8) | 70 (58.3) | 72 (58.5) | 51(65.4) | 34 (44.7) | 318 (56.1) | |
| Total | | 170(30.1) | 120(21.1) | 123 (21.7) | 78 (13.7) | 76 (13.4) | 567 (100) | |

⁰figures in parenthesis are percent.

< 0.05). Brahman cows had the overall highest PPA across all parities (P < 0.05). The average PPA for all breeds was 138, 99 and 55d for Brahman, Mashona and Nguni respectively, while the average PPA was 110, 102, 102, 112, and 148d for heifers, first calvers, second calvers , P3 and P4 respectively. There was a breed by parity interaction on PPA interval; Mashona cows in their second parity had the least PPA while Brahman cows in their first parity had the least PPA (P < 0.05) and Nguni cows in their third parity had the longest PPA.

Discussion

The global population is ever increasing, hence its demand for animal derived products has enormously increased [30,54]. In this regard reproductive efficiency of a beef cow herd is fundamental to meeting the protein requirements. In order to attain a high level of reproductive efficiency, producers should meet a plethora of targets along the production cycle and this unfortunately requires significant technical competency [18]. The pregnancy rate in the current study fall below recommendation for these breeds [34,48,57,58] or for any other beef type of 60-70% , however they are above those reported by Pfeifer [37,53] when the same protocol was used. A number of factors could be responsible for these low rates including but not limited to nutrition, diseases, season, relative humidity, rainfall and environmental conditions particularly temperature, body condition, live weight, [2,41,47] and their interactions.

The effect of parity on conception has been reported [17,18,24,31,47,51]. Surprisingly Khan et al. (2015) reported that cows in parity 2 and 3 had higher conception rates than nulliparous animals. This is against the generally acceptable practice that heifers are better to conceive than especially first calvers [33,58].

Bull breed effects on conception rate in the current study have also been reported earlier [26]. Boran bulls are known to produced small volume of highly concentrated semen with highest sperm motility when compared to Holstein dairy bulls [26]. On the other hand different cow breeds exhibit different conception rates as reported by Khan [55], no wonder the breed differences observed in the current study. Among other factors, temperament of cows has been implicated in pregnancy rates for beef cows [10,41]. showed that aggressive cows have reduced reproductive rates. This explains the low pregnancy rates observed in Brahman cows in the current study; these cows were reported to be aggressive by paravets. Other possible causes for low conception rates in the current study could be the prevalence of reproductive disease and nutrition. The level of pregnancy particularly for heifers in the current study is worrisome.

| Category | | Empty | Pregnant | Total | | |
|----------|-------------|------------|-----------|-------|--|--|
| | Boran | 69 (42.1) | 95 (57.9) | 164 | | |
| Droad | Brahman | 66 (45.8) | 78(54.2) | 146 | | |
| Breed | Mashona | 47 (40.9) | 68(59.1) | 114 | | |
| | Tuli | 67 (46.2) | 78 (53.8) | 142 | | |
| | < 3 years | 41 (57.7) | 30 (42.3) | 71 | | |
| Sire Age | 3 – 5 years | 143 (45.4) | 172(54.6) | 315 | | |
| | 5 years | 65 (35.7) | 117(64.3) | 182 | | |

Table 5: Breed and sire age related conception rates of beef cattle in Mwenezi.

Ofigures in parenthesis are percent.

Table 6: LS means for Post-partum period of breeding cows in Mwenezi.

| Cow breed | Parity | | | | | | |
|---|---------------------------|--------------------------|--------------------------|--------------------------|----------------------------|--|--|
| | Heifers | 1 st calvers | 2 nd calvers | P3 | P4 | | |
| Brahman | 128.8ª±16.33 | 99.5 ^b ±21.38 | 148.0ª±20.59 | 125.0ª±22.45 | 194.4ª±24.64 | | |
| Mashona | 105.4 ^b ±17.46 | 116.2ª±18.11 | 61.4 ^b ±14.82 | 113.4ª±18.99 | 102.1 ^b ±17.219 | | |
| Nguni | 30.0°±76.98 | 30.0±°76.98 | ND | 80.0 ^b ±57.38 | ND | | |
| abcontume means with different superparints were significant at $R < 0.05$ ND = no data | | | | | | | |

^{abc}column means with different superscripts were significant at P < 0.05), ND = no data.

These animals are generally expected to have higher pregnancy rate compared to any other parity of both beef and dairy cows [17,18]. It is highly likely that these animals had been exposed to one of the many reproductive diseases hence the low rates. If not, prenatal conditions associated with pregnancy resorption and deaths [39,42] could have caused the low pregnancy rates. [33] Affirms that nutritional management for controlled weight gain, identification of reproductive maturity by physiological and morphological indicators should be done to improve conception on heifers at first breeding. Particularly for such communities these practices will improve conception rates and are therefore recommended. It is also possible that the low fertility was associated with cow size [52].

There is a dichotomy as to the effects of cow size to fertility. It is generally accepted that smaller cows are more fertile under extensive grazing conditions [52]. This is so because small body size is an adaptive attribute, on the other hand larger cows produce more milk and, therefore wean heavier calves, thus the productivity of cows could be premised on adaptive capability. In the same vein larger cow body size translate into heavier culling weights which also impacts productivity. It is evident from our study that larger cows showed low reproductive rates possibly due to lack of adaptive capability, no wonder it is now generally recommended to encourage rural communities to rare small body sized cows [56]. The low conception rates could also be as a result of under nutrition. It is no secret that protein nutrition is limited in communal cattle [4,9,12,52,53,58]. The effects of proteins on reproductive capabilities have been alluded [9,12,23,38,39]. The endocrine effects on the PPI in beef cows has been reported [1,17].

Indeed the hypothalamic–anterior pituitary– ovarian axis recovers 30 days Post-Partum (PP) and becomes able to synthesise LH. At the same time ovaries are expected to respond to the increased LH pulse in the majority of suckled beef cows. However, the synthesised LH is largely sequestered in the anterior pituitary; consequently normal resumption of ovulation is prevented by inadequate LH pulse frequency [17]. The suckling– maternal bond that exists between the cow and her calf and/or her nutritional status are believed to affect the GnRH/LH pulse frequency [1,8,9,18,28,35,38]. In addition, it does appear that cyclicity and fertility of *Bos indicus* cows is more likely to be impacted by environmental conditions [53] hence It is certainly conceivable that higher rates of anoestrus may be observed postpartum . Furthemore, *Bosindicus* cattle are more susceptible to stressors compared to *Bostaurus*, since cortisol, a dominant stress hormone, was reported to be higher in temperamental cattle compared to cattle that are calm [53].

The major factors affecting the duration of the Postpartum Interval (PPI) in suckled beef cows are maternal bond, nutrition, parity and season [17]. Results from the current study are a clear indication to these interactions since most of the animals exhibited long PPI exceeding 30 days. Additionally it is understood that communal grazing animals suffer mostly from inadequate nutrition [7]. In a number of studies nutritional stress has been demonstrated to reduce resumption of ovarian cyclicity [16,20,21,32,50,60]. Furthermore an ovulation is observed in beef cows managed in pasture-based systems as they display a prolonged PPI [43]. The effects of nutrition on post-partum animals have also been extensively studied [13,17,22,27,31,32,38,43,58]. Reported that over-feeding during the far-off dry period leads to metabolic profiles which negatively affect reproduction [13]. It is believed that energy restrictions in the last trimester improve early post-partum metabolic profiles [44] hastening resumption of ovarian cyclicity.

In view of the many challenges faced by *Bos indicus* cattle, [53] suggest that oestrus synchronization protocols have to be modified to optimize results. In this regard we propose that the level of circulating progesterone concentrations be measured when designing protocols for *Bos indicus*-influenced females since most of the protocols have been developed using *Bos Taurus* cattle [59]. coined that reducing endogenous progesterone production or limiting exogenous progesterone administration during oestrus synchronization enhances ovarian follicular development in cattle [43]. Reported that interval to first ovulation was longer in 2-yr old or primiparous cows

compared with 3-yr old animals or multiparous cows. This probably reflects the nutritional stress imposed on younger cows due to their growth and lactation requirements. Results from the current study are not consistent with these observations particularly for Brahman and Mashona breeds.

Furthermore, the temperament of animals is also a critical factor influencing cow fertility [10]. In addition to minimizing stress placed upon animals, producers of *Bos indicus* cattle should consider opportunities to improve temperament through management that acclimates animals to human contact. Acclimatisation of heifers to human handling has been reported to improve temperament, reduced cortisol, and hasten puberty [53]. Although animals in the current study would be presumed acclimatised to human handling, it was clear during inseminations that they were temperamental and proper animal handling sessions can be recommended for the study area.

It was anticipated that oestrus synchronisation of Zebu cows in the current study should translate into higher percentage of animals participating in sexually active groups. This was supposed to lead greater number of animals into oestrus, due to social facilitation and sexual stimulation [21]. The results are partially a testimony to this effect, however the conception rates can still be improved especially through nutrition management or provision of supplements to improve body condition score [25]. Stated that Body Condition Score (BCS) can have an effect on post-partum date and subsequent pregnancy rates. As reported by Orihuelaand Galina [21] changes in body reserves are the driving force for a prompt restoration of ovarian activity and pregnancy rather than the time postpartum. Maqhashu, (2016) pointed out that BCS may be used as a management tool for assessing physiological state and, is the most practical method of evaluating energy reserves. [1] Avers that in beef cows Body Condition Score (BCS), is particularly the most crucial element conditioning the length of the PPA. Furthermore, [9,35] affirm that if beef cows calve in good BCS, they will not experience severe negative energy balance after parturition, hence the first postpartum ovulation should occur around days 35-40. In comparison, cows in poor BCS would require multiple follicular waves before ovulation occurs, usually 70-100 days on average. The results from the current study are a testimony to this fact. Therefore it can be suggested that communally managed cows have to be in a BCS above 3 for them to conceive within the expected time frame post-partum.

Conclusion

 $PGF2\alpha$ and EB could be successfully used to induce and synchronize ovulation in cattle undergoing TAI, sire age significantly influenced conception rate as well as that the post-partum interval is dependent on breed and parity.

Data Availability

Data will be made available on request through the corresponding author.

Conflict of Interest

All authors declare that there are no present or potential conflicts of interest among the authors and other people or organizations that could inappropriately bias their work.

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Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [Washaya Soul], and [Tavirimirwa Bruce]. The first draft of the manuscript was written by [Washaya Soul] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript."

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Soul W

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