An investigation of factors influencing synchronization response, conception and calving rate of communal cows in Limpopo Province, South Africa

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Abstract

A study was designed to assess factors affecting response to oestrus synchronization, conception and calving rate of communal cows following timed artificial insemination (AI) in Limpopo province, South Africa. A total of 140 cows were selected from communal villages based on; body condition score (BCS) of 2 and above (1-5 scale), not pregnant (excluding heifers), 90 days' post-partum, negative of contagious abortion (CA). A 9 day CIDR® protocol was used to synchronize the selected cows. The AI was done at 36 and 48 hours post synchronisation using Nguni frozen thawed semen. Pregnancy diagnosis was performed 90 days following AI. Data was analysed using logistic regression procedure of SAS. Cows that responded were 75%; of which 41% conceived and 36% calved. None of the factors such as district, age, parity, frame size and breed type significantly affect synchronization response and conception. However, BCS significantly affected calving rates. Small framed Nguni and Bonsmara type cows in their first parity with a BCS ≥3 had greater odds of conceiving to timed AI. In conclusion, acceptable oestrus synchronization response, conception and calving rate was achieved in communal setup regardless of BCS affecting calving. It is recommended that superior genetic materials can be successfully introduced through ARTs in organized communal production systems in South Africa.

Keywords: assisted reproductive technologies, Nguni, oestrus synchronization, timed artificial insemination

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Introduction

Livestock production has a potential to alleviate household food insecurity and poverty in rural households of the world, including South Africa (Musemwa *et al.*, 2008). For sustained agricultural production, there is a need to use all emerging technologies especially assisted reproductive technologies (ART) (Verma *et al.*, 2012). Of all the ART, oestrus synchronization and artificial insemination (AI) are among the most used and applicable techniques to increase the reproductive efficiency and genetic improvement of a cattle and most applicable to organized communal farmers (Belay et al 2016; Maqhashu *et al.*, 2016).

Previous studies reported that reproduction rates and growth rates are very low in communal cattle herds of South Africa (Nengovhela & Nedambale 2012). A high level of mortality (30.7%), low reproduction rates (48%) (Scholtz & Bester 2010), low weaning rates (45%) (Bembridge & Tapson, 1993) and a low turnover have been reported in the communal cattle sector. Moreover, this contributes to poor economic returns from this sector. The introduction of exotic breeds in the communal sector (Botsime, 2006) has led to an increase in a number of non-descriptive breeds which cannot prevail in the harsh conditions in communal villages (Musemwa *et al*., 2008). Hence, cattle kept in communal areas need to be improved through modern breeding programs in order to become adaptive and survive under these conditions (Tyasi *et al*., 2015).

The study posits that these reproductive techniques may be useful in introducing superior gametes in the communal areas. The aim of this study was to assess oestrus synchronization response, conception and calving rate when implementing oestrus synchronization and timed AI in communal cows. The influence of factors such as district, age, parity, frame size, breed type and body condition score (BCS) were evaluated.

Methodology

The study was conducted in the communal lands of Limpopo province, South Africa. Farmers' cows were selected as follows:(1) cows that have calved at least once, (2) cows should be above 90 days' post-partum, (3) should have BCS of above 2 on a sale of 1-5 (Nicholson and Butterworth 1986), (4) free from any physical disabilities and (5) test negative for contagious abortion. Pregnancy status was assessed by rectal hand palpation and ultrasound scanner. During the screening process, a total of 140 communal cows were successfully selected. All the selected cows were ear-tagged following selection and categorized per selected district (Capricorn, Mopani, Vhembe, Waterberg), BCS out of 5 (<3 and ≥3), age in years, breed (Nguni type, Brahman type, Bonsmara type), parity (first-P1, second-P2, third-P3, fourth+ -P4+) and frame size (small, medium). The cows were separated from the herd until after AI was done.

A total of 26 Stud Nguni bulls were used as sires. The bulls were maintained on natural pasture and water was provided *ad libitum*. Bulls were tested for reproductive diseases before collection. Semen was collected once from the 26 stud Nguni bulls. The collection, analysis and semen cryopreservation were done as previously reported (Seshoka *et al.*, 2016). Briefly, semen was collected using an electro ejaculator onto 15 mL graduated Falcon® tube (352099, USA). The semen was then analysed for concentration (spectrophotometer JENWAY® 6310), motility (Sperm Class Analyzer® system Microptic, Spain) and morphology (eosin nigrosine stain) using Olympus, Corporation BX 51FT, Tokyo, Japan.

Semen samples were then diluted (1:1) with egg yolk citrate extender, without cryoprotectant (fraction A) and stored at 5°C for 2 h. Thereafter, a second (fraction B) dilution (1:2) with egg yolk citrate extender supplemented with 12% of glycerol and further equilibrated for 2 h at 5°C. Following equilibration, semen was loaded into 0.5 mL polyvinyl chloride straws, sealed and placed into a controlled programmable freezer. The semen straws were then frozen using a controlled programmable freezer from 5 to -5°C at 0.008 °C per min and from -5 to -130 °C at 6 °C per min. When the target temperature of -130 °C was reached, the straws were plunged into a liquid nitrogen tank (-196 °C) for storage until thawing. Semen straws were thawed at 37°C and analysed for motility using computer aided sperm analyser. Semen straws with motility of above 40% was used for AI.

The selected cows went through a 9-day oestrus synchronization program using CIDR® protocol. Briefly, controlled internal drug release (CIDR®) device (1.9g progesterone: Pfizer™, New Zealand [Ltd]) was inserted intravaginally on day 0 and oestradiol benzoate (2mL) administed intramuscularly. The CIDR® was removed on day 8 and PGF2α was injected intramuscularly. Oestradiol benzoate (1mL) was injected on day 9 followed by heat observation using Kamar® heat mount detectors. The patch is white in colour when placed at the tail head of a cow. This turns red when the cow has been mounted by other cows during standing heat.

Timed AI was done at 36 and 48 hours following a second oestradiol benzoate injection using frozen-thawed Nguni bull semen. Pregnancy diagnosis was done 90 days following timed AI using a potable ultrasound scanner (Ibex pro™, E.I. Medical Imaging, USA) and also by rectal hand palpation methods. Data on the number of cows that calved was collected one month following the expected calving date of the last group where AI was done.

Data was analysed using the logistic regression procedure of SAS (9.4, 2013) with synchronization response, conception and calving being treated as binary response variables given a set of explanatory variables. Thus, the logistic regression model predicted the probability that a given factor would affect the response to oestrus synchronization, conception and calving. The prediction of the probability that an event had occurred (probability of success) was done by fitting data to a logit function. It makes use of several predictor variables where Y=1 for an occurrence and Y=2 for a non-occurrence (Agresti, 1996). The goal of the logistic regression was to find the best fitting (biologically reasonable) model to describe a relationship between the dichotomous character of interest and a set of predictor variables (Mafukata, 2012). Data was considered significant at $p<0.05$ level of significance.

The logistic regression model used in this study was expressed as:
Logit (p) = b0 + b1X1 + b2X2 +…bnXn
Where p is the probability of success or failure. And X1…Xn is;
X1 = District (Capricorn, Mopani, Vhembe, Waterberg)
X2 = Parity (P1, P2, P3 and P4+)
X3 = Age in years (4, 5, 6. 7 and 8+)
X4= Breed (Bonsmara type, Brahman type, Nguni type)
X5= Body condition score out of 5 (≤3 and >3)
X6= Frame (medium and small)

Results

Table 1 presents the frequency, proportion and overall response of cows to oestrus synchronization and timed AI in different districts. The overall oestrus synchronization response, conception and calving recorded in this study was 75%, 41% and 36%, respectively. Table 1 also shows the oestrus response, conception and calving rate of cows in subdivided categories.

Table 1 The simple frequencies and overall oestrus synchronization response, conception and calving of communal cows

Variable	N	Oestrus synchronization (%)	Conception (%)	Calving (%)
District				
Capricorn	44	84.1	37.5	32.4
Mopani	20	65	28.4	23.1
Vhembe	64	73.4	46	43.8
Waterberg	12	66.7	50	25
Parity				
First (p1)	40	75	50	40.7
Second (p2)	27	74.1	40	40
Third (p3)	30	73.3	43.8	39.1
Fourth+ (p4+)	20	75	50	46.2
Age in years				
4	62	75.8	40	43.3
5	28	75	37.5	38.1
6	30	73.3	43.8	39.1
7	15	73.3	33.3	33.3
8+	5	80	75	75
Breed				
Bonsmara	47	85.1	30.3	31.7
Brahman	14	64.3	37.5	22.2
Nguni	79	71	51.2	41.1
Body condition score (1-5)				
≤3	129	74.4	37.3	30.9
>3	11	81.8	85.7	75
Frame size				
Medium	109	74.3	36.5	32.9
Small	31	77.4	57.9	45.9
Overall results				
Yes	105	75	41.5	35.9
No	35	25	58.5	64.2

Capricorn district had the highest oestrus synchronization response followed by Vhembe, Waterberg and Mopani. Waterberg had the highest conception followed by Vhembe then Capricorn and lastly Mopani.

However, cows in Vhembe had the highest calving followed by Capricorn. Mopani and Waterberg had the lowest calving rate.

The P1 and P4+ parity cows had the highest oestrus synchronization response followed by the P2 and P3. The P1 and the P4+ had the highest conception of 50% each followed by P3 and P2. This trend was similar to the oestrus synchronization response. Conception rate decreased for P2 cows and tended to increase with an increase in parity. Cows in their P4+ were recorded to have the highest calving rate when compared to P1, P2 and P3.

Older cows aged eight+ years had the highest oestrus synchronization response as compared to other age groups. Cows aged seven had the lowest conception and cows aged eight+ had the highest conception. However, cows ages eight+ had the highest calving rate followed by four, six, and five. Cows ages seven recorded the lowest calving rate.

The probabilities of oestrus synchronization response are shown in Table 2. All the variables were not associated with oestrus synchronization response ($p>0.05$). However, it was noticeable that cows at the Capricorn district had better odd of responding as compared to other districts followed by Mopani and Vhembe district when compared with Waterberg district. P1 cows had higher probability of responding followed by P2 and P3 groups were less likely to respond when compared to P4+. Bonsmara type cows had 4.013 odds of oestrus success as compared to Nguni type cows. Cows with a body condition score of ≤3 had only 0.50% odds ratio of success compared to those with a body condition score of >3. Small framed cows had lower odds of oestrus synchronization response as compared to medium framed cows.

Table 2 The intercept, odd ratio and the significance of the different explanatory variables when modelling the probability of oestrus synchronization response in cows

Parameter		**Estimate**	**Standard error**	**Pr > chi square**	**Odds ratio**
Intercept		1.4314	0.7501	0.0564	
District	Capricorn	0.4510	0.5896	0.4443	2.070
District	Mopani	-0.0152	1.0571	0.9885	1.698
District	Vhembe	0.1092	0.5373	0.8390	1.923
District	Waterberg	0.00	0.00		
Parity	P1	0.5016	0.4592	0.2747	2.063
Parity	P2	-0.1892	0.5092	0.7102	1.035
Parity	P3	-0.0887	0.4825	0.8541	1.145
Parity	P4+	0.00	0.00		
Breed	Bonsmara	0.9524	0.5155	0.0700	4.013
Breed	Brahman	-0.5151	0.6044	0.3941	0.925
Breed	Nguni	0.00	0.00		
BCS	≤3	-0.3396	0.7877	0.6664	0.507
BCS	>3	0.00	0.00		
Frame size	Small	-0.1333	0.3797	0.7255	0.766
Frame size	Medium	0.00	0.00		

Note: P1= first parity, P2= second parity, P3= third parity and P4+= fourth parity and above. BCS= body condition score (1-5)

Table 3 presents the probability of conception following timed AI in communal cows. None of the parameters (district, parity, age, BCS, breed type and frame size) were associated with conception following timed AI. However, it was noticeable that cows in the Capricorn, Mopani and Vhembe districts had less odds ratio of conceiving when compared to cows in the Waterberg district. Cow in P1, P2 and P3 had only 0.55, 0.90 and 0.77 odds ratio of conceiving when compared with P4+ cows. Cows with a body condition score ≤3 had only 0,28 probability of conceiving compared to those with a BCS of >3. Bonsmara and Brahman type cows had a 0.28 and 0.20 odds ratio of conceiving when compared to Nguni type cows. Additionally, medium frames cows had lesser odd of conceiving to timed AI when compared with small frames cows.

Table 3 The intercept, odd ratio and the significance of the different explanatory variables when modelling the probability of conception in cows.

Parameter		**Estimate**	**Standard error**	**Pr > chi square**	**Odds ratio**
Intercept		0.5662	0.8169	0.4882	
District	Capricorn	0.0717	0.6300	0.9094	0.602
District	Mopani	-0.6740	1.2481	0.5892	0.285
District	Vhembe	0.0224	0.5949	0.9700	0.573
District	Waterberg	0.00	0.00		
Parity	P1	0.2682	0.4586	0.5587	1.840
Parity	P2	-0.0782	0.6241	0.9003	1.301
Parity	P3	0.1513	0.5268	0.7740	1.637
Parity	P4+	0.00	0.00		
Breed	Bonsmara	-0.3258	0.5478	0.5520	0.280
Breed	Brahman	-0.6208	0.8259	0.4522	0.209
Breed	Nguni	0.00	0.00		
BCS	≤3	-1.0486	0.9762	0.2828	0.123
BCS	>3	0.00	0.00		
Frame size	Medium	-0.6396	0.3824	0.0945	0.278
Frame size	Small	0.00	0.00		

Note: P1= first parity, P2= second parity, P3= third parity and P4+= fourth parity and above. BCS= body condition score (1-5)

The intercept, odds ratio and the significance of different explanatory variables when modelling the probability of calving are shown in Table 4. The odds ratio of a cow in Capricorn, Mopani and Vhembe districts to calve was higher when compared to Waterberg. The odds ratio of a cow at P4+ to calve was similar to cows in other parities. The odds ratio of Bonsmara and Brahman type cows to calve was only 0.38% and 0.09, respectively as compared to that of Nguni type. Cows with ≤3 body condition score had only 0.02 odds ratio of calving compared to those with >3 body condition score and the difference was p=0.0042. The probability of a medium framed cow to calve was 0.40 more compared to that of small framed cow.

Table 4 The intercept, odd ratio and the significance of the different explanatory variables when modelling the probability of calving.

Parameter		Estimate	Standard error	Pr > chi square	Odds ratio
Intercept		0.6526	0.5582	0.2424	
District	Capricorn	0.2169	0.5739	0.7054	3.584
District	Mopani	0.7231	0.8315	0.3845	5.946
District	Vhembe	0.1196	0.5109	0.8149	3.252
District	Waterberg	0.00			
Parity	P1	-0.0634	0.4086	0.8766	1.028
Parity	P2	0.2693	0.5062	0.5948	1.434
Parity	P3	-0.1144	0.4292	0.7899	0.977
Parity	P4+	0.00			
Breed	Bonsmara	0.1512	0.5319	0.7762	0.389
Breed	Brahman	-1.2469	0.9642	0.1960	0.096
Breed	Nguni	0.00			
BCS	≤3	-1.8287	0.6382	0.0042	0.026
BCS	>3	0.00			
Frame size	Medium	-0.4571	0.3298	0.1658	0.401
Frame size	Small	0.00			

Note: P1= first parity, P2= second parity, P3= third parity and P4+= fourth parity and above. BCS= body condition score (1-5)

Discussion

The study demonstrated that 75% of communal cows responded to oestrus synchronization. These results are comparable to those reported by Colazo & Ambrose (2013), who reported 68% and 67% of oestrus synchronization response, respectively when the same protocol was employed in dairy cows. Approximately one quarter of cows in the present study did not show signs of heat similarly to the report by Colazo & Ambrose (2013).

Mounting behaviours can also affect the response rate (Perry, 2004). Oestrus synchronization response in the present study was recorded using heat mount detectors which require a cow to stand immobile and be mounted by another in order to change colour. The number of mounts increases as the number of cows in oestrus increases (Smith *et al*., 2012). Therefore, cows that were recorded to not have responded to the oestrus synchronization protocol may have lacked a partner to mount them during standing heat. Also, cattle raised on pasture spend more time grazing thus have less time to engage in oestrus behaviour (Orihuela, 2000).

Calving rate has been used to measure reproductive efficiency of cows in communal areas. This is because techniques such as ultrasonography requires the presence of good cattle handling facilities and trained personnel (Ngeno *et al.*, 2010). In the present study, appropriate handling facilities were made available by the government and farmers for these technique to be used. As such, pregnancy was assessed 90 days following timed AI using ultrasound scanner and rectal hand palpation. Conception rates in the present study was 41%. This is higher when compared with the previous documented reproductive rate of 27% (Scholtz *et al*., 2008; Simela, 2012), 35.7% (Nowers *et al*., 2013) and 37.7% (Mokantla *et al*., 2004) from natural breeding in communal villages. The improvement may have been the results of proper cows selected for this study rather than the entire population used in previous studies. Also, quality of semen used in lieu of communal bulls may have contributed to favourable results.

The conception results are comparable to 37.97% observed by Zeuh *et al.* (2014) on African indigenous breeds and 44.3% (Kaoamo & Sawadogo 2012) when using AI in Gobra zebu cattle. These results are lower than those reported by Tebug *et al*. (2015) in Cameroonian zebu cattle (47.6%) and 48.30% by Woldu *et al.* (2011) using AI in indigenous Ethiopian cattle. Moreover, Belay *et al.* (2016) reported conception of 60.4% when indigenous and crossbred village dairy cows were synchronized for oestrus and inseminated in Ethiopia. This difference might be due to different hormones and management systems. Also, timed AI was employed in the present study whereas the AM/PM rule was used to inseminate cows in the other studies.

Extended periods of low rainfall (Burns *et al*., 2010) or extremely high temperatures (Rao *et al*., 2013) can potentially cause fertilization failure and embryonic loss. Heat stress is a major cause of low fertility and pregnancy due to embryonic death following AI (Caraba & Valicevici 2013). The lower results in the present study might have been caused by environmental factors such as heat stress, since the present study was conducted in summer with moderate to extreme drought (Ledet, 2015). Infertility or subfertility of the communal cows may also have caused the low conception rate in the present study when compared to Tebug *et al.* (2015) and Woldu *et al*. (2011). Mokantla *et al.* (2004) recorded the reproductive patterns of communal cows monthly for a year and observed that 54% of the cows remained open throughout the study which indicated infertility.

An overall calving rate of 36% was recorded in the present study. This is comparable to those obtained through natural service in South Africa (35.7%; Nowers *et al*., 2013). With a conception rate of 41% recorded, calving results showed a 5% decline in pregnancy. This is better when compared to the 12% pregnancy loss in communal villages reported by Mokantla *et al*. (2004). Munyai (2012) noted that drought in one year leads to lower calving rates in the following year. Therefore, drought conditions prevailed in communal lands of Limpopo province in the year before the study (2015) may have been the cause of pregnancy losses.

There was quantitative difference of response for various levels of variables tested (district, parity, age, breed type and frame size). Cows aged eight+ years and parities P1 as well as P4+ had higher oestrus synchronization response, conception and calving compared to other groups. Also cows in the P1 had the highest odds of responding to oestrus synchronization and conceiving to timed AI compared to other parities. Moreover, both P1 and P4+ had comparable conception rate. These results are constant with those reported previously by Belay *et al*. (2016), Tebug *et al.* (2015) and Tanhagen *et al.* (2004). Primiparous cows are younger, have low energy requirements (Tebug *et al.*, 2015) and have less reproductive problems (Tanhagen *et al.*, 2004), whereas multiparous cows are more mature. However, the age difference in the present study is contradicting with this fact. This contradiction might be explained by the fact that communal cows have calving intervals exceeding 2 years (Gaudex, 2014).

According to Ngeno (2008), there might be possible fertility differences among cow breeds under communal conditions. Bonsmara type exhibited higher oestrus synchronization response, whereas Nguni type cows had the highest conception and calving rate. According to Orihuela (2000), cattle breed differs with oestrus duration which may in turn affect the mounting duration between individuals in a herd. Also, zebu cattle do not exhibit obvious oestrus signs like exotic breeds; perhaps because oestrus is short and subdued (Woldu *et al.*, 2011). Tada *et al*. (2010) reported a high number of silent oestrus in indigenous Tuli cattle in an oestrus synchronization program. Therefore, low oestrus synchronization response in Nguni type compared to other breeds may have been an effect of short or silent oestrus.

Small framed indigenous cattle such as the Nguni have low maintenance feed requirements (Mapiye *et al.*, 2010). In the present study small framed cows had better odds of responding to the oestrus synchronization protocol, conceiving and consequently calving when compared with medium framed cows; this is consistent with the results of Tylor *et al.* (2008). According to Tylor *et al*. (2008), a small body frame is an adaptive attribute in stressful environments. These results are also in agreement with those observed in breed comparisons where Nguni type cattle which are naturally small performed better than other breeds.

The quantitative difference in oestrus synchronization response and conception of different body condition score was in agreement with previous studies (Moreira et al 1999; Mokantla *et al*., 2004; Woldu *et al*., 2011; Tebug *et al*., 2015). According to Ngeno *et al.* (2010), in natural breeding the nutritional status of the animal affects ovarian function, which in turn affects their reproductive performance. Calving rate was affected by body condition of the cows. Cows with body condition of less than 3 out of 5 had a lower calving (p=0.0042) with only 0.02 odds ratio of calving compared to those in the other category. Body condition at calving and change in body condition from calving to insemination are important for reproductive response (Alnimer *et al.*, 2002). It is therefore inferred that body condition of a cow not only affects the ability to maintain herself, but also the ability to maintain a pregnancy.

Conclusion

Communal cows had acceptable response to oestrus synchronization and timed AI was applied successfully with low but acceptable conception rates. Noteworthy, is that a calving rate with AI in this study was comparable to natural service. Therefore, this study demonstrated an opportunity to improve the production of communal cattle using superior genetic material though assisted reproductive technologies. Small framed cows performed better throughout the study. Nguni type cows had average response to synchronization, but interestingly had the highest conception and calving rate. Body condition score influenced calving percentage. Cows in communal areas had greater probability of calving when their condition score was above 3 out of 5. Small framed Nguni and Bonsmara type cows in their first parity with a BCS of more than 3 had a greater odd of conceiving to timed AI and calving.

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Author contributions

Conception and design RZC, NTL, NKA, NNB; Data collection RZC; Data analysis: NKA; Critical revision: NKA, NTL.

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