

Research Article

Evaluation of Reproductive Performance Using Sexed and Conventional Semen at Mruazi Heifer Breeding Unit, Tanga Tanzania

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Abstract

The crossbreeding program at Mruazi Heifer Breeding Unit in Korogwe, Tanga region, was launched in 2010, initially using conventional semen. In 2019, sexed semen was introduced into the program. Since then, no comprehensive evaluation has been conducted to compare the efficiency of sexed semen with conventional semen under the farm's operating conditions. This study analyzed retrospective data from 735 heifers. Information collected from reproductive record books and heifer cards included heifer ID, age at first service (AFS), breed, first insemination details, season of insemination, semen type, first service conception (FSC), number of services per conception (NSC), and inseminator's identity. The General Linear Model (GLM) assessed the impact of categorical factors on NSC, while a binary logistic regression identified factors influencing FSC among crossbred dairy heifers. A Chi-square test evaluated the relationship between categorical variables and the binary FSC outcome. Results showed that breed group 2 had 1.10 ± 0.11 NSC lower than other breeds, wet season showed 1.25 ± 0.03 NSC compared to 1.32 ± 0.04 NSC in the dry season, sexed semen had 1.39 ± 0.04 NSC higher than that of conventional semen, inseminator 3 had 1.25 ± 0.08 NSC lower than inseminator 4, and cattle under 18 months had 1.20 ± 0.07 NSC lower compared to 1.41 ± 0.05 in 25 – 36 months age group ($P < 0.05$). Logistic regression revealed semen type as the only significant factor affecting FSC ($p = 0.0011$), with heifers inseminated using sexed semen showing 63% lower odds of FSC ($OR = 0.37$; 95% CI: 0.20–0.67). Additionally, Chi-square analysis indicated a significant association between breed and FSC ($\chi^2 = 23.83$, $p = 0.0002$), as well as between semen type and FSC, with sexed semen linked to a considerably lower FSC rate. The study concluded that under the prevailing environment, conventional semen is more effective than sexed semen.

Keywords

Sexed Semen, Conventional Semen, Conception Rate, Number of Services per Conception

1. Introduction

Background

Regardless of having a larger number of cattle, Tanzania's per capita milk consumption is 67.5 liters per person per year [1]. This amount is less than 50% of the 200 liters recom-

mended by FAO [1]. One of the reasons is the low annual milk production of 3.97 billion liters compared to the annual demand of 12 billion liters [1]. The milk underproduction is largely attributed to the predominance of cattle with low

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genetic potential for milk yield, mainly the indigenous Zebu cows [2]. Artificial Insemination (AI) is among the methods adopted in Tanzania to improve the genetic potential of low milk-yielding cattle. This approach is significantly faster than natural selection, as a single ejaculate can be divided into multiple doses to inseminate many females [3]. In practice, the AI technique utilizes either sexed or conventional semen to enhance the genetic composition of the herd. In sex sorting technology, sperm damage incurred during the sorting process is a key factor reducing the fertilizing capacity of sexed semen. Flow cytometric sorting, followed by cryopreservation, has been shown to lower both in vitro and in vivo fertility, and this reduction cannot be fully offset by increasing the insemination dose. Although recent processing improvements have been made, fertility remains lower in part due to sorting-induced sperm damage, and the higher cost of sexed semen further limits its economically viable applications. Moreover, even a slight reduction in fertility compared with conventional semen can diminish much of its potential economic advantage [23, 39-41]. Regardless of the semen type used, its efficiency is a key consideration and can be evaluated using parameters such as the number of services per conception (NSC) and conception rate (CR) [4]. Several studies have shown differences in CR between sexed vs conventional semen. For example, conception rates of 56% with conventional semen compared to 39% with sexed semen in heifers have been documented [42]. Conception rates for sexed semen have been reported to range from 31.6% to 48.3%, compared with 39.6% to 63.8% for conventional semen, with model-based predictions indicating even lower values of 21.3% and 32.1% for sexed and unsexed semen, respectively [23, 26]. Generally, lower NSC and higher CR contribute to rapid herd improvement, increased net returns, and overall farm profitability [5]. Conversely, higher NSC and lower CR lead to increased operational costs, reduced income, and ultimately decreased farm profitability [6]. At Mruazi HBU, artificial insemination (AI) has been practiced since 2010 using both conventional and sexed semen. However, there is limited information on the comparative efficiency of these semen types, highlighting the need for the present study.

2. Material and Methods

2.1. Description of the Study Area

The study was conducted at Mruazi HBU, located in Mnyuzi Korogwe in the Tanga region, latitudes 4°15' and 5°15' South and longitudes 38°0' and 38°45' East [7]. It is about 5 km from Hale town along the Tanga - Dar es Salam Road. Its climatic condition comprises two seasons: A dry season from July to November and a wet season from December to June, with an annual mean rainfall of 900 mm, and an annual temperature range between 24.6 °C - 30 °C [8]. The relative humidity ranges from 72 – 75% [9]. The center produces milk and distributes heifers to smallholder farmers.

2.2. Data Collection

In this study, five-year retrospective data from 735 cross-bred heifers at Mruazi HBU were used. The heifers were categorized into four genetic groups, and for description, the crossbreds are here referred to as “breed” (Table 1). Age at first service (AFS), breed type, 1st inseminations, season of insemination, semen type, first service conception (FSC), number of services per conception (NSC), and inseminator's name were obtained from reproduction record books and heifer cards. Season was categorized into: Dry season from July to November and a wet season from December to June, while semen type comprised of two categories i.e., conventional or sexed.

Table 1. Cattle breeds and numbers used for the study.

Breed	Number
1	640
2	34
3	44
4	17
Total	735

Breed: 1 = Holstein Friesian x Sahiwal (FS 50:50), 2 = Holstein Friesian x Zebu (FZ 50:50), 3 = Holstein Friesian x Gyr (FG 62.5:37.5), 4 = Backcross of Holstein Friesian x Sahiwal (FS 75:25)

2.3. Data Processing and Analysis

All statistical analyses were performed using SAS software (Version 9.2, SAS Institute Inc., Cary, NC, USA). The analysis focused on two key reproductive outcomes: First Service Conception (FSC), a binary variable indicating whether a cow conceived after the first artificial insemination, and Number of Services per Conception (NSC), a continuous variable representing the number of inseminations required to achieve conception.

2.4. General Linear Model (GLM)

To evaluate the effect of various categorical factors on NSC, General Linear Model (GLM) was used. The model included the fixed effects of breed group, season of insemination, semen type, inseminator, and age at first service.

$$Y_{ijklo} = B_i + S_j + T_k + I_l + A_o + e_{ijklo}$$

$$Y_{ijklo} = \text{Performance on NSC}$$

$$B_i = \text{effect of the } i^{\text{th}} \text{ breed}$$

$$S_j = \text{effect of the } j^{\text{th}} \text{ season of insemination}$$

$$T_k = \text{effect of the } k^{\text{th}} \text{ type of semen used (sexed semen or conventional semen)}$$

$$I_l = \text{effect of the } l^{\text{th}} \text{ Inseminator}$$

Ao = effect of o^{th} age at first service (AFS)

$eijklo$ = Random error term

The continuous variable age at first service was categorized into four biologically meaningful groups: 1. (< 18 months); 2. (18 - 24 months); 3. (25 - 36 months); 4. (Above 36 months).

2.5. Chi-square Tests

Chi-square test of independence was conducted to assess the association between categorical explanatory variables (e.g. breed, semen type, insemination season) and the binary outcome FSC. The frequency procedure was used to generate cross-tabulations, and Pearson's Chi-square test was used to test for significant associations at the 5% level.

2.6. Logistic Regression

To identify factors associated with first service conception (FSC) among crossbred dairy heifers, a binary logistic regression model was applied using the GENMOD procedure in SAS. The dependent variable was FSC, coded as 1 for conception at first insemination and 0 otherwise. Independent variables included breed group, insemination season, semen type, inseminator, and age at first service category.

$\log(p/1-p) = \beta_0 + \beta_1(\text{Breed}) + \beta_2(\text{Season}) + \beta_3(\text{Semen Type}) + \beta_4(\text{Inseminator}) + \beta_5(\text{AFS Category}) + \epsilon$

Where:

p is the probability of conception at first service (FSC = 1),

β_0 is the intercept,

β_1 to β_5 are coefficients for the explanatory variables,

ϵ is the error term.

3. Results

3.1. Number of Services per Conception (NSC)

The number of services per conception (NSC) was significantly affected ($P < 0.05$) by factors such as breed group, insemination season, semen type, inseminator, and age at first service. Heifers in age group (18 - 24 months) recorded the lowest NSC, while differences among the other breeds were not statistically significant (Table 2). The wet season showed a significantly lower NSC (1.25 ± 0.03) compared to the dry season (1.32 ± 0.04) ($P < 0.05$). Sexed semen also resulted in a significantly higher NSC compared to conventional semen ($P < 0.05$). Concerning inseminators, the most notable variation was between Inseminator 3, who achieved the lowest NSC, and Inseminator 4 who recorded the highest NSC (1.50). Differences among the other inseminators were not statistically significant. Age at first service also had a significant effect ($P < 0.05$) on NSC, with heifers inseminated before 18 months showing a lower NSC; although their difference was not statistically significant when compared to heifers in age

groups (18–24 months) and (>36 months). Heifers in age group (25–36 months) tended to have a higher NSC, though the difference was insignificant relative to those heifers in age groups (18–24 months) and (>36 months). Overall, there was no consistent trend in the influence of Heifer's age on NSC (Table 2).

Table 2. Least Squares Means $\pm$ SE for Number of Services per Conception (NSC) of crossbred cattle.

Factor	Group	NSC (Mean $\pm$ SE)
Breed	1	1.36 ± 0.05^a
	2	1.10 ± 0.11^b
	3	1.37 ± 0.09^a
	4	1.47 ± 0.14^a
Insemination season	Dry	1.32 ± 0.04^a
	Wet	1.25 ± 0.03^b
Semen type	Conventional	1.20 ± 0.03^a
	Sexed	1.39 ± 0.04^b
	Inseminator 1	1.27 ± 0.05^{ab}
	Inseminator 2	1.35 ± 0.19^{ab}
Inseminator	Inseminator 3	1.25 ± 0.08^b
	Inseminator 4	1.50 ± 0.07^a
	Inseminator 5	1.31 ± 0.05^{ab}
	Inseminator 6	1.29 ± 0.05^{ab}
	Inseminator 7	1.38 ± 0.06^a
Age at first service	1	1.20 ± 0.07^b
	2	1.29 ± 0.05^{ab}
	3	1.41 ± 0.05^a
	4	1.33 ± 0.04^{ab}

Note: Breed: 1 = Holstein Friesian x Sahiwal (FS 50:50), 2 = Holstein Friesian x Zebu (FZ 50:50), 3 = Holstein Friesian x Gyr (FG 62.5:37.5), 4 = Backcross of Holstein Friesian x Sahiwal (FS 75:25) Groups: 1 = (< 18 months), 2 = (18 - 24 months), 3 = (25 - 36 months), 4 = (Above 36 months)

3.2. First Service Conception (FSC)

A binary logistic regression analysis was conducted to identify the factors influencing first service to conception (FSC) in the crossbred dairy heifers. The model included breed group, insemination season, and semen type as predictors. The analysis showed that semen type was the only factor that was significantly associated with first service conception rate ($p = 0.0011$), with heifers inseminated using sexed semen having lower odds of conception compared to

those inseminated with conventional semen (OR = 0.37, 95% CI: 0.20–0.67). This means that the likelihood of successful conception at first service was 63% lower when sexed semen was used. The factors of insemination season and breed showed no significant association with the outcome (Table 3).

Chi-square test showed that breed was significantly associated with first service conception rate, with Holstein Friesian x Zebu (FZ (50:50) (breed 2) having a higher conception rate (91.20%), and the least being breed 4 (Backcross of Holstein Friesian x Sahiwal (FS (75:25). Figure 1).

Table 3. Factors Associated with First Service Conception in Crossbred Dairy Cattle.

Predictors	Variable	β Coefficient, (95% CI)	p-value
Breed	Breed 1	1.08 (0.34, 3.44)	0.898
	Breed 2	0.17 (0.02, 1.71)	0.132
	Breed 3	0.84 (0.23, 3.01)	0.787
	Breed 4	2.54 (0.57, 11.23)	0.220
Insemination Season	Dry	0.90 (0.63, 1.30)	0.574
	Wet (Ref)		
Semen Type	Sexed	0.37 (0.20, 0.67)	0.001*
	conventional (Ref)		

* Significant at $P < 0.05$

Breed: 1 = Holstein Friesian x Sahiwal (FS 50:50), 2 = Holstein Friesian x Zebu (FZ 50:50), 3 = Holstein Friesian x Gyr (FG 62.5:37.5), 4 = Backcross of Holstein Friesian x Sahiwal (FS 75:25)

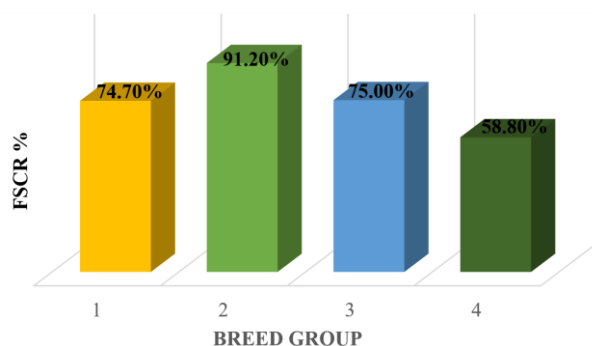


Figure 1. First Service Conception Rate (FSCR) in Different Heifer Crosses.

Breed: 1 = Holstein Friesian x Sahiwal (FS 50:50), 2 = Holstein Friesian x Zebu (FZ 50:50), 3 = Holstein Friesian x Gyr (FG 62.5:37.5), 4 = Backcross of Holstein Friesian x Sahiwal (FS 75:25).

Table 4. Association between Semen Type and First Service Conception Rate (FSCR) by Breed.

Breed	Semen Type	N	FSCR (%)	Chi-Square Value	P-Value
1	Conventional	592	70.71	1.5208	0.2175
	Sexed	43	4.57		
2	Conventional	28	84.38	0.1475	0.7010
	Sexed	4	12.50		
3	Conventional	35	65.12	1.1173	0.2905

Breed	Semen Type	N	FSCR (%)	Chi-Square Value	P-Value
4	Sexed	8	11.63	7.4725	0.0063 ★
	Conventional	13	58.82		
	Sexed	4	0.00		

★ Statistically significant at $p < 0.05$.

N = number of heifers inseminated with each semen type within the breed.

Breed: 1 = Holstein Friesian x Sahiwal FS (50:50), 2 = Holstein Friesian x Zebu FZ (50:50), 3 = Holstein Friesian x Gyr FG (62.5:37.5), 4 = Backcross of Holstein Friesian x Sahiwal FS (75:25).

Chi-square analysis was performed to determine whether there was a significant association between semen type (conventional vs. sexed) and first service conception (FSC) rate within each breed. As shown in Table 4, the relationship between semen type and FSC rate differed among breeds. In all cases, conventional semen resulted in higher conception rates compared to sexed semen. A statistically significant difference was observed among breeds where sexed semen was linked to a markedly lower FSC rate, notably in breed 4. Although the same trend was noted in the other breeds, these differences were not statistically significant, possibly due to the smaller sample sizes for sexed semen.

4. Discussion

4.1. Number of Services per Conception (NSC)

Number of services per conception is one of the widely used indicators of fertility in dairy cattle [10]. There are many factors, such as breed, insemination season, semen type, inseminator and the age at first mating, that may affect the NSC as discussed below:

4.2. Effect of Breed on Number of Services per Conception (NSC)

It has been classified that the fertility of nulliparous heifers as excellent, good, poor, or very poor when the NSC is 1.1, 1.5, 2.5, or greater than 3, respectively [11]. In the present study, NSC values ranged from 1.10 to 1.47, placing them within the good to excellent fertility category. The excellent fertility observed in the F1 crosses of Holstein Friesian × Zebu (FZ 50:50) may be attributed to their genetic composition, with 50% local genes likely contributing to improved tolerance to environmental stresses. For example, it has been reported that the HF50 are better heat tolerant compared to HF75 [43]. Furthermore, it has been reported that local breeds of cattle are more tolerant to heat than the crosses while exotic breeds are highly affected by heat stress [40, 41].

The NSC for FZ 50:50 is also lower than the values of 1.56 and 1.7 reported by [12, 13]. Conversely, the NSC of Holstein Friesian × Sahiwal (FS 50:50), Holstein Friesian × Gyr (FG

62.5:37.5), and the backcross Holstein Friesian × Sahiwal (FS 75:25) did not differ significantly. Their NSC values were slightly higher than the 1.23 ± 0.08 reported by [14]. On the other hand, they are lower than the 2.0 ± 0.08 reported by [15].

4.3. Effect of Insemination Season on Number of Services per Conception (NSC)

In the current study, the effect of season on NSC was found to be significant, with lower NSC values observed during the wet season compared to the dry season. This difference could be attributed to the fact that heifers inseminated in the wet season were mostly born in the dry season, benefiting from better maternal nutrition. This likely resulted in higher birth weights, improved weaning weights and earlier onset of puberty [16, 17]. Additionally, the reduced heat stress during the rainy season, where the temperatures in the study area range between 18 °C and 20 °C may have contributed to improved fertility outcomes [18]. Similarly, other researchers noted higher conception rates during the rainy season, implying a lower NSC relative to the dry season [19]. In contrast, studies by others have found no significant effect of insemination season on NSC [15, 20-22].

4.4. Effect of Semen Type on Number of Services per Conception (NSC)

The highest NSC was observed with sexed semen (1.39) compared to 1.20 for conventional semen in the present study, which can be explained by a small sample size of heifers inseminated with sexed semen on one side, but also, sperm damage that is encountered during the sorting process is among the reasons that can lower the capacity of sexed semen to fertilize an ovum [23, 39]. These findings are in agreement with many studies. For example, it has been reported NSC of 2.0 versus 1.5, 3.0 versus 1.3, 2.5 versus 2, and 3.1 versus 2.5 with sexed versus unsexed semen in Holstein heifers, respectively [23-26]. To the contrary, it has also been reported an NSC of 1.67 ± 0.63 versus 1.86 ± 0.74 for sexed versus unsexed semen in Holstein heifers, respectively [27].

4.5. Effect of Inseminator on Number of Services per Conception (NSC)

The NSC among inseminators ranged between 1.2 to 1.5, which is characterized as good. The reason for this achievement could be explained by the incentives given to the Mruazi HBU inseminators when insemination is successful. Researchers, once reported the significance of incentives and motivation to inseminators, which, if less considered, can result in variations of NSC because the inseminators pay less attention to their work, waiting for a monthly salary [28]. However, the differences observed among inseminators align with the findings of other researchers and may be influenced by factors beyond their skill level and dedication [26, 29].

4.6. Effect of Age at First Service on Number of Services per Conception (NSC)

Results displayed in Table 2 showed that there were differences among the ages at which the heifers were inseminated on NSC. However, small sample size for some of the age categories may skew the results and therefore lack a distinct trend. Overall, no significant differences were observed among the other age groups, though the NSC values fell within the ranges reported by other studies such as [21, 30].

4.7. First Service Conception (FSC) and First Service Conception Rate (FSCR)

First-service conception refers to a female animal conceiving after first AI or natural mating, while, first-service conception rate is the percentage of females that become pregnant after the first service, which, if above 60%, indicates a good reproductive efficiency in a dairy herd [31, 32].

4.8. Effect of Semen Type on FSC and FSCR

In the current study, both FSC and FSCR were low when using sexed semen compared to conventional semen (Tables 3&4). The reasons for this result are probably due to the small sample size of heifers inseminated by using sexed semen and, a damage encountered during sorting process [23, 39]. Other researchers reported 51.9% FSCR with sexed semen compared to 62.4% with conventional semen [33]. In some cases, conventional semen may have higher percentages of FSCR than sexed semen. For example, it has been reported an FSCR with conventional semen of 67.1% compared to its counterpart, sexed semen with 49.58% [34]. Moreover, a non-significant ($P > 0.01$) higher FSCR of 49.32% obtained by using conventional semen as compared to 40% when using sexed semen has been reported by other researchers [25].

The notably lower FSCR associated with sexed semen as observed in Breed 4 (Table 4), was caused by small sample

size. Other breeds' effects, though not significant, had higher FSCR with conventional semen outperforming the sexed semen. The finding is in agreement with other researchers who reported FSCR of 61.47 vs 51.45, 63 vs 49, and 67.1 vs 49.58 for conventional and sexed semen in Holstein Friesian Heifers, respectively [27, 34, 35].

4.9. Effect of Breed Group on FSCR

In this study, crossbreds FS (50:50), FZ (50:50), and FG (62.5:37.5) demonstrated higher conception rates than FS (75:25) (Figure 1). This indicates that an increase in exotic genetic proportion within a breed combination tends to reduce conception rates, while a higher proportion of indigenous genes appear to improve them. Similar findings have been reported elsewhere, with indigenous and non-descriptive heifer breeds showing superior first-service conception rates (FSCR) compared to exotic breeds [36, 37]. For example, researchers recorded FSCRs of 45.68% and 47.58% in indigenous and non-descriptive breeds, while Friesian and Jersey crosses exhibited slightly lower rates of 42.82% and 44.62%, respectively [36]. Higher conception rates of 49.17% and 45.96% for indigenous and non-descriptive breeds have been reported, compared to 41.36% and 43.59% in Friesian and Jersey breeds [37]. Other researcher also observed higher conception rates in native breeds (64%) relative to Friesian (57%) and Sahiwal crosses (53%) [38].

Environmental and management factors can significantly influence fertility, as poorly managed heifers often exhibit unacceptably low conception rates and animals with poor growth require more services to conceive [11, 44].

5. Conclusion

The findings from the study on reproductive efficiency indicators-NSC and FSC-across crossbred dairy heifers at Mruazi HBU indicate the following:

Breed had a significant effect on both NSC and FSC. FZ (50:50) crossbred heifers demonstrated the best reproductive efficiency, showing the lowest NSC and highest FSC rate (91.2%). In contrast, the backcross of Holstein Friesian $\times$ Sahiwal (FS 75:25) had the lowest FSC rate, indicating reduced conception success at first service. NSC was significantly affected by insemination season, with lower NSC observed during the wet season than the dry season. However, season of insemination had no significant influence on FSC in logistic regression, suggesting limited impact on conception at first service. Semen type was a major determinant of reproductive efficiency. Sexed semen resulted in significantly lower FSC and lower odds of successful first-service conception (OR = 0.37), compared to conventional semen. Chi-square tests confirmed that conventional semen consistently outperformed sexed semen in terms of FSC across all breed groups. Significant variation in NSC was found among

inseminators, with Inseminator 3 achieving the lowest NSC and Inseminator 4 the highest, indicating operator-related differences in insemination success. Although statistically significant differences in NSC were reported across age groups, no consistent trend was established. Cows mated before 18 months had slightly better NSC, but the differences were not statistically significant when compared to other age groups.

6. Recommendations

Based on the study findings, the following recommendations are proposed: FZ (50:50) and FS (50:50) crossbreds be promoted for herd reproductive improvement, as they showed the higher first service conception success and lowest insemination attempts per conception.

Caution should be taken when breeding backcrosses such as FS (75:25) due to their lower reproductive efficiency.

Conventional semen be used where maximizing first service conception is a priority. If sexed semen is used (e.g., for targeted heifer production), it should be accompanied by enhanced reproductive management (e.g., strict heat detection, timing of AI) to counteract its lower success rate.

Consider planning inseminations for the wet season, as it is associated with improved conception outcomes (lower NSC).

Further research should be conducted to understand seasonal influences on hormonal profiles and heat detection efficiency.

Differences in NSC among inseminators highlight the need for refresher training and performance evaluation of AI technicians to ensure consistent insemination practices and optimal results.

While results were inconclusive regarding optimal age for insemination, it is advisable to target 18–24 months as a practical age window, with close monitoring of heifer maturity and readiness to breed.

Additional studies with larger sample sizes, especially for sexed semen, are needed to validate these findings and support decision-making in AI protocols; and, to investigate hormonal, nutritional and environmental factors that may interact with semen type and breed to influence conception outcomes.

Abbreviations

AFS	Age at First Service
FSC	First Service Conception
FSCR	First Service Conception Rate
NSC	Number of Services per Conception
FAO	Food and Agriculture Organization of the United Nations
URT	United Republic of Tanzania
CR	Conception Rate
HBU	Heifer Breeding Unit
AI	Artificial Insemination

FS	Holstein Friesian x Sahiwal
FZ	Holstein Friesian x Zebu
FG	Holstein Friesian x Gyr

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Author Contributions

Wilirk Paul Mmeku: Conceptualization, resources, data collection and writing the original draft

Athumani Shabani Nguluma: Data Analysis and supervision

Said Hemed Mbaga: Supervision

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Data Availability Statement

The data is available from the corresponding author upon reasonable request.

Conflicts of Interest

We certify that there is no conflict of interest with anyone or any organization concerning the resources used in the preparation of this manuscript.

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