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Effectiveness of Sexed Semen for Producing Replacement Heifers: A Controlled Trial in Boran Breed Cattle

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Abstract

A longitudinal study design was used to conduct this trial after selection of the study population from September 2023 to November 2024. The breeding protocol primarily utilized sex-sorted semen or conventional semen randomly in Boran heifers. A total of 85 heifers were inseminated in the study herd, of which 66 were confirmed pregnant, giving an overall pregnancy rate of 77.65% with an average services per conception of 1.95. Heifers with BCS < 3 had a pregnancy rate of 52.17% (12/23), which was significantly lower compared to BCS ≥ 4, which had the highest pregnancy rate of 87.5% (21/24). Conception rate to the first service (48.24%) showed a significant effect (P = 0.028) as compared to consecutive services. Out of a total 56 calves born, 46 (82.14%) were female and 10 (17.86%) were male, resulting in a substantial sex ratio difference in favor of female calves. Further analysis of calves produced using sexed semen (N = 47), 41 calves (87.23%) were female, while only 6 (12.77%) were male, yielding a sex ratio difference of 74.47%. Herd fertility was relatively high, with 75% (66/88) of animals achieving pregnancy, indicating acceptable reproductive capacity at the population level. Among the recorded reproductive disorders, dystocia was identified in 3 cases (4.36%), reflecting a modest level of calving difficulty within the population where only 1(1.79%) still birth occurred in the population. Overall, animal management to improve body condition for the efficiency of artificial insemination with sexed semen would effectively meet the needs of smallholders in producing replacement Heifers.

Introduction

Ethiopia, among many Sub-Sahara African countries, has been engaged in delivering AI service to dairy farmers since long time (Delesa et al., 2014). Studies by (Tesfu et al., 2014) reported that many of the small scale and modern dairy farm owners in different areas of Ethiopia have been complaining about the increment of the probability of male calves born following artificial insemination in addition to its lower efficiency (Gebremedhin et al., 2009). The performance of AI is mainly affected by the heat detection efficiency, effectiveness and efficiency of the inseminator, fertility of the cow, and semen quality (Gebremedhin et al., 2009).

Dairy farmers are engaged in the production of milk, thus preferring female calves and tend to consider male calves as byproducts (Naniwa et al., 2019).The main goal in the use of sexed semen is to produce calves of a specific sex (Razmkabir, 2018). In livestock production, the possibility to modify the sex ratio of the calf can result in a substantial increase in the production in livestock farms (Mora et al., 2010). In this respect, the integration of sex-selected semen into artificial insemination programs has been reported to have positive economic effects, as it increases the proportion of female offspring, which can lead to improved herd genetics and potentially higher profitability (Carvalho et al., 2022; Joezy-Shekalgorabi et al., 2017).

Sexed semen enables farmers to increase the number of available replacement heifers; however, to optimize conception rates and the return on investment, sexed semen is best reserved for use in younger first-service heifers, by more experienced technicians (Healy et al., 2013). Reduced fertility from using

sorted sperm is linked to sperm damage during the sexing process. Possible damage includes tail stretching and Hoechst binding that delays the first cell cycle (Seidel et al., 2012). However, factors such as bull fertility, improper timing and site of insemination, poor confirmation of estrus, and inseminator skill in cows are the major factors influencing the conception rate of sexed semen in dairy cattle (Ali et al., 2024). Increasing sperm numbers only partly compensates for these effects (Seidel Jr, 2014). Despite ongoing improvements, sperm viability and quality still decline during sorting (Razmkabir, 2018)

In Ethiopia, where most cattle are low-yielding indigenous breeds, sexed semen technology supports breed improvement, genetic gains, herd control, profitability, sustainability, and animal welfare (Boneya, 2021). Despite its advantages, the use of sexed semen remains largely confined to commercial dairy herds in developed countries. In developing nations such as Ethiopia, adoption is constrained by low conception rates and high costs (Karakaya et al., 2014). An average pregnancy rates decline to 21%–65% with sexed semen compared to Conventional semen (Correa-Calderón et al., 2020; Healy et al., 2013; Joezy-Shekalgorabi et al., 2017; Oikawa et al., 2019).

Study by (Gebremedhin, 2008) reported that, at the national level, conception rates for first inseminations in Ethiopian dairy cows ranged between 7.14% and 40.23%. Furthermore, several studies have shown that the efficiency of field artificial insemination (AI) in most developing countries averages between 2.5 and 3 inseminations per conception (Gebremedhin, 2008; Teferal, 2013) which is considered an indication of poor fertility and low efficiency requiring urgent improvement (Schuster et al., 2020). Poor fertility in cattle has been identified as one of the major factors limiting their longevity (De Vries & Marcondes, 2020). Research findings suggest that only a small proportion of cows survive beyond the fifth parity due to fertility problems (Schuster et al., 2020; Wathes et al., 2008) resulting in fewer calves born per cow and reducing the number of potential replacement animals within a herd (De Vries & Marcondes, 2020).

The Ethiopian government promotes genetic improvement through increasing farmers' use of crossbreeds program. Currently, limited data are available on the performance of sexed semen in dairy herds in Ethiopia. This study hypothesizes that using sexed semen would satisfy smallholders in generating replacement females. The objective of this control trail study was to evaluate sex ratio, conception rate to sexed and conventional semen in Boran heifers, and to determine key reproductive performance parameters under the study.

Study Methodology

Study area

This study investigated conception rates and sex ratio outcomes achieved with artificial insemination (AI) of Boran heifers using sexed and conventional semen of Holstein Friesian bulls in Holeta Livestock Development Center from September 2023 to November 2024. The study area is Holeta town administration. Holeta town is found in central Ethiopia located at 30 km from Addis Ababa on the way

to Ambo town. Holeta town administration is potential dairy area where different federal government organizations working on agricultural researches are found. Holeta is a town in the Central Oromia Region, Ethiopia. It has a latitude and longitude of 9°3'N 38°30'E and an altitude of 2391 meters above sea level. It experiences a bimodal rainfall pattern, with the main rainy season and a short rainy season.

Management of Study animals

Indigenous Boran heifers were utilized in the present study. All selected animals were confirmed to be cyclic and exhibited a body condition score (BCS) ranging from 2 to 4 on a five-point scale, where 1 denotes emaciation and 5 denotes obesity. The heifers were managed as a single experimental cohort and maintained under a semi-open housing system. Uniform management conditions were ensured for all animals, with unrestricted access to clean drinking water provided throughout the experimental period. Prophylactic health management was rigorously implemented, including routine deworming against prevalent parasitic infections and systematic vaccination against major infectious diseases such as Lumpy Skin Disease (LSD), Foot-and-Mouth Disease (FMD), and Pasteurellosis, in addition to other regionally significant pathogens.

A total of 85 Boran heifers were selected from Holeta Livestock development Center herd based on the presence of regular estrous cycles. Their reproductive soundness was further verified through rectal palpation to exclude any anatomical or physiological abnormalities. All animals were maintained under standardized feeding and reproductive management protocols. The heifers were housed in tie-stall systems and provided with regular exercise in a designated paddock. Nutritional management consisted of a mixed feeding regimen, with hay serving as the basal diet and supplemented by a formulated concentrate mixture. The concentrate ration comprised maize, wheat bran, wheat middling, noug cake, and a mineral premix to ensure adequate micronutrient supply. Comprehensive records were systematically maintained for each animal, including identification details, breed, date of birth, insemination history, pregnancy diagnosis outcomes, and calving data.

Breeding protocols

A longitudinal study design was used to conduct this trial after selection of the study population. Artificial insemination (AI) was performed once daily following the detection of estrus in the study group of herd. Initial and subsequent services were conducted upon the observation of natural standing estrus. Estrus was identified through visual observation of clinical signs, including restlessness, vulvar hyperaemia, mounting behavior, standing to be mounted, and vaginal mucus discharge. Animals were allocated for insemination according to the AM/PM rule. Prior to each procedure, sample from each batch of sperm motility was evaluated to ensure viability. The breeding protocol primarily utilized sex-sorted semen or conventional semen randomly. Insemination was administered into either the uterine body or uterine horns via random allocation.

Pregnancy Diagnosis

Pregnancy was diagnosed via transrectal palpation between 40 and 50 days post-insemination. Detection of an embryonic vesicle fetal slippery memebrane, presence of CL and amniotic fluid was used as a positive indicator of pregnancy. Heifers identified as non-pregnant were re-inseminated upon the next observed estrus. In instances where previously confirmed pregnant heifers exhibited recurrent estrus, a secondary palpation was performed; if found non-pregnant, the case was recorded as an embryonic or fetal loss.

Data analysis

All collected data was managed in Microsoft excel sheet. Statistical analysis were performed using STATA-12 software for the data recorded on Heifers information and response to treatment with respect to pregnancy/conception. The t-test was used to compute gestation length and Birth weight of data. The effect of factors on conception rate was seen using binary logistic regression (OR) by considering $P < 0.05$ as statistically significant value.

Results

A total of 85 heifers were inseminated in the study herd, of which 66 were confirmed pregnant, giving an overall pregnancy rate of 77.65% with anaverage services per conception of 1.95. Pregnancy rates varied across different body condition scores. Heifers with BCS < 3 had a pregnancy rate of 52.17% (12/23), which was significantly lower compared to the reference category (BCS ≥ 4), which had the highest pregnancy rate of 87.5% (21/24). In contrast, age did not have a statistically significant influence on pregnancy outcomes, although younger heifers (< 3 years) tended to have higher pregnancy rates (Table 1).

Table 1
Proportion of pregnant heifers in the study herd

Factors	No. Inseminated	No. Positive	Percentage (%)	OR	P-Value	95%CI
BCS						
< 3	23	12	52.17	.110	.010	.020-.595
3–4	38	33	86.84	.736	.721	.137-3.942
>=4	24	21	87.5		-	
Total	85	66	77.65			
Age/year						
< 3	13	12	92.31	2.866	.387	.264-1.139
3–4	33	23	69.70	.521	.339	.137-1.985
> 4	39	31	79.49			
Overall pregnancy	85	66	77.65			

Table 1: **Proportion of pregnant heifers in the study herd**

The study assessed conception rates across different artificial insemination service protocols, techniques, semen deposition sites, and semen types that yielding an overall conception rate of 51.16%. Overall, only the first service (48.24%) showed a significant effect (P = 0.028) on conception, while other factors had no statistically significant influence (Table 2).

Table 2
Conception rate across different protocols of service

Services	No. served animal	No. positive	Percentage (%)	OR	P-Value	95%CI
1st service	85	41	48.24	10.378	.028	1.288–2.620
2nd service	30	22	73.33	4.116	.186	.506 – 1.480
3rd service	7	3	42.85			
Overall AITs efficiency						
AIT-1	77	44	57.14	1.167	.776	.403-3.379
AIT-2	52	22	42.30			
Site of semen deposition						
Bilateral uterine horn (BUH)	74	41	55.41	.897	.841	.313-2.577
Body of Uterus (BU)	58	25	43.10			
Overall	129	66	51.16			
Type of semen used						
Sexed	114	57	50	.686	.682	.112-4.178
Conventional	15	9	60			
Overall	129	66	51.16			

Table 2: **Conception rate across different protocols of service**

The study presents the newborn sex ratio and associated characteristics of calves obtained in the study population. Out of a total of 56 calves, 46 (82.14%) were female and 10 (17.86%) were male, resulting in a substantial sex ratio difference of 64.29% in favor of female calves. Further analysis of calves produced using sexed semen (N = 47) revealed an even greater skew toward female offspring. Among these, 41 calves (87.23%) were female, while only 6 (12.77%) were male, yielding a sex ratio difference of 74.47%. Of 56 newborn crossbreed calves, an average birth weight and gestation length was 23.8 ± 2.2 and 277.3 ± 4 respectively (Table 3).

Table 3
Newborn Sex ratio and other Characteristics

Over all Sex ratio (N = 56)	No. of calf	Percentage /Mean
Female	46	82.14
Male	10	17.86
Sex ratio difference	-	64.29
Sex ratio among sexed semen (N = 47)		
Female	41	87.23
Male	6	12.77
Sex ratio difference	-	74.47
Gestation length and Birth weight		
Average birth weight	56	23.8 ± 2.2
Average gestation length	56	277.3 ± 4

Table 3: **Newborn Sex ratio and other Characteristics**

The reproductive performance parameters presented in Table 4 indicate a moderate level of breeding efficiency within the herd. A total of 129 services resulted in 66 conceptions, yielding a number of services per conception (NSPC) of 1.95. The non-return rate (NRR) was 48.24% (41/85), indicating that less than half of the inseminated animals failed to return to estrus. Herd fertility was relatively high, with 75% (66/88) of animals achieving pregnancy. The annual calving rate was 65.88% (56/85) (Table 4).

Table 4
Reproductive Performance parameters

Parameter	No services	No conceived	Percentage (%)
NSPC	129	66	1.95
NRR	85	41	48.24
Conception Rate	129	66	51.16
Total Fertility of herd	88	66	75.00
Calving rate/year	85	56	65.88
Pregnancy Loss Rate	66	1	1.75

Table 4: **Reproductive Performance parameters**

The incidence of reproductive performance parameters among the studied population (n = 56) revealed that anterior fetal presentation was overwhelmingly predominant, occurring in 52 cases (92.86%), indicating that most parturitions followed the normal orientation of the fetus. In contrast, posterior presentation was observed in 4 cases (7.14%). Among the recorded reproductive disorders, dystocia was identified in 3 cases (4.36%), reflecting a modest level of calving difficulty within the population (Table 5).

Table 5
Incidence of reproductive problem (n = 56)

Incidence type	Rate of occurrence	Percentage
Anterior presentation	52	92.86
Posterior presentation	4	7.14
Dystocia	3	4.36
Stillbirth	1	1.79
Abortion	1	1.79
Calf Death	1	1.79

Table 5: **Incidence of reproductive problem (n = 56)**

Discussion

A total of 85 heifers were inseminated in the study herd, of which 66 were confirmed pregnant, giving an overall pregnancy rate of 77.65% with an average NSPC of 1.95. The lower efficiency of AI were reported by different researchers in different parts of Ethiopia 72.9% (Befkadu et al., 2019), 29% (Yehalaw et al., 2018) an higher report of 86% (Jemal et al., 2016). According to (Müller-Sepúlveda et al., 2020), number of cows in the herd, experience of the inseminators and type of insemination and semen used affects the success of the pregnancy. The success of AI is also influenced by endometrial thickness, artifcial insemination timing, insemination frequency, and ovarian stimulation protocols (Wang et al., 2021).

Conception at first service (48.24%) was significantly associated with pregnancy outcome of second services (73.33%). Higher report of first service conception rate of 60.4% (Belay et al., 2016) in Ethiopia and 62.86% (Sharifuzzaman et al., 2015) in Bangladesh were documented. Variation in conception rates across studies is primarily attributed to management and technical factors. Key determinants include the skill of the inseminator, semen quality and accuracy of heat detection, reproductive health status of cows/heifers, site of semen deposition and timing of insemination (Belay et al., 2016; Mollal, 2011).

Pregnancy rates varied across different body condition scores. Heifers with BCS < 3 had a pregnancy rate of 52.17% (12/23), which was significantly lower compared to the reference category (BCS ≥ 4), which had the highest pregnancy rate of 87.5% (21/24). In contrast to the current study, BCS did not influence the pregnancy rate as reported by (Endebu et al., 2025)

A slightly higher pregnancy rate when conventional semen used than when sexed semen was reported in this study. This was further confirmed by the study of (Endebu et al., 2025) as the pregnancy rate was significantly higher in using conventional semen (72.9%) than in sexed semen (47.9%). As a result, the average pregnancy rates are reduced to 21% in sexed semen as compared to conventional semen (Correa-Calderón et al., 2020; Joezy-Shekalgorabi et al., 2017; Oikawa et al., 2019). This could be due to fewer numbers of sorted sperm per straw (Frijters et al., 2009), damage followed by process of sperm sorting (Garner, 2006; Karakaya et al., 2014), breeding season and estrus detection type, heifer age at breeding (Oikawa et al., 2019), and AI technician efficiency (Correa-Calderón et al., 2020) are also affecting the pregnancy rate using sexed semen.

Conception rates did not differ significantly by semen deposition site (BUH: 55.41% vs BU: 43.10%). Higher pregnancy rate 66.3% using bilateral deep intracorneal insemination was reported (Kirks, 2020), The differences observed in the current and previous works could be due to selection criteria used to select the most fertile cows, dam parity and season when AI was performed, semen dosage, insemination techniques used, and overall management system (Endebu et al., 2025).

The study presents the newborn sex ratio and associated characteristics of calves obtained in the study population. Out of a total of 56 calves, 46 (82.14%) were female and 10 (17.86%) were male, resulting in a substantial sex ratio difference of 64.29% in favor of female calves. Further analysis of calves produced using sexed semen (N = 47) revealed an even greater skew toward female offspring. Among these, 41 calves (87.23%) were female, while only 6 (12.77%) were male, yielding a sex ratio difference of 74.47%. Previous research by Tesfu et al. (2014) utilized surveys, observations, and secondary data to gather information. Their findings indicated a 55:45 female-to-male sex ratio among calves; however, this difference was only numerical and lacked statistical significance ($P > 0.05$). Probability and evolutionary equilibrium theories indicate that the secondary sex ratio, which is defined as the ratio of male-to-female offspring at birth, should be 50:50 (Roche et al., 2006). (Berry & Cromie, 2007) reported that artificial insemination (AI) increases the probability of male calves born in beef cattle. On the contrary, (Bekele, 2005) observed that AI did not significantly affect calf sex ratio. The present study pronounced bias toward female births demonstrates the effectiveness of sexed semen technology in increasing the proportion of female calves. This has important implications for herd management, particularly in dairy production systems where female calves are economically more valuable for replacement and milk production purposes.

The reproductive performance parameters in this study indicated a moderate level of breeding efficiency within the herd. A total of 129 services resulted in 66 conceptions, yielding a number of services per conception (NSPC) of 1.95, which is slightly in the optimal range and suggests good efficiency in service utilization while using sexed semen. The non-return rate (NRR) was 48.24% (41/85), indicating that less than half of the inseminated animals failed to return to estrus, a value that may reflect suboptimal estrus detection, insemination timing, or fertilization success. The overall conception rate was 51.16%, demonstrating moderate success of insemination practices. Herd fertility was relatively high, with 75.00% (66/88) of animals achieving pregnancy, indicating acceptable reproductive capacity at the

population level. The annual calving rate was 65.88% (56/85), suggesting that approximately two-thirds of eligible animals produced calves, which may be influenced by conception efficiency and reproductive management practices. Notably, the pregnancy loss rate was very low at 1.75% (1/66), indicating effective maintenance of established pregnancies. Collectively, these findings suggest that while pregnancy retention and overall fertility are satisfactory, improvements in breeding management could enhance conception efficiency and non-return rates.

The incidence of reproductive problems among the studied population (n = 56) revealed that anterior fetal presentation was overwhelmingly predominant, occurring in 52 cases (92.86%), indicating that most parturitions followed the normal orientation of the fetus. In contrast, posterior presentation was observed in only 4 cases (7.14%). Among the recorded reproductive disorders, dystocia was identified in 3 cases (4.36%), reflecting a modest level of calving difficulty within the population. Other adverse reproductive outcomes were comparatively rare: stillbirth, abortion, and calf death were each reported in 1 case (1.79%), indicating low but notable incidences of reproductive loss. Overall, the findings suggest that while normal anterior presentation predominates, a small proportion of cases involve complications such as dystocia and fetal loss, which may have implications for herd reproductive efficiency and require targeted management interventions.

Conclusion

Analysis of the newborn produced using sexed semen showed a substantial sex ratio difference in favor of female calves. Herd fertility remained relatively high, indicating an acceptable reproductive capacity at the population level. Among the recorded reproductive disorders, dystocia was identified in only three cases, reflecting a modest level of calving difficulty, with only one stillbirth recorded. Overall, maintaining good animal management to improve body condition for the efficiency of artificial insemination with sexed semen would effectively meet the needs of smallholders in generating replacement females.

Declarations

Ethical approval:

This research followed animal experimental ethics as approved by reference number of hldc00123.

Competing Interests:

No competing Interest

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Author contributions:

AD: conceptualization; Design, analysis; writing original draft and editing; DW: Data collection and organization, writing review and editing; GF: Conceptualization, Methodology, Supervision, HM: Conceptualization; investigation; GA: Data collection and organization, GT: Data collection and investigation.

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Data availability:

The data will be available upon request following publication of this manuscript

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Figures



Figure 1

Boran Breed Dams and Crossbreed Replacement Heifers Produced Using Sexed Semen in the Study