Zoological and Entomological Letters

E-ISSN: 2788-8428
P-ISSN: 2788-8436
ZEL 2024; 4(1): 08-13
Received: 12-10-2023
Accepted: 15-11-2023

Sayid Ali
National Animal Biotechnology Research Program, Debre Zeit Agricultural Research Center, Ethiopian Institute of Agricultural Research, Bishoftu, Ethiopia

Tamrat Degefa
National Animal Biotechnology Research Program, Debre Zeit Agricultural Research Center, Ethiopian Institute of Agricultural Research, Bishoftu, Ethiopia

Asnaku Funga
National Animal Biotechnology Research Program, Debre Zeit Agricultural Research Center, Ethiopian Institute of Agricultural Research, Bishoftu, Ethiopia

Mosisa Dire
National Animal Biotechnology Research Program, Debre Zeit Agricultural Research Center, Ethiopian Institute of Agricultural Research, Bishoftu, Ethiopia

Ayda Mohamed
National Animal Biotechnology Research Program, Debre Zeit Agricultural Research Center, Ethiopian Institute of Agricultural Research, Bishoftu, Ethiopia

Asmarech Yeshaneh
National Animal Biotechnology Research Program, Debre Zeit Agricultural Research Center, Ethiopian Institute of Agricultural Research, Bishoftu, Ethiopia

Correspondence
Sayid Ali
National Animal Biotechnology Research Program, Debre Zeit Agricultural Research Center, Ethiopian Institute of Agricultural Research, Bishoftu, Ethiopia

Reproductive biotechnology tools as a game changer for the dairy sector: The case of Sexed Semen and estrus synchronization to produce Seedstock dairy heifers

Sayid Ali, Tamrat Degefa, Asnaku Funga, Mosisa Dire, Ayda Mohamed and Asmarech Yeshaneh

Abstract
For thousands of years, livestock owners wanted to find a suitable way to predict the sex of the progeny to be born for their herds on their farm. Thanks to the development of semen sexing technology, animal breeding has undergone a revolution, which allows farmers to control the sex of their offspring. Sexed semen enhances overall productivity and also assists farmers in meeting the rising demand for high-quality dairy animals by enabling them to selectively generate seedstock female calves. The technology is a one-stop solution to enhance breed improvement especially in a country like Ethiopia where 97 percent of the cattle population is indigenous, which is not selected for milk production and results in undernourishment for animal source origin diet, including milk and meat. The benefits of using sexed semen include sex selection, improved genetic selection, enhanced breeding effectiveness, better control over herd dynamics, greater financial gains, environmental sustainability, and improved animal welfare. Hence, with the finding of this study, an overall result of a 98% response rate 95% female skewness, and 79% conception rate was recorded after being inseminated with sexed semen. The finding of the response rate is closer to the results reported by another researcher, while the average conception rate is comparatively higher. This could be due to careful animal selection, the use of fertile quality semen, proper heat detection, and inseminating at an optimum window of time. In general, from the piece of this study, it is possible to foresee and conclude that the adoption and application of sexed semen technology is a groundbreaking and game-changer technology for the dairy industry in a country like Ethiopia, which overcomes the limitations of improved crossbred dairy cattle, replacement heifers, and high milk prices on the one hand, a shortage of grazing land, and environmental destruction due to a decreasing number of unwanted cattle population on the other side.

Keywords: Dairy industry, game changer, seedstock dairy heifers, sexed semen

Introduction

Human curiosity about the sexual orientation of living things has a long history. For thousands of years, livestock owners wanted to find a suitable way to predict the sex of the progeny to be born for their herds on their farm. This dates back to the early Greeks, specifically Democritus (470-402 BC), who believed that the right testis produces males while the left testis produces females.

However, the advancement in the biological sciences, particularly genetics, led to many discoveries during the first half of the 20th century, one of which was the identification of the sex chromosomes. Accordingly, improvement in livestock productivity could be achieved through selective breeding and control of production (Stear *et al.*, 2001) [26]. Artificial insemination (AI), the oldest and most effective reproductive technology with estrous synchronization, is a regular practice on large-scale animal farms, and desired-sex animals are in high demand in such animal forms (Robertson, 1954) [21]. Dairy animal keepers demand female cattle, whereas male calves are required for meat products for the beef sector. The ideal method for sex control in farm animals is the separation of "X" and "Y" chromosome-bearing live sperm cells from the natural mixture of semen samples.

Even though numerous traditional methods of sperm sorting have been developed, A recently developed flow cytometry technique increases the potential of commercially available sex-sorted semen. Normally, while using conventional semen, on average over thousands of animals, 49% of calves born will be heifers, and a few will be sterile freemartins (Seidel Jr., 1999) [23]. Currently, the supply of replacement heifers on dairy farms is a major issue in commercial ventures.

Sex-sorted bovine semen became commercially available in the early 2000 s. The application of sexed semen technology has gained importance in the bovine (beef and dairy) industry due to the high demand for milk and meat by the growing population of the world (Rath *et al.*, 2009) [19]. This technology is used to reduce animal-derived food scarcity on the one hand and to improve the economy of animal farmers on the other (Garner & Seidel Jr., 2008; Rath & Johnson, 2008; Holden & Butler, 2018) [9, 18, 11]. On the other hand, sexed semen technology is important in reducing the number of animals with unwanted sex in the beef or dairy sector, and this will greatly reduce the maintenance cost of the animal farm (Seidel Jr. 2007) [24].
The flow cytometric approach of the sperm cell sorting technique is sorting the X chromosome-bearing sperm and Y chromosome-bearing sperm based on differences in their DNA content (Weigel, 2004; Seidel Jr., 2007) [29, 24]. The differences in DNA content in domestic animals between X and Y chromosome-bearing spermatozoa range from 3% to 4.5% (Johnson, 2000) [12], and while using a fluorescent dye that binds to DNA, male and female cattle sperm that carry different chromosomes can be electrically charged. This makes it possible for a fluorescence-activated cell sorter to separate them (Seidel Jr., 2007) [24]. About 90% of the sperm contain the desired sex, indicating that the procedure is reasonably accurate (Garner and Seidel, 2003 [8]; DeJarnette *et al.*, 2008) [4].
In Ethiopia, where 97% of the total cattle population is indigenous and is not selected for milk production, the introduction of assisted reproductive technologies like sexed semen and estrus synchronization technologies will be considered a game changer for the sector. Therefore, keeping all facts into consideration, the objectives of this research work are to assess the efficacy of the CIDR-based estrus synchronization technique and ascertain the potential of sexed semen technology in producing seedstock heifers for the dairy sector.

Materials and Methods

Description of Study Area: This study was carried out from August 2020 to November 2023. The experiment was conducted both at a research station, the Debre Zeit Agricultural Research Centre (DZARC), with the National Animal Biotechnology Research Program of the Ethiopian Institute of Agricultural Research (EIAR), and at a private dairy farm found in Bishoftu City, Dukem, Bishoftu and Oda Bultum University located in Oromia regional state, is located about 45 km east of Addis Ababa, the capital city of Ethiopia (8°46′13.57"N, 38°59′50.45"E) at an altitude of 1920 M.A.S.L.

Experimental animals: Indigenous Boran breed and high-grade Boran crossed Holstein Friesian heifers and cows were used for this study. All selected animals are cycling and had a body condition score (BCS) of 3- 8 on a scale of 1 to 9; (when 1=emaciated; 9 =obese). All the experimental animals were maintained as a group and were housed in a semi-opened housing system. Experimental animals were provided with a feed of different mixes: tef (*Eragrostis tef*) straw and grass (*Andropogon abyssinicus*) hay as a basal diet and supplemented with commercially prepared concentrate, mineral salts, and alfalfa green fodder. Management of these animals was nearly similar and sometimes DZARC experimental animals were released extensively for free grazing. Water was provided ad libitum. The experimental animals were regularly dewormed against a common parasitic disease and vaccinated for lumpy skin disease (LSD), foot and mouth disease (FMD), and other common infections.

Experimental Protocol: In the first trial, from the research center, a total of 104 animals were gynecologically examined with transrectal palpation for the presence of a well-developed and functional corpus luteum (CL) in either ovary, from the research center, university, and private farms. A total of 80 animals were technically selected and Control Intravaginal Drug Release (CIDR) devices were implanted for seven days for synchronization. A Zoetis product, luteolytic hormone (Lutalyse® injection dinoprost tromethamine), was injected deep intramuscularly on day six, post-CIDR implantation. Crayon livestock paint and ESTROTECT, a red or orange fluorescent color-coated heat detector, were applied on day seven during CIDR removal on the halfway point between the hip and tail heads perpendicular to the spine after brushing the hair thoroughly to create an optimal condition for adhesion. Hence, with each mount, the surface will gradually turn from silver to its indicator color, indicating true standing heat. As per the manufacturer's recommendation, approximately 50% of the silver rub-off coat removed should indicate standing heat. Sexed semen from ABS Global was used for insemination. Since sexed semen remains fertile for a shorter period in the female reproductive tract following insemination, insemination was conducted approximately 18 to 24 hours (Thomas, J., 2021) after the start of that female's standing heat behavior (Figure 1). In the second trial, a total of 40 heifers born from the first batch at the research center and private farm were gynecologically examined with transrectal palpation for the presence of a well-developed and functional corpus luteum (CL) in either ovary, and 35 heifers were technically selected. Control Intravaginal Drug Release (CIDR) devices were implanted for seven days for synchronization (table 2).

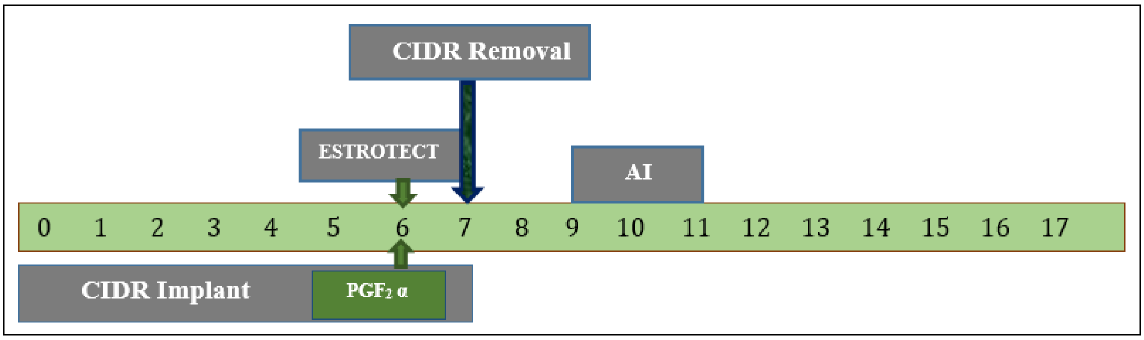

Fig 1: Synchronization and artificial insemination protocol

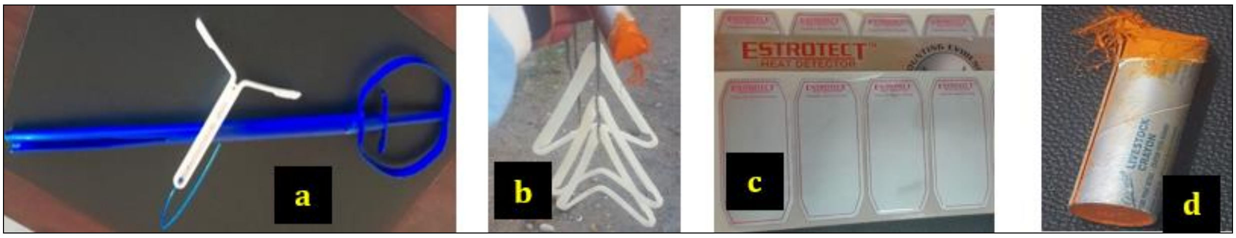

Fig 2: a & b) CIDR and its applicator and c) ESTROTECT d) Crayon livestock paint for heat detection

Fig 3: First batch synchronized animals manifesting behavioral estrus sign

Results

During the first shoot of this study, in the research center, governmental university, and on a private farm, a total, of 104 animals were gynecologically examined, and screened animals were selected and synchronized. From the synchronized animals, those responded animals were inseminated using sex-sorted semen. The breed, conception rate, sex of newborn calves, or gender skewness at all farms were recorded. The overall response rate, conception rate, and sex skewness was 98%, 79%, and 95% respectively.

Table 1: Estrus response, conception rate, and calve sex ratio in cattle inseminated with sexed semen at the research center's private dairy farm and universities.

Farm category		Gynecologically examined animals	Synchronized (CIDR) implanted animals	Animal responded	Inseminated animals	Pregnant animal	Abortion and calve mortality encountered	Female Skewness (%)
Research center	HF	31	25	24	24	20	2	100% (18/18)
	B	7	5	5	5	3		0 67% (2/3)
Private farm	HF	31	26	26	25	22	0	91% (20/22)
University	HF	35	24	23	23	18	0	100% (18/18)
Total		104	80	78 (98%)	77	61(79)	2	95% (58/61)

HF: Holstein Friesian breed; B=Boran Breed

Fig 4: Calves born on research station and private dairy farm using sexed semen (F1) at DZARC.

During the second phase of the experiment, heifers born from the first batch were synchronized once they reached puberty and inseminated using sexed semen following the same procedure as in the first phase experiment and given female calve crop (Figure 5a and b).

Table 2: Estrus response, conception rate, and calve sex ratio in heifers born from sexed semen and inseminated with sexed semen at the research center, private dairy farm, and universities

Farm category		Gynecologically examined animals	Synchronized (CIDR) implanted animals	Animal responded	Inseminated animals	Pregnancy rate	Abortion and calve mortality encountered	Female skewness (%)
Research center	Holstein Friesian	18	18	18	18	(18/18)100%	3	100% (18/18)
	Boran	7	5	4	4	(3/4) 75%	0	67% (2/3)
Private Farm	Holstein Friesian	15	12	11	11	(10/11) 91%	-	-
		40	35	33 (94%)	33	(30/33) 91%		-

Fig 5 a: Heifers born from sexed semen give female calve crops (seedstock) after estrus synchronized and inseminated with sexed semen (F2)

Fig 5b: Collective figure for female calves born from whose mothers were born with sexed semen (F2) at DZARC.

Discussions

In a country like Ethiopia, where about 97% of the total cattle population is indigenous and is not selected for milk production due to low milk yielders which is below 1.5 liters per day, producing crossbred heifers through sexed semen technology is not a choice rather mandatory.

In several large-scale studies with the use of sexed semen in cows and heifers as well as within heifers in dairy and beef heifers, the conception rates are different. Rhinehart and co-workers (2011) reported a 4% to 38% reduction in pregnancy rates when using sexed semen compared to conventional semen in beef heifers. Similarly, Meyer *et al.*, (2012) [15] reported that the pregnancy rate of heifers inseminated with sexed semen resulted in a 17% decrease in pregnancy rates compared to heifers inseminated with conventional semen. This is mainly due to two main reasons, these are, 1). Due to the effects of the sorting procedure, and 2). Low number of sperms in a standard dose of sorted semen is responsible for the reduction in fertility. According to (Frijters *et al.* 2009) a low number of spermatozoa accounts for two-thirds of the reduction in the pregnancy rate whereas damage caused by the sorting accounts for one-third.

Regarding the finding of this study, In the first trial, of 104 gynecologically examined and screened animals from research centers, private farmers, and universities, 80 animals were selected and synchronized with the CIDR plus prostaglandin-based synchronization protocol. An overall result of a 98% response rate 95% female skewness and 79% conception rate was recorded after being inseminated with sexed semen. The finding of the response rate is closer to the results reported by Dhami, *et al.*, 2015 [5]; Kumaravel and Sendur Kumaran, 2017 [14], which is about a 100% response rate but in contrast to other reports like Chebel *et al.*, 2006 [2], Kalwar Q *et al.*, 2015 [13] where 30 and 76.47% estrus expressions were reported respectively. With regards to female skewness, the current result is higher compared to Nishant Sharma *et al.* (2018) [18], findings that are about 82.14% of female calves, and De Jarnette *et al.* (2009) [3], Borchersen and Peacock (2009) [1], Norman *et al.* (2010) [17], Healy *et al.* (2013) [10], Djedovic *et al.* (2016) [6], and Shekalgorabi *et al.* (2017) [25], where 85-90% of desired sex calves were born by sexed semen.

The average conception rate of sexed semen was found to be 79%, which is comparatively higher than many previous reports, which stated that the fertility of sexed semen reached about 75-85% of the unsexed semen (Garner and Seidel, 2003 [8]; DeJarnette *et al.*, 2009 [3]; Borchersen and Peacock, 2009) [1] and some trials of Seidel *et al.* (1999) [23]. This could be due to the cows being fertile, which are selected purposefully, the semen being fertile, which is obtained from ABS Global, proper heat detection using herd attendants and ESTROTECT heat detectors, and researcher-based inseminations being conducted at the optimum window of time.

For the second trial, about 40 heifers born from the first batch of sexed semen insemination at the research center, private farm, and university (Figure 4) were gynecologically examined and 35 were synchronized and inseminated with sexed semen, resulting in a 94% response rate (table2), which is similar to the result of the first trial.

The main application of sex-sorted semen is producing a potential dairy heifer (seedstock) for future quality and highly productive herd replacement. Beyond this, there are several advantages to using sexed semen in the dairying system. 1st the fertility of heifers is superior to that of lactating cows. 2nd the ease of calving smaller and lighter female calves and thereby the occurrence of less dystocia compared with giving birth to male calves (Tubman *et al.* 2004). 3rd steadily producing the replacements from heifers, shortens the generation interval. 4th, herd expansion and the sale of heifers to others. 5th Economic aspect (raising replacement heifers at a lower cost than purchasing them). 6th building known genetics and 7th biosecurity issues (including managing Johne's disease).

Since the best genes in a herd are among the youngest animals, the genetic progress is accelerated when they are used to produce the next generation. Furthermore, one interesting advantage of using sexed semen and also realized in this particular study is a heifer born from sexed semen gives a heifer through sexed semen insemination which has created a stir in the dairy industry.

Conclusion

The dairy industry is ever-changing as technology alters management practices. At present, purebred and commercial seedstock producers will receive the most benefit from the use of sexed semen. In addition, the use of sexed semen by commercial producers to generate replacement heifers or to breed replacement heifers is a viable option. In this article, scenarios for using sexed semen have been critically evaluated.

In general, from the piece of this study, it is possible to foresee the adoption and application of sexed semen technology as a groundbreaking and game-changer technology for the dairy industry in a country like Ethiopia where there is an insignificant number of improved crossbred dairy cattle, which results in skyrocketing prices for replacement heifers, high milk prices, and the shortage of heifers for replacements. Furthermore, rapid population growth, and urbanization, result in a shrinkage of grazing land, and environmental destruction due to the greater number versus low productive cattle population.

In conclusion, sex-sorted semen has revolutionized animal breeding by providing farmers with the desired sex of calve offspring, better control over herd dynamics, and greater financial gains. Overall, semen sexing offers tremendous potential for genetic advancement, improved herd management, and meeting the demands of the improved livestock potential specifically and agricultural industry at large.

References

1. Borchersen S, Peacock M. Danish AI. Field data with sexed semen. Theriogenology. 2009;71:59-63.
2. Chebel RC, Santos JEP, Cerri RLA, Rutigliano HM, Bruno RGS. Reproduction in dairy cows following progesterone insert pre-synchronization and resynchronization protocols. J Dairy Sci. 2006;89:4205-4219.
3. De Jarnette JM, Nebel RL, Marshall CE. Evaluating the success of sex-sorted semen in US dairy herds from on-farm records. Theriogenology. 2009;71:49-58.
4. DeJarnette JM, Nebel RL, Marshall CE, Moreno JF, McCleary CR, *et al.* Effect of sex-sorted sperm dosage on conception rates in Holstein heifers and lactating cows. J Dairy Sci. 2008;91:1778-1785.
5. Dhami AJ, Nakrani BB, Hadiya KK, Patel JA, Shah RG. Comparative efficacy of different estrus synchronization protocols on estrus induction response, fertility and plasma progesterone and biochemical profile in crossbred anestrous cows. Vet World. 2015;8(11):1310-1316.
6. Djedovic R, Bogdanovic V, Stanojevic D, Nemes Z, Gaspardy A, Cseh S. Involuntary reduction in vigor of calves born from sexed semen. Acta Veterinaria Hungarica. 2016;64:229-238.
7. Frijters AC, Mullaart E, Roelofs RM, van Hoorne RP, Moreno JF, Moreno O. What affects the fertility of sexed bull semen more, low sperm dosage, or the sorting process? Theriogenology. 2009;71:64-67.
8. Garner DL, Seidel GE. Past, present, and future perspectives on sexing sperm. Can. J Anim. Sci. 2003;83:375-38.
9. Garner DL, Seidel GE Jr. History of commercializing sexed semen for cattle. Theriogenology. 2008;69(7):886-895.
10. Healy AA, House JK, Thomson PC. Artificial insemination field data on the use of sexed and conventional semen in nulliparous Holstein heifers. J Dairy Sci. 2013;96:1905-1914.
11. Holden SA, Butler ST. Applications and benefits of sexed semen in dairy and beef herds. Animal. 2018;12(s1):s97-s103.
12. Johnson LA. Sexing mammalian sperm for production of offspring: The state-of-the-art. Anim. Reprod. Sci. 2000;60:93-107.
13. Kalwar Q, Memon AA, Bhutto Md B, Kunbhar HK, Mirani AH, Anwar Md. Estrus response and fertility rate in Kundhi buffaloes following estrus synchronization in the breeding season. J Adv Vet Anim Res. 2015;2(3):362-365.
14. Kumaravel V, Kumaran SS. Estrus synchronization in crossbred dairy cows under field conditions. Int. J Appl. Pure Sci Agric. 2017;03(8):2394-5532.
15. Meyer TL, Funston RN, McGrann JM. Evaluating Conventional and Sexed Semen in a Commercial Beef Heifer Program. 2012 Nebraska Beef Cattle Report. 2012;pp 20-21.
16. Sharma N, Chand DK, Rawat S, Sharma M, Verma H. Effect of sexed semen on conception rate and sex ratio

under field conditions. J Entomol. Zool. Stud. 2018;6(1):702-705.
17. Norman HD, Hutchison JL, Miller RH. Use of sexed semen and its effect on conception rate, calf sex, dystocia, and stillbirth of Holsteins in the United States. J Dairy Sci. 2010;93:3880-3890.
18. Rath D, Johnson LA. Application and commercialization of flow cytometrically sex-sorted semen. Reprod Domest Anim. 2008;43:338-346.
19. Rath D, Moench-Tegeder G, Taylor U, Johnson LA. Improved quality of sex-sorted sperm: A prerequisite for wider commercial application. Theriogenology. 2009;71(1):22-29.
20. Rhinehart JD, Arnett AM, Anderson LH, Whittier WD, Larson JE, Burris WR. Conception rates of sex-sorted semen in beef heifers and cows. J Anim. Sci. 2011, 89 (Suppl. 2).
21. Robertson A. Artificial insemination and livestock improvement. In Advances in Genetics. 1954;6:451-472.
22. Seidel GE Jr, Schenk JL, Herickhoff LA, Doyle SP, Brink Z, Green RD. Insemination of heifers with sexed sperm. Theriogenology. 1999;52:1407-1420.
23. Seidel GE Jr. Sexing mammalian spermatozoa and embryos - state of the art. J Reprod. Fertil. Suppl. 1999;54:477-48.
24. Seidel GE Jr. Overview of sexing sperm. Theriogenology. 2007;68(3):443-446.
25. Shekalgorabi SJ, Maghsoudi A, Mansourian MR. Reproductive performance of sexed versus conventional semen in Holstein heifers in various semiarid regions of Iran. Ital. J Anim. Sci. 2017;16(4):666-672.
26. Stear MJ, Bishop SC, Mallard BA, Raadsma H. The sustainability, feasibility, and desirability of breeding livestock for disease resistance. Res. Vet. Sci. 2001;71(1):1-7.
27. Thomas J. Sexed Semen for Artificial Insemination: Recommendations and AI approaches, University of Missouri Division of Animal Sciences; c2021.
28. Tubman LM, Brink Z, Suh TK, Seidel GE Jr. Characteristics of calves produced with sperm sexed by flow cytometry/cell sorting. J Anim. Sci. 2004;82:1029-1036.
29. Weigel KA. Exploring the role of sexed semen in dairy production systems. J Dairy Sci. 2004;87(E. Suppl.):E120-130.