Zygote

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Review Article

Cite this article: Kumar S *et al.* (2024) Semen sexing and its impact on fertility and genetic gain in cattle. *Zygote*. **32**: 109–118. doi: 10.1017/S0967199424000066

Received: 19 September 2023
Revised: 3 February 2024
Accepted: 16 February 2024
First published online: 19 March 2024

Keywords:
Fertility; Genetic progress; Pregnancy rate; Sexed semen; Sperm concentration

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Semen sexing and its impact on fertility and genetic gain in cattle

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Summary

Semen sexing is among one of the most remarkable inventions of the past few decades in the field of reproductive biotechnology. The urge to produce offspring of a desired sex has remained since traditional times. Researchers have tried many methods for accurate semen sexing, but only the flow cytometry method has proved to be effective for commercial utilization. However, there were always concerns about the effects of sexed semen, especially on fertility and the rate of genetic gain. Some concerns were genuine because of factors such as low semen dosage in sexed semen straws and damage to sperm during the sorting process. Various researchers have conducted numerous studies to find out the effect of sexed semen on fertility and, in this article, we reflect on their findings. Initially, there were comparatively much lower conception rates (~70% of conventional semen) but, with refinement in technology, this gap is bridging and the use of sexed semen will increase over time. Concerning genetic gain with use of sexed semen, a positive effect on rate of genetic progress with the use of sexed semen has been observed based on various simulation studies, although there has been a mild increase in inbreeding.

Introduction

In the past few decades, the world has witnessed several groundbreaking technological advancements, and reproductive biotechnology is no exception. There have been noteworthy advances in reproductive biotechnology in fields such as embryo transfer, *in vitro* fertilization (IVF), multiple ovulation and embryo transfer (MOET), embryo sexing, and semen sexing. Semen sexing is the process of separating X-bearing sperm from Y-bearing sperm. The main aim of semen sexing is to produce the offspring of the desired sex, which has always been a human craving. The curiosity of men about controlling the sex of progeny is not new and is as old as the time of Hippocrates (Amann, 1989).

Now it is a well established fact that, in mammals, primary sex is determined by a combination of sex chromosomes: XX determines a female, whereas XY combination results in male progeny (Kasimanickam, 2021). Jost (1953) demonstrated that after removal of gonads in rabbit foetuses before sexual differentiation, regardless of XX or XY chromosome, the resulting rabbits had a female phenotype indicating that development of femaleness represents the 'default' state. Wilhelm *et al.* (2007) have reviewed the various molecular and cellular events related to the sex determination and gonadal development in mammals. A gene called *SRY* (sex-determining region of the Y chromosome), on the mammalian Y chromosome triggers differentiation into a male offspring. Many other potential genes such as *SOX9*, *DAX1*, and *WNT4* might play a crucial role in sex determination. In contrast, various hormones viz., testosterone, anti-Müllerian duct hormone (AMH) and oestrogen also play an important role in secondary sex determination (Gilbert, 2000).

However, during earlier times, people were not aware of sex determination. Scientists, particularly Greek philosophers, have proposed various theories about the determination of the sex of offspring. Parmenides believed that the sex of an embryo was determined by its location in the womb: males develop on the right side of the womb, while females develop on the left, whereas Anaxagoras believed it was determined by the father's semen from the right testis developing into a male child, and from the left testis developing into a female child (Mittwoch, 2013). McCartney (1922) also summarized many other such theories. But all these theories were mere assumptions or derived from folklore and lacked any scientific basis. In modern times, the science behind sex determination is pretty much known, but to produce offspring of the desired sex is another aspect that is of great importance to scientists and animal rearers for a profitable industry. Based on various properties of X- and Y-bearing sperm, different methods have been tried by different researchers to separate the two types of sperm to produce offspring of the desired sex and these methods are described briefly in the following section of this paper.

Furthermore, people tended to compare existing technology with the latest one regards pros and cons. The same is the case with the sexed semen technique and, since its inception,

Table 1. Drawbacks of various semen sexing techniques

Technique/Principle	Drawback	References
Albumin gradient (difference in motility)	In domestic animals, this technique does not effectively separate X- and Y-bearing spermatozoa	Seidel and Garner (2002), Jain *et al.* (2011) and the references therein
Free flow electrophoresis (surface charge differences)	Reduced sperm motility and successful separation of sperms of other mammalian species has not been reported	
Gradient swim-down procedure (swimming pattern differences)	Conflicting results	
Immunoassay (surface antigenic differences)	Unconvincing results	
Centrifugal countercurrent distribution	May not be successful in species with lesser difference in DNA content (>4.2%)	
Volumetric differences	Purity cannot exceed 80%	

numerous studies have been conducted on the effect of sexed semen on various aspects for comparison and, therefore, utility. This paper reviews the results of available studies conducted by researchers on various methods of semen sexing and regarding the effect of sexed semen usage on fertility and its probable effects on genetic progression.

Methods of semen sexing

Amann (1989) discussed that offspring of the desired sex can be obtained using two approaches: first, by separating X- and Y-bearing sperm, and second, by altering the function of either of the two types of sperm. From time to time, various scientists have tried different approaches for separation of X- and Y-bearing sperm based on parameters such as mass and motility (albumin gradient and Percoll gradient), swimming patterns, surface charges (free flow electrophoresis), volumetric differences, centrifugal counter current distribution, and immunologically relevant properties, but most of these techniques did not meet expectations and are, therefore, not in commercial use. These methods have been comprehensively reviewed by some scientists (Seidel and Garner, 2002; Jain *et al.*, 2011), and various drawbacks of these techniques, such as conflicting or unconvincing results in addition to reduced sperm motility, were observed, therefore techniques other than flow cytometry did not have that much success. Previously studied techniques along with their drawbacks are listed briefly in Table 1.

Scientists are still trying to find other novel techniques for the separation of X- and Y-bearing sperm. Recently, a new method that does not use traditional sorting technology has been developed to get sperm of the desired gender by destroying the sperm of the undesired gender. In this technology, sperm are stained, and based on DNA content, the unwanted cells are destroyed (Faust *et al.*, 2016). ABS Global (Genus plc) produces Sexcel using their proprietary IntelliGen technology (Thomas, 2021). In this sexing technology, a laser ablation process was used to destroy sperm cells carrying the undesired chromosome. Perry *et al.* (2020) recommended careful use of Sexcel gender-ablated semen among animals that do not exhibit oestrus, while it can successfully be used among animals that exhibit oestrus in fixed-time AI protocols.

In another recent study in Japan, Umehara *et al.* (2019) reported that ~50% of the round spermatids in the testis and a similar percentage of the epididymal sperm expressed Toll-like receptors 7/8 (TLR7/8), coding the X chromosome in mice. Furthermore, ligand activation of Toll-like receptors 7/8 (TLR7/8) in mice suppressed the mobility of X-bearing sperm but not of Y-bearing sperm, and this difference in sperm motility allowed for the separation of X- and Y-bearing sperm. The use of ligand-selected, high-mobility sperm resulted in ~90% male embryos. In another novel approach, Chowdhury *et al.* (2019) used a monoclonal antibody (WholeMom) in cattle to separate the two kinds of sperm, which binds with the plasma membrane of the heads of Y-bearing sperm. The study gave optimistic results with cryopreserved sexed semen, leaving hope of improvement with fresh semen. Soleymani *et al.* (2021) also reported high reactivity of the mouse anti Rb-SRY monoclonal antibody in cattle against Y sperm compared with X sperm. For pigs, new genome editing technologies such as zinc finger nucleases (ZFN), transcription-activator like endonucleases (TALENs), and the CRISPR/Cas system have caught the attention of researchers and offer unlimited prospects regarding sex determination at the genome level and can be used for the investigation of genes such as *SRY* (Kurtz and Petersen, 2019).

But, for the time being, the only commercially viable method is flow cytometry or fluorescence-activated cell sorting (Garner *et al.*, 2013).

Flow cytometric sorting of semen

The basic principle of sexing X and Y chromosome-bearing sperm through flow cytometric technique is based on the DNA content, i.e. X sperm have more DNA compared with Y sperm. Even if this difference is very small, it is possible to measure the DNA content of each sperm with sufficient accuracy to discriminate between X and Y sperm with ~90% precision (Seidel, 2007). Pinkel *et al.* (1982) reported the first flow sorting of sperm with the goal of separating X- from Y-bearing spermatozoa. The sperm sorter can currently sort bull sperm at 6000 live sperm per second for each sex (Johnson, 2000). A fluorescing dye called Hoechst 33342 is used to measure the DNA content of sperm (Seidel, 2007). A specific wavelength of light must be present for this dye to fluoresce. A detector and computer are used to measure and analyze fluorescence. X sperm has ~4% more DNA than Y sperm in cattle and consequently binds to more dye and, therefore, emits ~4% more fluorescence (Seidel, 2007). A flow cytometer comprises a pump that pushes sperm-containing fluid past a fluorescence detector. The system's cell sorting component operates as follows: once the fluid stream leaves the flow cytometer, a vibrator breaks it into tiny droplets at a rate of 70,000–80,000 per second (Seidel, 2007). A positive electrical charge is added to a droplet if the

https://doi.org/10.1017/S0967199424000066 Published online by Cambridge University Press

computer determines that it contains an X sperm; a negative electrical charge is added to a droplet if the computer determines that it contains a Y sperm; and no electrical charge is applied to a droplet if it contains damaged sperm, no sperm, and multiple sperm based on the DNA content (Seidel, 2007). When droplets leave the flow cytometer's nozzle, they move across electric fields. The droplets that have a positive or negative electrical charge move toward the negative or positive part of the field, respectively. Those with no electrical charge continue to fall straight down (Rahman and Pang, 2019). Three streams of droplets are produced and can be collected into three test tubes to separate the X from the Y sperm (Garner *et al.*, 2013).

Applications of sexed semen

In dairy cattle, sexed semen has many applications, such as producing surplus heifer calves for herd replacement or helping more bulls produce progeny, tested by allowing the requisite number of daughters to be produced more rapidly and economically (Hohenboken, 1999). In the beef industry, concentrated Y-bearing sperm can be used to get more efficient male calves (Hohenboken, 1999). There are other advantages, such as a role in efficient livestock production and management systems (Hamano, 2007) and in the conservation of endangered species (Garner, 2006). Kumar *et al.* (2016) have summarized the usage of sexed semen in the replacement and expansion of herds, as a control measure of unproductive stray cattle, in progeny testing, in the production of superior with breeding bulls, in combination with assisted reproductive technologies, and in meeting the increased demand for milk. Other notable applications of sexed semen include improved biosecurity, an increased rate of genetic gain, reduced problem of dystocia, facilitation of faster and more profitable herd expansion, and also increased milk production (Butler *et al.*, 2014). Seidel and DeJarnette (2022) have also suggested future uses of sexed semen for 'The all heifer/no cow system' and 'Selection for increased sexual dimorphism in beef cattle'.

Impact of semen sexing on fertility and genetic gain

Fertility

There were concerns about the effect of using sexed semen on the fertility rate, and these were obvious as the dose of sexed semen is usually lower than conventional semen and, additionally, there remain chances of wear and tear on sperm during the sorting process, resulting in reduced sperm fertility. These two factors account for approximately two-thirds and one-third of sexed semen fertility reduction, respectively (Frijters *et al.*, 2009). Various researchers have conducted research on the effect of using sexed semen on fertility (conception rate/pregnancy rate) by comparing their results in heifers and/or cows. Researchers have tried to compare the effect of sexed semen with conventional semen in their normal concentration (Borchersen and Peacock, 2009; DeJarnette *et al.*, 2010), increased sexed semen concentration (DeJarnette *et al.*, 2010), or equal dosage in both types of semen (Bodmer *et al.*, 2005). Studies to determine whether the use of fresh or frozen sexed semen has different results on conception rates have also been conducted (Maicas *et al.*, 2019). The results of these studies depicting conception rate/pregnancy rate using conventional semen and sexed semen are discussed in detail in the following sections.

Table 2. Comparison of fertility in heifers and cows inseminated with sexed semen and conventional semen having equal sperm concentration

Category of animals	Fertility (%) Conventional semen	Fertility (%) Sexed semen	Semen dosage	References
Heifers	59.3	33.3	2×10^6	Bodmer *et al.* (2005)
Heifers	61.0	62.0	10×10^6	Schenk *et al.* (2009)
Heifers	55.0	38.0	2.1×10^6	DeJarnette *et al.* (2011)
Heifers	60.0	44.0	10×10^6	DeJarnette *et al.* (2011)
Cows	27.6	28.1	2×10^6	Bodmer *et al.* (2005)

Equal sperm concentration

One of the major issues with sexed semen is doubts over fertility due to the sorting process and less numbers of sperm cells per straw. The standard dose for a straw of sexed semen is ~2×10^6 sperm cells (Garner and Seidel, 2008; Healy *et al.*, 2013) while the same for conventional semen is 15–20×10^6 sperm cells. Many scientists have tried to find out whether this factor actually affected the fertility, as some studies (Andersson *et al.*, 2004) reported earlier significantly lower pregnancy rates using a 2×10^6 spermatozoa/dose compared with 15×10^6 spermatozoa/dose (31.3% and 44.9%, respectively) even for conventional semen. So, the number of sperm cells per straw was kept identical for both sexed and conventional semen. The differences in conception rate/pregnancy rate after using sexed and conventional semen straw at equal concentrations is given in Table 2.

In one of the earlier studies, Bodmer *et al.* (2005) in Switzerland evaluated fertility in dairy cattle after low-dose insemination (2×10^6 spermatozoa per straw) with sexed and conventional frozen–thawed semen under field conditions. They reported that the pregnancy rates of cows inseminated with sexed and conventional semen did not differ significantly. However, heifers receiving conventional semen had quite higher pregnancy rates compared with cows, and also heifers, who had been inseminated with sexed semen. Looking at all possible factors, DeJarnette *et al.* (2011) compared the conception rates of Holstein heifers after artificial insemination with 2.1×10^6 or 10×10^6 sperm dosages of sexed or conventional sperm and reported that the sperm concentration did not affect conception rates in ~80% of herds (41/51), perhaps due to technician proficiency, and affected only in ~20% of herds. Conception rates of AI with sex-sorted semen improved after using almost five-fold dosage but were still lower than conventional semen irrespective of dosage. However, they supported the fact that sperm dosage was not the major factor influencing conception rates of sex-sorted semen.

In one study conducted by Schenk *et al.* (2009), exciting results were obtained when pregnancy rates in Holstein heifers inseminated with sexed semen were similar to those of control unsexed group containing similar sperm number per dose (10×10^6). The experiment was conducted with an ideal design. Everything from oestrus detection, thawing to insemination, and pregnancy diagnosis was done quite perfectly by trained professionals, although the data size was smaller. Still the results can be called encouraging, when looking at the corresponding pregnancy rates.

https://doi.org/10.1017/S0967199424000066 Published online by Cambridge University Press

Table 3. Comparison of fertility in heifers and cows inseminated with sexed semen and conventional semen having normal sperm concentration

Category of animals	Conventional semen dosage	Fertility (%) Conventional semen	Sexed semen	Sexed semen dosage	Reference	Fertility indicator
Heifers	–	56.0	45.0	2.1×10^6	DeJarnette *et al.* (2009)	Conception rate
Heifers	15×10^6	61.9	49.3	2×10^6	Borchersen and Peacock (2009)	Conception rate
Heifers	–	56.0	39.0	–	Norman *et al.* (2010)	Conception rate
Heifers	15×10^6	60.7	43.9	2.1×10^6	DeJarnette *et al.* (2010)	Conception rate
Heifers	20×10^6	51.8	40.2	2×10^6	Chebel *et al.* (2010)	Pregnancy per AI
Heifers and cows	$>29 \times 10^6$	57.9	38.8	2.1 and 3.1×10^6	Dominguez *et al.* (2011)	Pregnancy rate
Heifers	–	58.4	41.0	2×10^6	Funston and Meyer (2012)	Pregnancy rate
Heifers	20×10^6	39.6	31.6	2×10^6	Healy *et al.* (2013)	Conception rate
Heifers	25×10^6	62.5	34.0	2.1×10^6	Abdalla *et al.* (2014)	Pregnancy rate
Heifers	15×10^6	51.6	41.7	2.1×10^6	Kurykin *et al.* (2016)	Pregnancy rate
Heifers	–	55.0	44.0	–	Djedović *et al.* (2016)	Conception rate
Heifers	20×10^6	63.8	48.3	2×10^6	Joezy-Shekalgorabi *et al.* (2017)	Conception rate
Heifers	–	56.9	47.3	–	Oikawa *et al.* (2019)	Conception rate
	15×10^6	66.4	52.8	2.1×10^6	Frijters *et al.* (2009)	Non-return rate
Cows	–	30.0	25.0	–	Norman *et al.* (2010)	Conception rate
Cows	15×10^6	31.5	23.0	2.1×10^6	DeJarnette *et al.* (2010)	Conception rate
Cows	12×10^6	40.9	31.8	2.2×10^6	Karakaya *et al.* (2014)	Pregnancy per AI
Cows	–	49.32	40	–	Sharma *et al.* (2018)	Conception rate

Increased sperm concentration

Scientists have also tried to see any difference in fertility if an increased sperm dose of sexed semen is used for insemination. In these studies, semen dose was increased from $1.5–2 \times 10^6$ sperm up to 6×10^6 sperm per inseminate. DeJarnette *et al.* (2008) found no significant increase in pregnancy rates with 3.5 or 5×10^6 sperm compared with 2.1×10^6 sperm per dose in heifers (across sires) or cows. Similar results were obtained by Seidel and Schenk (2008) when they compared pregnancy rates in heifers and cows after insemination with frozen–thawed sexed semen. They observed no difference in pregnancy rates when the semen dose was increased from 1.5×10^6 to 6×10^6 sperm per inseminate. Schenk *et al.* (2009) reported the pregnancy rates of Holstein heifers inseminated with 2×10^6 and 10×10^6 as 56% and 62%, respectively. In another study, DeJarnette *et al.* (2010) found that, despite increasing the sex-sorted semen dosage from 2.1×10^6 to 3.5×10^6, there was no such improvement in conception rates in either cows or heifers. In heifers, the conception rates were 43.9 and 45.7%, respectively. Similar results were reported in lactating cows (23.0 and 25.4%, respectively).

Similarly, when sexed semen dose was doubled from 2.1×10^6 to 4.2×10^6 in a single dose, or by performing AI twice with a 12-h interval, Sá Filho *et al.* (2010) did not observe any significant increase in pregnancy per artificial insemination in Jersey heifers. To see the results of two types of semen on heifers treated with the double Ovsynch protocol, Dawod and Elbaz (2020) subjected Holstein heifers to the said protocol and compared the results of sexed (4×10^6 live cells/straw) and conventional semen (25×10^6 live cells/straw). Similar to earlier reports, heifers receiving conventional semen had a significantly higher first-service pregnancy rate (61.47%) than those receiving sex-sorted semen (51.45%).

From the results of various studies, it can be concluded that, when the semen dose was increased in sexed semen straw for insemination, no such corresponding increase in fertility was observed. So, there was no need to increase semen dose, but other factors need to be studied to increase the fertility of sexed semen.

Normal sperm concentration

Different researchers have conducted many studies to determine the difference in fertility of cows and heifers after using sexed and conventional semen at their standard/normal sperm dose i.e. $\sim 2 \times 10^6$ and $\sim 15 \times 10^6$, respectively. The differences in conception rate/pregnancy rate after using sexed and conventional semen straws at normal concentrations are presented in Table 3.

Dominguez *et al.* (2011) compared the pregnancy rate of Nelore heifers and non-suckling cows for male-sexed semen and conventional semen from the same bulls. They reported that total pregnancy rate of females inseminated with conventional semen was significantly higher than those inseminated with sexed semen. However, no significant difference in the pregnancy rate of heifers with non-suckling cows (36.4 and 43.3, respectively) was observed after the use of sexed semen. Chebel *et al.* (2010) also reported similar differences in pregnancy rates of heifers inseminated with sexed and conventional semen in Holstein heifers and DeJarnette *et al.* (2010) in Holstein heifers and cows. Norman *et al.* (2010) also reported conception rates with sexed semen as ~70% of

https://doi.org/10.1017/S0967199424000066 Published online by Cambridge University Press

conventional semen. Healy *et al.* (2013) reported similar differences in conception rates from the use of sexed and conventional semen. However, these were affected significantly by other variables such as insemination sire, heifer age at breeding, temperature and humidity surrounding breeding, service number, and AI technician.

Borchersen and Peacock (2009) reported differences of 5–10% points in conception rate from sexed and conventional semen for breeding heifers of Holstein, Jersey, and Danish Red Dairy breeds in Denmark. In another study, Funston and Meyer (2012) synchronized heifers and evaluated the use of sexed and conventional semen in beef heifers. They reported that the pregnancy rate using sexed semen was ~70% compared with conventional semen. In Turkey, Karakaya *et al.* (2014) studied the effect of sexed semen on the fertility of Holstein cows. They found that the use of sexed semen resulted in lower fertility compared with conventional semen, despite using some selection criteria such as the animals with clean vaginal mucus and a synchronized ovulation with specified follicles, distinctive of cows having high fertility diameter at AI, to select the most fertile cows.

In a recent study in Japan, Umehara al. (2019) reported that the conception rate after AI with sexed semen was ~83% compared with the conception rate using conventional semen. Although the heifer age and time of the year had different effects on the conception rates of the two semen types. Furthermore, the conception rate using sexed semen was relatively low during warmer months. Contrary to the other studies, Abdalla *et al.* (2014) reported much lower pregnancy rates at 40 and 90 days post-insemination (54.4% and 55.70% of conventional semen, respectively) after comparing sexed and unsexed semen from different bulls.

From the findings of various studies, it can be interpreted that fertility is generally low when using normal concentrations of sexed semen compared with the use of normal concentrations of conventional semen. The fertility of sexed semen is generally 70–80% compared with conventional semen, with a few exceptions.

Fresh and frozen sexed semen

Another important aspect of sexed semen usage is the use of fresh semen instead of frozen and thawed semen to bypass the process of freezing and thawing, which causes some damage. Some scientists have tried to explore this by comparing the differences in fertility using fresh and frozen sexed semen. Xu (2014) evaluated the performances of Holstein cows in New Zealand over three seasons (2011–2013) using fresh sexed semen (1×10^6 sperm/straw) and conventional semen (sperm dose of 1.25, 1.75 or 2×10^6/straw) and reported non-return rates (NRR) as 69.1 and 73.1%, respectively, over the three seasons the NRR of sexed semen compared with conventional was 94.6%. In another study, Maicas *et al.* (2019) compared the reproductive performance of heifers and cows inseminated with fresh sexed semen (1×10^6 or 2×10^6 sperm/straw) or frozen sex-sorted semen (2×10^6 sperm/straw) or fresh conventional semen (3×10^6 sperm per straw). They reported that pregnancy per artificial insemination (P/AI) in heifers was significantly higher for inseminations with conventional semen (60.9%) than with frozen sex-sorted semen inseminations (52.8%), but not different from either of the dosages of fresh sex-sorted semen (54.2 and 53.5%, respectively). However, in cows, conventional semen was found to have a greater P/AI than sex-sorted semen, irrespective of fresh or frozen type. All sex-sorted treatments did not differ from each other in either heifers or cows for P/AI. The relative performance of sexed semen compared with conventional semen ranged from 86.7% to 88.9% in heifers and 78.4% to 84.7% in cows, which is quite higher than earlier studies, especially for frozen sexed semen.

From various studies conducted by different researchers, it transpires that the dose of sexed semen does not influence fertility to a great extent because, when sexed semen dosage was increased in some studies, there were either no differences or no striking ones. In addition, there can be several reasons affecting fertility as discussed earlier, such as insemination sire, heifer age at breeding, temperature and humidity surrounding breeding, service number, and AI technician. Cottle *et al.* (2018) observed that farms with better fertility performance are better suited for sexed semen usage.

Even if increasing the sperm dosage per insemination augments fertility, the issue of higher cost still remains (Naniwa *et al.*, 2019), not to forget that increasing the dose can only overcome compensable abnormalities (Kastelic, 2013). The comparable results of frozen with fresh sex-sorted semen indicated that the sex-sorting process alone is not that detrimental. But, perhaps when coupled with cryopreservation it causes compounded damage to sperm. The presence of reactive oxygen species might also be one of the reasons affecting sperm survival (Espinosa-Cervantes and Córdova-Izquierdo, 2013). Earlier also, a popular opinion was that it is the multiple processes during the sorting process that together caused damage to sorted sperm, resulting in reduced fertility and were a major reason for the low adoptability of sexed semen technology in the past (Seidel, 2014; Vishwanath and Moreno, 2018).

Vishwanath (2015) highlighted the differences between the processing of conventional semen and sex-sorted semen: the sex-sorted semen processing involves more than 20 steps compared with only three or four steps in conventional semen processing. It can be a reason for reduced fertility (Seidel and Garner, 2002). So, in all scenarios, the only meaningful way out is refinement in technology. With the advent of SexedULTRA™ sperm sorting technology (Sexing Technologies, Navasota, TX), some of the problems associated with the technique have been solved, as it involves a modified protocol comprising a new staining medium, and modified sheath fluid and freezing medium (Vishwanath and Moreno, 2018). Furthermore, compared with previous XY Legacy technology, SexedULTRA™ medium improved sperm motility and acrosome integrity, and at the same sperm concentrations (Gonzalez-Marin *et al.*, 2017). Studies using SexedULTRA™ sexed semen have also shown promising results, with conception rates nearly equivalent to those of unsorted semen (Lenz *et al.*, 2017). Naniwa *et al.* (2019) suggested that it would be beneficial if AI with sexed semen was done closer to ovulation time, as the optimal period of insemination with sex-sorted semen was less than that of standard AI. After conducting a meta-analysis, Reese *et al.* (2021) noticed that trials using sexed semen during the last 5 years attained significantly higher pregnancy rates compared with previously published trials. This might be attributed to refinements occurring in the technology. Heuer *et al.* (2017) also observed a similar pattern in the past few years and, particularly during 2014, the conception rates using sexed and conventional semen were almost similar.

Genetic gain

It is often speculated that the use of sexed semen in a breeding scheme can reduce the number of dams required for maintaining the population at a constant size. Moreover, it is expected that sex

https://doi.org/10.1017/S0967199424000066 Published online by Cambridge University Press

determination can result in improved genetic gain (Rendel and Robertson, 1950) as a result of giving large selection differential in producing replacements, therefore increasing the selection intensity on cow dams because, in a population, the annual genetic response depends on various factors, viz., the selection intensity, the accuracy and the generation interval.

But only a few studies have been conducted until now to determine the actual effect and, more importantly, the long-term consequences. Furthermore, most of these studies are based on stochastic simulation modelling (Abdel-Azim and Schnell, 2007; Bérodier *et al.*, 2019). We will discuss in the following paragraphs the estimated effect of sexed semen by various authors in different conditions and scenarios.

In one of the earliest studies, Van Vleck (1981) estimated that the rate of genetic gain in milk yield could increase by 15% if sexed semen was used in dairy cattle compared with the use of regular AI. However, Baker *et al.* (1990) suggested that the effect of semen/embryo sexing on rates of genetic change was relatively low. Montaldo *et al.* (1998) compared various technologies using deterministic modelling of dairy cattle nucleus herds. They reported that the usage of sexed semen had a minor effect (0.4–1.4%) on selection responses. However, it would enhance efficiency by reducing the number of embryo transfers needed for the same rate of genetic progress. Some other authors (Hohenboken, 1999; Weigel, 2004) have furthermore theorized that the use of sex-sorted semen in dairy herds may accelerate the rate of genetic gain. It can be achieved by selecting only the highest-ranking cows to breed replacements from with sexed semen. Butler *et al.* (2014) also accentuated the need to breed replacements only from virgin heifers to obtain genetic gain in a favourable direction.

Using stochastic simulation, Abdel-Azim and Schnell (2007) studied the effect of using sex-sorted semen by monitoring genetic progress in elite and commercial dairy herds over 20 years. They reported that the effect of sexed semen in commercial cows was large in the early stages but, despite using sex-sorted semen outside the nucleus, the nucleus contribution increased over time and sidelined the cow-to-cow contribution. Furthermore, while evaluating the effect inside the nucleus, no effect of sexed semen on genetic progress was found in juvenile schemes. The genetic advantage of sexed semen usage in combination with MOET in the nucleus was nominal except when marker-assisted selection (MAS) was also available (Abdel-Azim and Schnell, 2007).

However, certain scenarios, such as the use of sexed semen in commercial herds in combination with the use of MOET in the nucleus were not studied by them. In another stochastic simulation study, Sørensen *et al.* (2011) compared nine scenarios to examine the effect of using sexed semen for cow dams in a dairy cattle breeding scheme, with or without MOET to bull dams, on annual genetic gain at the population level by monitoring over 30 years. Different scenarios were obtained using three levels of sexed semen combined with three levels of MOET, viz., no sexed semen, sexed semen to the best cow dams and sexed semen to all heifers, combined with no MOET, MOET on all bull dams, and MOET randomly on 20% of the bull dams. The study revealed that there was a 2.7 % (significant) increase in the annual genetic gain when sexed semen alone was used on all heifers, and this change was 2.1% (non-significant) when used on the best cow dams. Compared of schemes without sexed semen and MOET on all bull dams, the use of sexed semen together with MOET on bull dams increased the annual genetic gain by 1.8–2.5%. Overall, annual genetic gain tended to increase with the use of sexed semen in the simulated population (Sørensen *et al.*, 2011).

Previous studies did not examine the effect of genetic gain using MOET with genomic selection (GS), therefore Pedersen *et al.* (2012) investigated the same. They compared three levels of MOET, viz., no use of MOET (0%), 50%, and 100%, i.e. half and full young bull candidates born as a result of MOET, respectively. Furthermore, sexed semen and conventional semen were used in the nucleus and production parts of the population. The usage of sexed semen in the nucleus and production population observed improved genetic gain of 4.5% and 5.7% in the best scenario compared with conventional semen usage. The results revealed that the genetic gain in the whole population increased when sexed semen (X) was used in the production population because GS exploited the higher selection intensity among heifers with great accuracy. But with the application of MOET in breeding schemes, the effect was much reduced. In addition, the effect of sexed semen usage on genetic gain was comparatively minor with the effect of MOET. It was not always favourable and also depended on whether CD or bull dams (BD) were inseminated and on the type of semen used on BD. Based on stochastic simulation programs, the findings of Hjortø *et al.* (2015) further supported that the combined use of genomic tests with the use of sexed semen and beef semen is very beneficial for decreasing genetic lag as genomic information increases the accuracy of selection and the latter increases selection intensity, resulting in an overall positive interaction. In a recent study, Bérodier *et al.* (2019) used a mechanistic, stochastic, and dynamic model to assess the effect of using sexed semen along with female genotyping on genetic progress in commercial dairy cattle herds. The use of sexed semen resulted in the highest genetic gain as it increased selection intensity by making more heifers available for replacement. Combined use of sexed semen and genotyping also resulted in higher genetic gain as high-merit heifers were available for use of sexed semen in mating plans.

In Iran, Hossein-Zadeh *et al.* (2010) used a stochastic bio-economic model to compare the effects of applying sexed semen with conventional semen in simulated Holstein dairy herds. They reported that the rate of genetic progress was significantly higher when artificial insemination was done with sexed semen. Ettema *et al.* (2011) studied the importance of including genetic progress in milk yield through dynamic simulation modelling while evaluating the use of sexed semen and other reproduction strategies in a dairy herd. They reported that after including genetic progress in the model with the use of sexed semen on 50% of the best heifers, milk yield was found to be 167 kg energy-corrected milk (ECM)/cow/year higher after 16 years compared with the default strategy (typical Danish dairy herd), while it was only 78 kg (4%) when genetic progress was not included. It was lower than reported by Abdel-Azim and Schnell (2007) that was 11.1% in 16–20 years; the reason being that Ettema *et al.* (2011) assumed use of sexed semen in only herds under study and not the nucleus, and the use was only on 50% of the best heifers.

Khalajzadeh *et al.* (2012) compared the effects of limited and widespread use of sexed semen. They observed the genetic progress through different pathways, i.e. active sires (AS), young bulls (YB), BD, and milking cows (CW) (Table 4). They reported that in the AS pathway, the use of sexed semen does not have any increasing effect on selection intensity. Similarly, using sexed semen had a low effect on the extra genetic merit of YB, although breeding values were significantly different compared with using unsexed semen. Genetic merit was increased in the BD and CW pathways, but the effect was less in BD and quite high in CW (3.5% and 19% genetic superiority, respectively). They further reported that instead of

https://doi.org/10.1017/S0967199424000066 Published online by Cambridge University Press

Table 4. Genetic superiority using sexed semen relative to unsexed semen

Path	Genetic superiority (%) in best strategy relative to use of unsexed semen	References
AS (Active sires)	1.19	Khalajzadeh *et al.* (2012)
YB (Young bulls)	2.69	
BD (Bull dams)	3.41	
CW (Milking cows)	19.2	
FLC (First lactation cows)	30 % (in year 11)	Abdel-Azim and Schnell (2007)

limited use, widespread use of sexed semen was more advantageous in increasing the genetic merit of animals. But there is a simultaneous decrease in reproductive performance of dairy cows due to the low conception rate in widespread use, and these negative effects can be minimized if sexed semen is used only in heifers. After comparing many scenarios, Kaniyamattam *et al.* (2016) also reported that the one involving the use of sexed semen in heifers and the culling of surplus heifers based on the net merit index resulted in the greatest progress in favourable direction. In another simulation study, Cottle *et al.* (2018) found that the rate of genetic gain in terms of increase in herd economic breed index (EBI) was considerably higher in scenarios using sex-sorted semen, mainly because of increased selection pressure in breeding herd replacements. Net profit was found to be higher in scenarios using sex-sorted semen compared with an unsorted one.

Sire of dam pathway

Most of the earlier studies were conducted to assess the effect of using sexed semen on the dam of dam pathway, but Joezy-Shekalgorabi and De Vries (2018) studied the effect of using sexed semen on the selection proportion in the sire of dam's (SD) pathway by various strategies, viz., continuous use of sexed semen (CS) and the other two being mixed semen strategies, i.e. use of sexed semen for the first two and for only the first insemination, respectively, and conventional semen for remaining inseminations (S2 and S1, respectively). Results indicated that there was an increase in selection proportion in all three strategies, and it was highest in CS, followed by S2 and S1, which means lower selection intensity and, therefore, reduced genetic improvement in the studied pathway. They reported that the use of sexed semen has a negative effect on the selection proportion of the SD pathway. However, using sexed semen in YB was not taken into account by them, and all of the available doses of sperm in the SD pathway were assumed to be consumed for producing sexed semen.

Inbreeding

There were apprehensions about an acceleration in the level of inbreeding due to sex-sorted semen usage without the use of neutralizing measures (De Vries *et al.*, 2008). Some studies analyzing the effect of sexed semen on inbreeding are also available. In one such study, Abdel-Azim and Schnell (2007) averaged inbreeding coefficients for all animals in the pedigree file and, after analyzing, found that after 20 years of simulation, sexed semen schemes had slightly higher inbreeding coefficients. However, the differences between the use of sexed and normal semen were not that pronounced. Norman *et al.* (2010) compared expected future inbreeding between bulls with sexed semen and conventional semen and reported that, for bulls with sexed semen, mean expected future inbreeding was marginally higher than for active bull with conventional semen (5.55 and 5.70%, respectively). In another study by Sørensen *et al.* (2011), sexed semen was not found to affect the rate of inbreeding; however, the rate of inbreeding increased with the use of MOET. The reason was that change in selection intensity was smaller while using sexed semen because of selection in BDs, whereas MOET increased the selection intensity in the selection of YB.

Similar to previous findings, Pedersen *et al.* (2012) reported that sexed semen was found to have a significant effect on the rate of inbreeding, especially when sexed semen was used on both cow dams and BD; only then was a significant effect observed. In different scenarios, sexed semen usage raised the rate of inbreeding by 16.0 to 20.3%, and it was highest when all young bull contenders were born through MOET and normal semen was used on top BD and sexed semen (X) on remaining BD and also on cow dams (Pedersen *et al.*, 2012). They further reported that the rate of inbreeding was found to be sensitive to the type of semen used in the remaining BD. With the application of MOET, the highest rate of inbreeding was observed when X-semen was used on BD, and the lowest rate of inbreeding occurred when Y-semen was used in the nucleus, as young bull candidates increased following use of Y-semen in the nucleus, while use of X-semen decreased their number.

Conclusion

From various simulation studies, sexed semen usage has been found to increase the rate of genetic gain in general, although other scenarios such as combined use with MOET and GS affect the outcome. With the use of sexed semen, more number of heifers are available for replacement, therefore causing increased selection intensity and, therefore, higher genetic gain. Other factors such as accuracy of selection further influenced genetic progress. In widespread use of sexed semen, there was some negative effect on reproductive performance of dairy cows. However, with use in heifers only, it can be managed. As far as the effect of sexed semen on inbreeding is concerned, based on available studies, it can be concluded that sexed semen usage does increase the level of inbreeding but it is not that pronounced. With the use of MOET, the chances of inbreeding are higher as it increases selection intensity in YB.

Acknowledgements. None.

Author contributions. Sunil Kumar: Conceptualization, Literature collection and review, Writing – original draft. Ankit Magotra: Revision and editing of original draft. Manoj Kumar and Sonu Kumari: Literature collection and proofreading. D.S. Dalal: Supervision, Discussion and critical revision, All authors read and approved the final manuscript.

Financial support. None.

Competing interests. The authors declare no conflicts of interest.

Ethical standard. None.

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