Animal (2018), **12:S1**, pp s97–s103 © The Animal Consortium 2018
doi:10.1017/S1751731118000721

Review: Applications and benefits of sexed semen in dairy and beef herds

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(Received 22 December 2017; Accepted 9 March 2018; First published online 10 April 2018)

The use of sexed semen in dairy and beef cattle production provides a number of benefits at both farm and industry levels. There is an increasing demand for dairy and beef products across the globe, which will necessitate a greater focus on improving production efficiency. In dairy farming, there is surplus production of unwanted male calves. Male dairy calves increase the risk of dystocia compared with heifer calves, and as an unwanted by-product of breeding with conventional semen, they have a low economic value. Incorporating sexed semen into the breeding programme can minimise the number of unwanted male dairy calves and reduce dystocia. Sexed semen can be used to generate herd replacements and additional heifers for herd expansion at a faster rate from within the herd, thereby minimising biosecurity risks associated with bringing in animals from different herds. Furthermore, the use of sexed semen can increase herd genetic gain compared with use of non-sorted semen. In dairy herds, a sustainable breeding strategy could combine usage of sexed semen to generate replacements only, and usage of beef semen on all dams that are not suitable for generating replacements. This results in increased genetic gain in dairy herd, increased value of beef output from the dairy herd, and reduced greenhouse gas emissions from beef. It is important to note, however, that even a small decrease in fertility of sexed semen relative to conventional semen can negate much of the economic benefit. A high fertility sexed semen product has the potential to accelerate herd expansion, minimise waste production, improve animal welfare and increase profitability compared with non-sorted conventional semen.

Keywords: sex-sorted semen, assisted reproductive technology, genetic gain, welfare, greenhouse gas emissions

Implications

Sex-sorted semen is a revolutionary technology for cattle breeding. Greater utilisation of sexed semen can increase the efficiency of both dairy and beef production, increase farm profitability and improve environmental sustainability of cattle agriculture.

Introduction

The use of sex-sorted semen in both dairy and beef production allows predetermination of calf sex with ~90% reliability. In cattle, an X-chromosome bearing sperm contains 3.8% more DNA than a Y-chromosome bearing sperm (Johnson, 1995), providing a feature that can be utilised to quickly identify X- and Y-chromosome bearing sperm. At present, the only reliable method of pre-determining offspring sex is by manipulating the relative abundance of viable X- and Y-chromosome bearing sperm. This is typically carried out via a specialised type of flow cytometry called fluorescence-activated cell sorting (Garner *et al*., 2013), but other methods such as laser splitting of the unwanted X- or Y-chromosome bearing sperm have recently been reported (Faust *et al*., 2016).

Despite the benefits associated with the use of sex-sorted semen, it currently represents a small (but rapidly growing) percentage of the artificial insemination (AI) market (<5%; Seidel, 2014). Sex-sorted semen is primarily used in dairy herds, and within dairy herds it was traditionally limited to use on heifers (Borchersen and Peacock, 2009; DeJarnette *et al*., 2009; Frijters *et al*., 2009) due to concerns over reduced pregnancy rates in cows (Seidel and Schenk, 2008; DeJarnette *et al*., 2011; Healy *et al*., 2013). More recent studies have demonstrated that sexed semen can be successfully used in both virgin heifers and lactating cows, and that targeted use of sexed semen should be employed (Butler *et al*., 2014b; Xu, 2014). Nevertheless, it should be noted that any reduction in fertility will reduce the financial benefits from implementing sexed semen usage on farm, and that usage of sexed semen is unlikely to be profitable in herds with poor fertility. This review addresses the potential applications of sexed semen, and identifies possible

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implications of increased use of sexed semen in dairy and beef farming systems.

Motivation for sexed semen usage

The primary reasons for mating cattle are to initiate lactation, produce replacements and to produce meat (Hohenboken, 1999). Each of these is a direct outcome of a successful pregnancy, highlighting the importance of fertility in dairy and beef production systems. The use of frozen-thawed semen for AI is the most common practice amongst dairy producers worldwide. The use of AI slightly increases the probability of producing a male calf compared to natural mating (Berry and Cromie, 2007), as does the use of frozen semen (Xu *et al*., 2000). The primary reason for incorporating sex-sorted semen in any dairy or beef system is to impose a desired sex bias in the resulting progeny. In dairy systems, dairy breed heifer calves are required for both replacements and herd expansion, and may also increase the value of calf sales where surplus heifer calves are produced and where there is a good market for such calves (De Vries *et al*., 2008). It has also been reported that gestation of a female calf results in increased milk production, especially if the daughter pregnancy occurs at the first parity (Hinde *et al*. 2014), but this needs to be verified in different populations of cows managed under diverse production systems. In beef cow systems, female calves with a high maternal index are required to generate replacements, whereas male calves with a high terminal index will achieve greater growth rates and carcass price.

Calf welfare

Inevitably, the use of conventional non-sorted semen leads to surplus calves of the non-desired sex (~50% of all calves), and this is a particularly important issue in the dairy industry. For example, in the United States, 0.1% of the bull calves produce sufficient sires for the dairy industry (De Vries *et al*., 2008), resulting in excess production of low value dairy bull calves. The fate of male dairy calves varies between countries. In some countries, male dairy calves are used for veal production, but most are used in some form of calf-to-beef production system. Any animal that has a small monetary value is a potential welfare concern, because their low value does not incentivise good husbandry. A survey carried out on 242 Brazilian farms reported that 35% of farms killed dairy bull calves on site (Hötzel *et al*., 2014). Utilising sexed semen for breeding replacements would minimise the production of unwanted male dairy breed calves, thus allaying potential welfare issues associated with male dairy calves.

Cow welfare

Sexed semen can reduce the occurrence of dystocia by an estimated 20% (Seidel, 2003; Norman *et al*., 2010), as heifer calves are smaller and easier to calve. Moreover, if dystocia does occur, mortality is about 57% greater with male calves than with female calves (Dematawena and Berger, 1997). Because of the importance of achieving a concentrated calving pattern in seasonal-calving systems (dairy and beef), it is vital that the cow quickly returns to high fertility potential after calving to maintain a 365-day calving interval. It is well established that dystocia is a risk factor for retained foetal membranes, uterine disease, delayed resumption of oestrous cyclicity and conception failure. Hence, reducing the incidence of dystocia has both immediate and subsequent health and welfare benefits for the dairy cow. In addition, utilisation of sexed semen to generate replacements can improve biosecurity, as a farm can more easily generate replacements and expand the herd from within (i.e. not reliant on purchasing stock of unknown disease status). This would facilitate biosecure herd expansion from dams of known genetic merit (Weigel, 2004). Finally, to extract maximum benefits from sex-sorted semen usage, animals must be well managed, which has associated benefits for all aspects of animal welfare.

Accelerating genetic gain

Additive genetic gain

The advent of genomic selection has allowed earlier identification of the next generation of sires (Calus *et al*., 2015). One of the major potential benefits associated with use of sexed semen, which is often overlooked, is the more efficient dam selection. With non-sorted semen use, ~90% of genetic gain in milk yield has occurred from sire selection (Wilcox *et al*., 1992). Sexed semen facilitates concurrent sire and dam selection, which has been estimated to increase the rate of genetic gain by 15% (Weigel, 2004).

Heterosis

Capitalising on the effect of heterosis (hybrid vigour) in the F1 offspring of two complimentary breeds can quickly improve herd health, fertility and longevity. Crossbreeding Holstein-Friesian cows with high genetic merit Jersey sires offers a rapid approach to deliver a type of cow that is ideally suited to many different production systems, especially seasonal pasture-based dairying: high yields of milk fat and protein, moderate size, excellent fertility, high intake capacity relative to their moderate size, and high productivity per unit area (Prendiville *et al*., 2010; Buckley *et al*., 2014). Because of the large genetic distance between the breeds, potential gains from heterosis are maximised, in addition to breed complementarity. One of the major barriers to the uptake of crossbreeding with Jerseys is the low value of the male calf. Hence, greater uptake and usage of Jersey genetics is reliant on sexed semen. While cull cow prices are also lower, the crossbred cow has already repaid the lower cull value through better fertility and milk solids production during a long productive life.

Sire allocation

Up until 2014, the majority of published studies that evaluated sexed semen highlighted that fertility performance was reduced, as sexed semen generally achieved conception rates (SS-CR) that were 70% to 80% of those achieved with conventional semen (reviewed by Butler *et al*., 2014b). In seasonal-calving systems, poor conception rates disrupt calving patterns resulting in financial loss (Dillon *et al*., 1995; Shalloo *et al*., 2004 and 2014). Therefore, any reduction in fertility negates some of the benefits that sexed semen offers. Since 2014, a number of publications have reported improved fertility performance with sexed semen (Butler *et al*. (2014a), SS-CR 87%; Xu (2014), SS-CR 94%; Vishwanath and Moreno (2018), SS-CR ~90%). These high SS-CR values are only observed in well-managed herds, and quickly deteriorate in herds with average or poor management. Availability of a sexed semen product with fertility equivalent to conventional semen requires a fundamental change in the strategy employed for herd breeding management. First, decide how many female calves are needed, and breed an appropriate number of the highest genetic merit dams with sexed semen from high genetic merit bulls to generate the required number of female offspring. Second, dams that were not included on the list of highest genetic merit dams should be inseminated with beef semen. Economic modelling of implementing this strategy indicated increased profitability compared with use of conventional dairy semen alone (Murphy *et al*., 2016).

Rearing heifer calves

In order to obtain maximum lifetime milk production, all replacement heifers should be first bred at ~15 months of age (to calve at ~24 months of age). This is particularly important in seasonal pasture-based systems, where it is desirable to have heifer calves (future replacements) born at the start of the calving period. This could be achieved by using the allocated quota of sex-sorted semen in the first 3 weeks of the breeding season. The resulting heifer calves would be born at the start of the subsequent seasonal-calving period, and thus be older at first insemination, which would favourably impact their productivity and longevity in the herd (Archbold *et al*., 2012; Butler *et al*., 2014b). In year-round calving systems, heifers could be inseminated 3 weeks before normal age at first breeding ('the early bird scheme' Weigel, 2004); breeding earlier would limit the impact of any reduction in fertility due to sexed semen, and birth of heifer calves would offset the occurrence of dystocia from calving at a younger age.

Currently, an estimated 60% of breeding aged dairy cows and heifers are needed to produce sufficient number of replacements (De Vries *et al*., 2008), but this number could be greatly reduced with targeted use of sexed semen in heifers and cows (Hutchinson *et al*., 2013; McCullock *et al*., 2013). Replacement heifers could be generated within the first 3 weeks of the breeding season in seasonal-calving systems, and by selecting only genetically superior animals in year-round calving systems. Crosson (2008) indicated that beef cow replacements sourced from dairy beef-cross heifers are more profitable than sourcing replacements from within a beef cow herd, partly due to loss of heterosis in the latter. This suggests that there is a market for beef-cross heifers from the dairy herd as future beef cows. Breeding lower genetic merit dairy cows with beef semen means their genes are being removed from the lactating herd, aiding herd genetic gain. McCullock *et al*. (2013) carried out simulations to compare the effect of using sexed semen on heifers and genetically superior cows to generate replacements and breeding the rest of the herd with beef semen versus using conventional dairy semen on all animals. The former strategy was more profitable, resulted in faster genetic gain, and increased the number of heifers born.

Sexed semen in beef production

The uptake and usage of AI is much less in beef production compared with dairy production. Consequently, the utilisation of sexed semen in beef production is also low. Nevertheless, a sexed semen product with high fertility could revolutionise beef production. Successful use of AI, especially with sexed semen, is highly dependent on accurate heat detection. This can be difficult in beef herds, especially in large beef herds managed under extensive rangeland systems. The development of high fertility timed AI protocols, although laborious to implement, facilitate synchronisation and whole-herd insemination at the start of the breeding period. In a simple change to traditional beef production systems that rely on conventional semen for AI, sexed semen could be used to preselect the offspring sex, facilitating generation of female offspring with strong maternal traits and male offspring with strong terminal traits.

Sexed semen could be utilised in a number of different beef production systems. First, with a single sexed heifer breeding system, as described by Taylor *et al*. (1985) beef heifers are inseminated with X-chromosome bearing sperm to produce replacements and are then sent for slaughter after her first parturition. In this system the price for a beef cow is not affected by the age of the animal and therefore more valuable. Another system that would benefit from sexed semen is the three breed terminal crossbred. Semen biased for female offspring from a sire with excellent maternal traits would be used to create a maternal crossbred, and this maternal crossbred would then be inseminated with Y-chromosome bearing sperm from a sire with excellent terminal meat production traits. This system would not only increase heterosis, but would also utilise complimentary traits from different breeds for maximum advantage. Beef production systems will only profit from the uptake of sexed semen when the monetary return from producing offspring of a desired sex is greater than the cost of implementing it (Hohenboken, 1999).

Assisted reproductive technologies using sex-sorted semen

Embryo transfer currently represents a small proportion of the total commercial market for assisted reproduction, but recent trends indicate that it is increasing. *In vitro* production of embryos saw a sevenfold increase between 2000 and 2012 (Stroud, 2012), and in 2014 *in vitro* and *in vivo* embryo production were roughly equal (Blondin, 2017). Embryo transfer utilising sexed semen for fertilisation increases the chances of producing multiple calves of the desired sex from a specific dam and sire coupling (Hayakawa *et al*., 2009). Currently, there are two methods that can be used to achieve a desired offspring sex bias with transferred embryos. In the first, a blastomere is collected by biopsy, PCR with Y-chromosome specific primers used to determine sex of the embryo, and embryos of the desired sex are transferred. In the second, sexed semen is used for the fertilisation step.

During the sorting process, sperm cells bearing an X- or Y-chromosome are differentiated based on their florescence intensity. The process also has the potential to select for other sperm specific physiological characteristics based on fluorescent staining techniques. For example, when sperm is co-incubated with propidium iodide, non-viable cells take up the fluorescent stain and can be removed from the sample during the sorting process through fluorescent gating. This is true for any sperm specific physiological biomarker that can be tagged by a fluorescent marker and identified by the cytometers' detectors (e.g. acrosome integrity, mitochondrial function).

The addition of a fluorescent marker to the sorting technique would undoubtedly slow down the process, but could be implemented for targeted use in reproductive technologies such as *in vitro* fertilisation (IVF). High rates of good quality blastocyst development can be achieved through the combined use of ovum pick up and IVF with sexed semen (Matoba *et al*., 2014). Technologies such as IVF require far less sperm per oocyte to achieve acceptable fertilisation rates compared with AI. Consequently, a process known as 'reverse' sorting has been developed for use in specialised *in vitro* embryo production facilities. This process involves sex sorting frozen-thawed conventional semen straws for use in IVF (Morotti *et al*., 2014). The success of IVF with reverse sorted semen would likely improve if additional sperm physiological markers could be added to remove non-viable, cryo-damaged and sub-fertile sperm during the sorting process.

Future environmental restrictions

To meet projected increases in food demand, Alexandratos and Bruinsma (2012) forecast that by 2050 global milk production would increase to 1075×10^6 tonnes (liquid milk equivalent; 62% increase relative to 2005 to 2007) and global beef production would to increase to 112×10^6 tonnes (76% increase relative to 2005 to 2007). These increases will require marked improvements in the efficiency of production, as arable land resources are not increasing (Bouwman *et al*., 2005). As the global demand for milk increases, dairy herd size is expanding in many countries. However, future environmental regulations may limit milk and meat outputs due to mounting concerns regarding greenhouse gas (GHG) emissions. For example, EU member states have targeted reductions in GHG emissions of 30% below 1990 levels by 2020 and 60% to 80% below 1990 levels by 2050 (Efforts Sharing Decision 406/2009/EC). Across the EU, the dairy and beef sectors account for 70% of total GHG emissions from agriculture (Lesschen *et al*., 2011). A Norwegian study, employing life cycle assessments, estimated that >45% of CO_2 and CH_4 emissions come directly from cattle, which combined with field emissions from forage production contribute significant burdens on the environment (Roer *et al*., 2013).

The opportunity to improve the efficiency of beef production by generating more beef from the dairy herd was investigated in a simulation study. At the outset of the simulation, 100 000 tonnes of beef carcass weight (CW) was being produced, 50% derived from the dairy herd and 50% derived from the beef cow herd. It was assumed that dairy cow numbers would increase by 50% over a 12-year period, but for the purposes of the simulation, total production of beef would be held constant. The purpose of the simulation was to determine the proportion of beef derived from the beef cow herd that could be displaced by beef derived from the dairy herd, and to determine the consequences for the total GHG emissions from beef production.

For the purposes of modelling the potential impact of sexed semen on beef output, the proportions of female dairy calves, male dairy calves and dairy beef-cross calves derived from the dairy herd were assumed to be 0.3, 0.3 and 0.4, respectively. Two scenarios were compared for dairy herd breeding management:

- Conv-2030: All replacements generated with conventional dairy semen (Holstein-Friesian), beef semen used to sire the remainder (calves born: 0.3 female dairy, 0.3 male dairy, 0.4 beef cross);
- SS-2030: sexed semen (fertility equal to conventional) used to generate all replacements, beef semen used to sire the remainder (calves born: 0.3 female dairy, 0.03 male dairy, 0.67 beef cross).

The implications of the two alternative future situations (Conv-2030 and SS-2030) on beef GHG emissions are summarised in Table 1. For this analysis, key assumptions were made:

- Beef output would remain the same as the current levels (100 000 tonnes CW).
- Annual cow culling rate was assumed to be 22%.
- 6% mortality was assumed for male dairy calves, 3% for beef-cross calves.
- The Economic Breeding Index in Ireland (as in other countries) is selecting for smaller cows, and hence cull cow carcase weight was assumed to get lighter by 2030. For the same reason, the carcase weight of male dairy calves at slaughter was also lighter by 2030.
- Selecting for beef bulls suited for use on the dairy herd could increase carcase weight of dairy beef offspring by 2030.
- The value of dairy beef was calculated based on current beef prices (Cows €3.25/kg, dairy steer €3.80/kg, beef cross €4.20/kg).

Table 1 *Simulation of the effect of utilising conventional (Conv) or sexed semen (SS) for breeding all dairy replacements on the economic value and greenhouse gas (GHG) footprint of beef production assuming an expansion in dairy cow numbers by 50% between 2018 and 2030 when total beef output is kept constant*

	Status quo-2018	Conv-2030	SS-2030
Total beef produced, tonnes CW	100 000	100 000	100 000
Dairy cows population	189 193	283 790	283 790
Dairy cows culled	41 622	62 434	62 434
Male dairy calves, population	53 352	80 029	8 003
Dairy beef calves, population	73 407	110 111	184 435
kg CW/culled cow	270	260	260
kg CW/male dairy calf to slaughter	300	290	290
kg CW/dairy beef calf to slaughter	310	320	320
Culled cow, total tonnes CW	11 238	16 233	16 233
Male dairy calves, total tonnes CW	16 006	23 208	2 321
Dairy beef calves to slaughter, total tonnes CW	22 756	35 235	59 019
Dairy beef, total tonnes CW	50 000	74 677	77 573
Value of dairy beef (€)	192 921 237	288 936 815	309 456 535
t GHG emission/t culled cow	7.6	6.9	6.9
t GHG emission/t male dairy calf to slaughter	13.0	11.9	11.9
t GHG emission/t dairy beef calf to slaughter	13.0	11.9	11.9
Dairy beef, total GHG emission (t)	588 846	805 726	840 105
Beef required from beef cow herd, tonnes CW	50 000	25 323	22 427
t GHG emission/t beef from beef cow herd	23	21	21
Beef from beef cow herd, total t GHG emission	1 150 002	531 793	470 970
Beef, total GHG emission	1 738 848	1 337 520	1 311 075
Change in total GHG emissions (%)		− 23.1	−24.6
Average t GHG emission/t beef	17.4	13.4	13.1
Beef from dairy herd (%)	50	75	78
Beef from beef cow herd (%)	50	25	22

CW = carcass weight; kg = kilograms; t = tonnes.

In the current status quo, beef was sourced equally from dairy herds and beef cow herds. Expansion of dairy cow numbers reduced the requirement for beef from the beef cow herd with or without sexed semen usage, but the absolute reduction was greater when sexed semen was used to generate dairy female replacements and more beef semen was used. The structural shift from male dairy calves (Conv-2030) to beef-cross calves (SS-2030) resulted in greater beef value (+ €20.5 M). Keeping total beef output constant, using sexed semen to generate dairy replacements and increasing the proportion of beef-cross calves reduced the requirement for beef from the beef cow herd from 50% in the status quo to 22% in 2030. Of note, even without sexed semen usage, the requirement for beef from the beef cow herd will decline to 25%. This assumes that a beef market exists (or could be established) for a 50% increase in the number of male dairy calves, and that most of the animals would make it to maturity before slaughter. As outlined in the section on 'Calf welfare', this is likely a gross overestimate. Because of the marked reduction in the requirement for beef from the beef cow herd, the carbon footprint of the beef produced also declined. Widespread usage of sexed semen in 2030 reduced the carbon footprint from 17.4 kg CO_2 eq/kg CW currently to 13.1 kg CO_2 eq/kg CW in 2030 (−24.6%). Hence, widespread sexed semen usage could facilitate a pronounced structural shift in the beef industry that would reduce the GHG emissions in the sector, and reduce the carbon footprint of beef.

Although CH_4 and CO_2 emissions are positively and significantly correlated with cow output, the percentage increase in emissions per animal are much lower than the percentage increase in productivity (Gerber *et al.*, 2011). Furthermore, many studies have reported between animal variation in feed efficiency and enteric CH_4 emissions (Herd *et al.*, 2002; Hegarty *et al.*, 2007; Yan *et al.*, 2010; De Haas *et al.*, 2011). Selection of sires and dams based on feed efficiency has the potential to have a large impact on reducing GHG emissions from livestock systems. Therefore, selective breeding for daughter traits should also take into account animal efficiency in minimising GHG production. Obviously, dispersion of genetics with a favourable environmental footprint could be accelerated with sexed semen.

The use of beef semen in dairy production systems is likely to further increase in the coming years because the requirement for replacements is finite, and the greater value of a beef-cross calf *v.* a male dairy calf provides some protection from volatile milk prices (McCullock *et al.*, 2013; Murphy *et al.*, 2016). This would obviously diminish the ability to expand herd size, and is most suited to herds that have already stabilised in size. This breeding strategy also has reduced operating costs, lower initial investment requirements and can still generate profit when milk prices are low

(€0.22/l; Murphy *et al.*, 2016). Similarly, in simulations carried out by McCullock *et al.* (2013) where only heifers and genetically superior cows were inseminated with sexed semen to produce replacements and the rest of the herd was bred with beef semen, profit, genetic gain and the ability to generate heifers were all increased. If production efficiency of milk and beef can be improved, there is large potential to reduce emissions while concurrently improving farm profitability (Place and Mitloehner, 2010) and also cater for more efficient land use.

Conclusion

The advantages of sexed semen over conventional semen are numerous and varied. The key criterion of importance for the farmer is the relative conception rate achieved with sexed semen compared with conventional semen. In recent years, this fertility gap appears to have been narrowed. A high fertility sexed semen product allows much greater flexibility in the breeding management programme: diminished numbers of low value male dairy calves, thereby eliminating a potential welfare concern; greater dairy beef production; reduced GHG emissions from beef production; greater selection intensity on the dam line; reduced barriers to crossbreeding with the Jersey breed; easier heifer rearing; and improved biosecurity. Societal concerns regarding animal welfare and GHG emissions can be at least partially addressed through widespread uptake and usage of sexed semen. The advantages conferred by sexed semen must be harnessed to improve production efficiency, and provide animal protein products that are economically, socially and environmentally sustainable.

Acknowledgements

Funding from the Irish Department of Agriculture, Food and the Marine under research grant 15S732 is gratefully acknowledged. The authors thank Dr Donal O'Brien (Teagasc) for assistance with the GHG simulation.

Declaration of interest

The authors declare no conflict of interest.

Ethics statement

There was no ethics committee review, as no animals were used to provide data for this manuscript.

Software and data repository resources

There was no software or data specifically generated for this manuscript.

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