Sexed semen

Introduction

Male dairy calves are/have been a by-product of dairying. As dairy cows are bred for milk production, (particularly the Holstein/Friesian), the perceived beef value of pure-bred male dairy calves is low, due to poor confirmation and low kill-out percentage. Hence, any reduction in the production of low value dairy bred calves, (accompanied by the supply of crossbred dairy/beef calves), gives better returns to the dairy farmer and a better value product for the beef industry. In the UK, approximately 50% of the beef supply comes from the dairy industry, (cull cows, male dairy calves and crossbred dairy/beef calves). By reducing the supply of male dairy calves and consequently increasing the supply of dairy cross beef calves via sexed semen, both industries benefit.

Male dairy calves increase the risk of dystocia, (calving difficulties), compared with heifer calves (particularly in 1st calving heifers) and also have higher mortality rates, (possibly because of the increased calving difficulties and less attention regarding colostrum intake, due to perceived low value).

By using sexed semen to breed herd replacements, (from the best animals, as determined by genetics) and beef semen on everything else, genetic gain of the replacements increases, as does the financial value of calves, (both replacements and surplus dairy cross beef calves.

The sorting of X- and Y-chromosome bearing sperm on the basis of DNA content differences is the most reliable, as well as repeatable technique for producing sex pre-selected animals. Other techniques have been tested, but the method based on 'quantitative flow cytometry' is the predominant method for this process and has many associated patents, (Yadav, H. et. al., 2018).

In principle, the sorting technique, (based on quantitative flow cytometry), spermatozoa after co-incubated and labelling with the DNA fluorescent dye, (Hoechst 33342), are hydro-dynamically focused in a discontinuous droplet stream, which passes an ultra-violent laser beam. X-bearing sperm shine brighter than the Y-bearing sperm when illuminated with a laser for fluorescence. A positive or negative charge is then applied to the droplets. Charged (+/-) droplets are deflected in opposite directions and uncharged droplets pass straight through, (not sorted). Sex selection accuracy is about 90%. The process has been refined and updated over time and especially the number of sperm that can be sorted in a given time. There are in excess of 20 different sub-processes involved in the sex-sorting procedure, including an extended holding time before staining, exposure to a laser beam to induce fluorescence, separation into X- and Y-sperm and finally exposure to an electrical field for drafting as a relatively pure population into an appropriate vessel, all of which may contribute to a reduction in field fertility, (Arruda, A. et. al., 2012).

To illustrate typical sorter performance at 90% accuracy with 10% membrane-compromised sperm, 32,000 sperm may be evaluated per second, with 3,000 discarded as dead or dying sperm, 1,000 discarded due to technical issues, such as two sperms per droplet, or being too close to each other in the stream to get accurate information, 8,000 not evaluated because of not being appropriately oriented for accurate measurement because the flat aspect of the sperm was not facing the detector and another 4,000 not being clearly X- or Y-sperm, due to biological and procedural variability. Thus, in this example, only 16,000 sperm actually get sorted per second, or 8,000 of each sex, which represents 25% of the ejaculate for each sex. Unfortunately, the problem does not end there because the sorted sperm are very dilute and must be concentrated

by centrifugation, and a few percent of sperm are lost in that process. Also, some sperm are lost in the 'dead space' of the equipment. Packaging 2 million sperm per insemination dose under the above ideal conditions would produce about 14 straws of sexed semen of each sex per hour, (Seidel, G. 2014).

It is important to highlight that populations of calves resulting from sexed semen do not differ from those derived from the use of conventional semen, including gestational length, birth weight, mortality rate and weight gain. No genotoxic effects on sexed semen were detected to date from using DNA fluorescent dye, (Hoechst 33342), Tubman, L. et al., (2004). There is some evidence of higher neonatal mortality in the bull calves that are of the 'wrong' sex when using X-sorted sperm, (DeJarnette, J, et al., 2009). A recent German study found that stillbirths for male calves from sexed semen was 2.86 times than that for unsexed male born calves and this warrants further investigation, Diers, S. et al., (2020). A Serbian study found no difference in gestation length in heifers following insemination with either sexed or unsexed semen. However, birthweights were 0.9 kg lower for sexed semen, ($P<0.001$), presumably due to sex ratio differences and twinning was significantly lower for sexed semen, (1.1% compared to 3.8% for unsexed). However, some other studies did not observe this difference in twinning, (Djedovid, R. 2017).

Conception/fertility rate with sexed semen

Conception rate with sexed semen is generally lower than conventional semen, (75-80%), in both cows and maiden heifers, for two main reasons. One of them is lower number of spermatozoa in the insemination dose, (usually ~ 2 million, compared to 10-20 million in conventional non-sorted semen straws) and the second one is spermatozoa damage that occurs during the sorting process, (Seidel, G. 2014).

In most bulls, an important role is played by these both factors, but it has not been established yet which one of them plays the greater role. There is evidence that the reduction in fertility due to the sexing process is not consistent across sires and anecdotal evidence would appear to support this finding. In a Dutch study with conventional and sexed semen from 7 bulls, using the 56-day non-return rate as the measure of pregnancy, it was determined that about two thirds of the difference in fertility was due to the low dose rate and about one third due to the sorting process. There were considerable differences between the bulls compared on the basis of sorting only, with some have improved conception rates after sorting, (Frijters, A. et. al., 2009).

The results of a recent study emphasize that the process of sex-sorting affects sperm in a manner which persists beyond fertilization and highlights the fact that sperm integrity plays a major role in embryo quality. The results suggest that the reduced conception rates after sexing are due to altered sperm morpho-kinetics, decreasing the chance of sperm reaching and fertilising the oocyte and also aberrant early embryonic development. Functional knowledge of the post-sorting alterations in sperm integrity and early embryonic development are pivotal in developing cost-effective remediation strategies to improve conception rates in vivo, which will enable widespread adoption of this technology, (Steele, H. 2020).

A study in New Zealand found that at 50% of the dose rate of frozen sex sorted semen, liquid sex sorted semen was able to achieve a performance of over 94% of conventional semen, which was between 15 and 25% better than the 70 to 80% achieved by frozen sex sorted semen relative to conventional semen. Therefore, the efficiency gains from liquid over frozen sex sorted semen is 2-fold: twice as many straws per unit time of sorting and 15 to 25% increase in conception rate.

(However, this technology is only applicable to countries with seasonal calving herds, with bulls for semen collection residing in the country where semen is used), (Xu, Z. 2014).

The lack of any previous records of field fertility for young sires increases the risk of processing a sire with poor fertility. To date, no diagnostic test, (or suite of tests), has been successfully developed to accurately predict sire fertility, or how a sire will respond to the sexing process. The identification and removal of these sires would ensure that only the most suitable sires are processed, thereby improving the relative performance of sexed semen compared with conventional semen.

In a recent Irish study, the researchers identified markers of sperm function and seminal plasma composition that were correlated with the fertility of both and sexed sorted sperm, highlighting the need for differing fertility markers for non-sexed or sexed sorted sperm, Holden, S. et al., (2017). In a 2021 study, a set of micro-RNAs detected in conventionally produced semen batches were linked to the fertilizing potential of bovine sperm after sex-sorting. These micro-RNAs should be further evaluated as potential biomarkers of a sire's suitability to produce sex-sorted sperm, (Keles, E. et. al., 2021).

Abortion rates were similar, but male stillbirths averaged 6.3% higher with sexed semen. Several possible reasons for this difference were postulated, (Reese, S. et al., 2021).

There is evidence that lowering the pressure in the sperm sorting system from 50 to 40 psi, (or less), improved conception for most bulls, (Schenk, J. et. al., 2009). These researchers also concluded that at least for heifers in a timed AI program, AI them approximately 12 hours later than that for unsexed semen was beneficial.

In a meta-analysis of 'the reproductive success of bovine sperm after sex sorting' between 1999 and 2021, the results showed than on average, non-return rates were 13% lower, pregnancy rates were reduced by 23%, (25% in cows and 21% in heifers) and calving rate reduced by 24% for sex sorted versus unsorted. Since 2015, with refinements in the processing and the development of SexedULTRA™ (see below), the gap between the two types of semen has narrowed.

When to AI

The literature is equivocal on when animals, (to receive sexed semen) should be inseminated. It seems that it may depend on whether a fertility programme is used (and what type), or whether animals are being served following a natural heat without intervention. The general recommendation is about 18 hours after the onset of heat, (normally 12 hours for unsexed semen).

Modelling studies

(Ireland, 2017) This study identified a significant profit advantage to using sex-sorted semen in the context of a high-output, spring-calving dairy system in Ireland. The financial advantage was largest in a scenario where both heifers and cows, rather than heifers only, were inseminated with sex-sorted semen. The financial advantage of sex-sorted semen was most sensitive to (1) base pregnancy rate, (2) sex-sorted semen pregnancy rate, and (3) standard deviation of the breeding selection index. The income contribution of increased genetic gain was quite modest, with the main financial advantage of sex-sorted semen arising from enhanced sales values for young stock, (Cottle, D. et. al., 2017).

(Ireland, 2021) The increased sale value of the beef calves more than compensated for the increased insemination costs of sexed semen, even when coupled with a reduction in pregnancy

rates. The use of dairy sexed semen to generate replacements, combined with beef breed AI, (or natural service beef breed bull), to generate non-replacements would lead to a marked increase in the beef merit of the calves derived from the dairy herd. The use of synchronization and targeting the best dams for AI with sexed semen would be a useful strategy to mitigate risk of poor conception. The use of sexed semen has significant potential to help drive the integration between the dairy and beef herds, if used correctly. The added advantage of increased genetic gain has the potential to further increase the potential of sexed semen to deliver increased profitability in the future, (Ruelle, E. et. al., 2021).

(Ireland, 2021) Adoption of timed AI and sexed semen have the potential to increase profitability in high-producing pasture-based dairy systems. Use of timed AI with sexed semen facilitates more rapid genetic progress through increased selection intensity. From a farm management perspective, timed AI alleviates the labour requirement for oestrus detection and has been shown to be most profitable in combination with sexed semen use in high-producing pasture-based dairy systems, (Walsh, D. et. al., 2021).

(USA, 2013) Three distinct scenarios were created to model 3 initiatives that a producer might take with sexed semen: (1) using sexed semen on heifers, (2) using sexed semen on heifers and a fraction of the genetically superior cows, and (3) using sexed semen on heifers and a fraction of the genetically superior cows, and breeding all other cows with beef semen. Profitability was very sensitive to the price of dairy heifer calves, relative to beef and dairy bull calves. Dairy heifer price proved to be the most influential variable, regardless of scenario. Targeting heifers first but also using sexed semen on cows is more profitable than trying to regulate sex only in heifers. Finally, a producer should target non-replacements to beef or dairy based on expected relative prices, (McCullock, K, et. al., 2013).

(Italy, 2020) The combined use of sexed semen to produce purebred replacement dairy heifers and conventional beef semen to produce terminal beef × dairy crossbred calves does not reduce herd fertility. This is because the possible, non-significant reduction in the conception rate associated with the use of sexed semen is compensated for by the significant increase in the conception rate associated with the use of beef semen. Taking into consideration the number of sexed semen doses needed per pregnancy and the welfare of the cows with regard to possible cases of dystocia, sexed dairy semen is particularly recommended for use in virgin heifers and beef semen is recommended for use in mature cows. A combination of sexed dairy semen and conventional beef semen could also be used in rotational dairy crossbreeding systems; this would exploit the significant increase in conception rate due to heterosis also in the production of replacement heifers and a further increase in beef production, because of the lower replacement rate of crossbred dairy cows. The quantitative and qualitative increase in beef production from dairy herds has the potential to reduce the ecological footprint of beef production and improve the profitability of dairy farms. The financial benefit will depend, in particular, on the (decreasing) cost of sexed semen and on the disparity in the price of purebred dairy bull calves and beef × dairy bull and heifer calves, (Bittante, G. et. al., 2020).

(Australia, 2017) Concurrent adoption of sexed semen and genomic testing offers an opportunity to increase net benefit more than genomic testing only, but is highly influenced by the individual parameters of a farm. Currently, benefits from using sexed semen, in conjunction with genomic testing, are most likely to be seen when usage is restricted to heifers. Wide-scale use of sexed semen, in conjunction with genomic testing, will largely depend on achieving conception rates that are comparable to those of conventional semen and the sale price of these surplus heifers, (Newton, J. et. al., 2017).

New developments:

SexedULTRA™/ Sexcel®

This is a system devised to simplify and optimize the media used for the sex-sorting protocol, so that the stressors are removed and the sperm are retained in a medium that is far more benign. Also, straws contain 4 million spermatozoa, rather than the normal 2 million per straw. The end result is conception rates that are on a par with conventional unsexed semen. This is marketed as SexedULTRA 4M by the Cogent Breeding Company. A German study, (Lenz, R., et. al., 2016) compared conventional semen, (15 million sperm/dose), with normal sex-sorted sperm and also SexedULTRA at 2.1, 3.0 and 4 million sperm/dose. They found that the SexedULTRA 4 million sperm had a similar non-return rate at day 56, compared to conventional semen at 15 million sperm and also that moving from 2.1 to 4 million sperm/dose significantly improved conception rates.

Genus ABS have a similar technology, marketed as Sexcel®, with a claimed 90% conception rate for sexed compared to conventional semen. (Cannot find any scientific papers for dairy cows/heifers to back up these claims from Genus ABS).

Summary/Conclusions

Sexed semen is now increasingly used in the dairy cattle industry and it is likely that this trend will continue.

Replacement heifers that should have the best genetics and the highest conception rates are the obvious place to start with sexed semen.

Young fit/health cows with no calving issues, which should have the best genetics (after maiden heifers), are the next animals to target.

All animals with poorer genetics/late calvers should be targeted with beef semen.

The genetics of each animal should be ascertained, either from milk recording (PLI, EBI) and/or genomic testing, to aid selection for breeding/sexed semen.

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