

Appendix 1: Guidelines for sexed semen usage in dairy herds

The current sexed semen product produced by Sexing Technologies and Cogent is marketed under the tradename Sexed ULTRA 4M. This product was evaluated in field trials conducted in Ireland in 2018 (AI after detected heat) and 2019 (fixed-time AI). In both trials, using bulls that were resident in a stud at the sex-sorting laboratory, the mean conception rate for sexed semen was poorer than conventional semen (2018: 50.2% vs. 60.3%; 2019: 50.1 vs. 61.1% for sexed and conventional, respectively). The performance of sexed semen versus conventional is often expressed as the relative conception rate $[(CR \text{ for SS} \div CR \text{ for CONV}) \times 100]$. In 2018 and 2019, the mean relative conception rate ranged from 82% to 84%. This means that the conception rates achieved with sexed semen, on average, were 82% to 84% of those achieved with conventional semen. Maximising conception rate with sexed semen requires careful animal selection, appropriate timing of AI, and attention to detail regarding straw handling.

Bull selection

Identify the bulls with the highest EBI that are available sexed, and within that list, identify the bulls that are suited to the herd's breeding objectives. At present, it is not possible to predict which bulls will have good field fertility and which bulls will have poor field fertility after the sorting process. To mitigate the risk, use sexed semen from a large team of bulls (≥ 5) in equal proportions.

Dam selection

Sexed semen use must be targeted towards the dams with the expected best fertility to maximise the likelihood of conception.

- Heifers
 - Have achieved the target live-weight for breed and BCS ≥ 3.25 .
 - Regularly cycling.
- Cows
 - Parity 1 to 4.
 - >50 days in milk on day of AI.
 - BCS ≥ 3.00 on the day of AI.
 - Regularly cycling.
 - Free of postpartum disorders and uterine disease.

Timing of AI

Sperm cells are damaged during the sorting process, and consequently the viability of the sex-sorted sperm cells in the reproductive tract is shorter (<12 h) than for conventional sperm cells (>24 h). Hence, the timing of AI is more important when using sex-sorted semen straws than conventional semen straws. When heifers/cows are being inseminated with sexed semen after observed heat, AI should be conducted 14 to 20 h after heat onset. In the

table below, the dams that are best suited for AI with sexed semen are indicated based on time since onset of heat. If AI is being conducted once a day, some dams will be at the optimum time for sexed semen, and all others should receive conventional semen. If AI is being conducted twice a day, most dams will be at the optimum time for sexed semen either in the morning or in the evening.

First obs. heat	Hours since heat onset at 7 AM	Suitable for sexed	Suitable for conv	Hours since heat onset at 3 PM	Suitable for sexed	Suitable for conv
06:00	1 h	-	++	9 h	+	++++
10:00	21 h	++++	++	5 h	-	+++
14:00	17 h	++++	++++	1 h	-	++
18:00	13 h	+++	++++	21 h	++++	++
22:00	9 h	+	++++	17 h	++++	++++

Fixed time AI is costly, but provides some advantages when using sexed semen. It facilitates targeting of sexed semen usage on the dams that it is desirable to get replacements from, and importantly, this can be scheduled to be completed on the farm mating start date. Conducting AI with sex-sorted semen straws on the farm mating start date advances the submission of the targeted heifers/cows, and mitigates the risk of poor conception rates causing a deterioration in the calving pattern.

Straw Handling

It is important to be fully aware that the sex-sorting process causes some damage to the sperm cells. Hence, careful handling of the straws from the time of removal from the AI tank to deposition of sperm cells into the uterus is more critical for sexed semen than for conventional semen.

- Organise sexed straws into one goblet on the tank, and minimize the frequency that the goblet is lifted.
- Change water in the thawing unit daily, and clean the thawing unit weekly.
- Check that the temperature in the thawing unit is 35 to 37 °C.
- Thaw a maximum of two sexed semen straws at a time.
- Using a timer, thaw the straws for 45 seconds.
- Load straws into pre-warmed AI guns.
- Keep AI guns warm after loading straws, and ensure that inseminations are promptly completed (<5 mins after loading).
- Deposit semen in the uterine body.

How many sexed semen straws do I need to use?

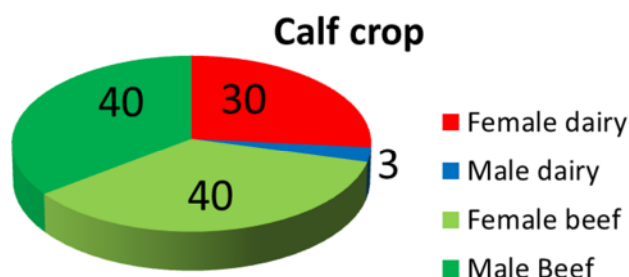
The exact figure depends on herd fertility, so better fertility means fewer sexed semen straws to get the same number of pregnancies. If we take the example of a herd of 100 lactating cows with 25 maiden heifers available for breeding, the goal is to use the number of sexed semen straws required to achieve 30 female dairy calf births. This is done by prioritising sexed semen first for heifers, and then for selected cows using the criteria previously outlined under dam selection. All remaining dams are bred to beef semen (AI for first 6 weeks, AI or natural service bulls thereafter). The assumed conception rates for heifers and cows with conventional and sexed are outlined in Table 1, which equates to approximately 85% relative conception rate.

Table 1. Assumed conception rates in heifers and cows with conventional and sexed semen.

	Heifer conception rate	Cow conception rate
Conventional	70	60
Sexed (observed)	60	50
Sexed (TAI)	60	50

We assume that all 25 heifers are suitable for sexed semen. With 60% conception rate and 90% sex bias, this will result in 13.5 heifer calves ($25 \times 0.6 \times 0.9$). It will also result in 1.5 male calves ($25 \times 0.6 \times 0.1$). Any heifers that repeat are bred to an easy-calving beef stock bull for the remainder of the breeding season. If we assume 95% final in-calf rate, this will mean 8.75 beef cross calves.

How many to sexed semen straws need to be used on the lactating dairy cows? We want to achieve 30 female dairy calf births, and 13.5 are coming from the heifers, meaning that 16.5 are required from the lactating cows ($30 - 13.5 = 16.5$). To achieve 16.5 female calves using sexed semen straws with 50% conception rate and 90% sex bias will require 37 straws ($16.5 / (0.5 \times 0.9) = 37$). This will result in 16.7 female dairy calves ($37 \times 0.5 \times 0.9$) and 1.9 male dairy calves ($37 \times 0.5 \times 0.1$). After the allocation of sexed semen straws have been used, all remaining cows and all repeats are bred to high DBI beef AI followed by a mop-up easy-calving beef stock bulls. If we assume 90% final in-calf rate, this will mean 71.4 beef cross calves.



The calf crop that arises highlights that the required number of female dairy calves was achieved, the number of male dairy calves was greatly reduced, and the number of readily marketable beef calves was markedly increased.