The use of sex-sorted semen in Irish dairy farming

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explores sex-sorted semen, its benefits, the factors that influence its success, and herd implementation

Selection of offspring sex with sex-sorted (SS) semen has the potential to dramatically change the landscape of the dairy industry. Historically, high cost and reduced fertility rates have restricted SS semen use. Technological advancements, commercial availability and ongoing research have made the application of SS semen a profitable endeavour for most herds.[5,15] The aspirational benchmark of comparable fertility of SS semen to conventional semen has yet to be fully achieved. Teagasc has worked extensively to identify and overcome SS semen fertility obstacles and help vets and farmers apply this technology to the Irish dairy industry.[2]

BENEFITS OF SEX-SORTED SEMEN

The benefits of SS semen are far-reaching and impactful. The Irish dairy industry has experienced tremendous growth since the 2015 abolition of milk quotas. The national herd size increased 3% between 2017 and 2019, with a projected 200,000-cow increase by 2027.[1] Increasing the proportion of heifer births facilitates rapid herd expansion that aims to maximise profits. Herd expansion using SS semen will eliminate the need to purchase costly replacement heifers while maintaining biosecurity on the farm. Risks of the introduction of Johne's disease, leptospirosis, and other infectious diseases into the herd are eliminated when closed herd expansion can be achieved.[3]

Entrant herds are steadily joining the Irish dairy industry due to profitability of the sector. These new herds, coupled with farmers wishing to rapidly expand their existing herds, are driving a market for heifer sales. Farmers can benefit from this additional economic opportunity by fueling the use of SS semen.

The pasture-based, seasonal calving pattern in Ireland relies on heifers achieving body-weight targets and breeding at 15 months of age to achieve a timely calving at 24 months of age and benefit from peak pasture nutrition.[3] The use of SS semen at the beginning of the breeding season ensures replacement heifers are calved and reared early in the following season. These heifers can then reasonably achieve their target body weights and conception by 15 months, thus reinforcing the ongoing seasonal calving pattern.

Dairy bull calves are, sadly, an undesirable by-product of the industry and they raise significant welfare concerns. The use of SS semen to achieve desired heifer yields while strategically breeding dairy cows to beef sires to produce calves with improved feed conversion for the beef sector will create a profitable market for unwanted dairy calves while maintaining animal welfare.[7] Additionally, improved feed conversion for beef-cross calves and reduced time to target body weight for slaughter can reduce greenhouse gas emissions.[10]

Using SS semen with heifers and elite cows will increase annual genetic gain within the herd.[12] Genetic gain is primarily driven by bull selection using artificial insemination (AI). SS semen enables additional gain to be achieved annually through targeted dam selection of replacement heifers.[12]

Finally, it has been concluded through bioeconomic models that SS semen benefits translate to increased herd profitability.[5,15] Farm economic profit can be increased by as much as €50 per cow when SS semen is used in herds with good fertility.[15] However, this financial advantage evaporates with herds that have poor conventional semen fertility rates as SS semen has reduced relative fertility. Herd fertility is the primary factor influencing the financial advantage of adopting SS semen in a pasture-based dairy production system.[15] Therefore, selecting herds with high conventional semen fertility and focusing efforts on minimising risks of poor fertility with SS semen will help maximise profitability for herds.

SEX-SORTED SEMEN TECHNOLOGY

A commercial laboratory to produce SS semen is currently unavailable in Ireland. Teagasc and Irish commercial herds utilise SexedULTRA 4M™ technologies operated by Cogent Breeding Ltd, in the UK.[13] SexedULTRA™ technology capitalises on the female (X-bearing chromosome) containing 3.8% more DNA than the male (Y-bearing chromosome). The difference in DNA is detected through flow cytometry, enabling differentiation of female and male sperm so they can be further deflected into different populations.[13]

On average, a Cogent SS semen straw contains over 90% female sperm.[13] Quality-control measures include morphological assessment, proportion of dead sperm in the sample, sperm membrane integrity, and fertilisation ability. The concentration of SexedULTRA™ semen is $4x10^6$ sperm per straw.[13]

In February 2021, the *Irish Farmers Journal* reported a deal

between Sexing Technologies and Teagasc Moorepark to potentially locate a sex-sorting laboratory at Fermoy in 2022. This is a positive development for the Irish dairy industry, and more details should emerge this year.

FACTORS INFLUENCING SUCCESS OF SEX-SORTED SEMEN

Six key factors influence the success of SS semen use. These factors have been established through Teagasc large field trials in 2018 (AI after heat detected) and 2019 (fixed-time AI). In these trials, the mean conception rate for SS semen was poorer than conventional semen.[6,8,9] The performance of SS semen versus conventional (CONV) is often expressed as a relative conception rate [(CR for SS ÷ CR for CONV) x 100]. Conception rate is then often expressed as pregnancy achieved per artificial insemination, or P/AI.[2]

1 **Timing within the breeding season**

Timing is an important success factor when SS semen is used in a herd. SS semen should always be used in the first three weeks of the breeding season, ideally in the first 10 days.[2] This will then ensure replacement heifers are produced early in the calving season as well as prevent reduced fertility from deteriorating the calving pattern.

2 **Herd variability**

Herd variability is another significant factor influencing the relative P/AI of SS semen documented in both the Teagasc 2018 and 2019 field trials.[6,8] In the 2019 field trial, cow selection was balanced for parity and days in milk (DIM), and they were subjected to the same nutrition and management factors across all herds. The mean relative P/AI was 83.3%, with a range from 48-121% between herds.[6] It is important to note that the tertile of herds with the poorest relative P/AI for SS semen achieved P/AI with CONV semen higher than the intermediate and best tertiles of herds. This demonstrates the fertility of cows in the herds was acceptable and the fixed-time AI (FTAI) protocol and AI procedures were properly implemented.[6] Herds with high fertility rates with CONV semen are not necessarily guaranteed to have acceptable fertility rates with SS semen.

Also, of significance, the tertile of herds with the best relative P/AI achieved with SS semen were similar to CONV.[6] In the 2018 field trial, approximately one third of herds achieved a relative P/AI of ≥90%, with the majority of herds in that subset achieving ≥100%.[8] This demonstrates a significant opportunity to enhance SS semen success if future research can determine the underlying factors contributing to a third of herds achieving relative P/AI near 100%. Currently, however, the factors influencing herd variation are still unknown. It is reasonable to help mitigate this risk by only selecting herds that have achieved fertility benchmarks with CONV semen including ≥60% conception rates, 90% calved in the first six weeks of the calving season, and a 365-day calving interval.[4]

3 **Dam selection**

Dam selection for SS semen usage is an integral part of programme success. Heifers should always be selected first as they, generally, represent the highest genetic gain and greatest fertility. By mating start date (MSD), heifers should all have reached their target body weights, be regularly cycling, and have body condition score (BCS) ≥ 3.25.[2] Similarly, cows selected following heifers should also be regularly cycling and have strong general health (free of reproductive disease, lameness, etc.). Factors influencing elite cow selection for SS semen usage have been determined through Teagasc 2018 and 2019 field trials[6,8,9]:

1. BCS ≥ 3.0
2. DIM > 50 on day of AI
3. Parity 1-4
4. Highest economic breeding index (EBI)

BCS ≥3.0 is the most influential factor on relative P/AI. Maicas et al (2019) demonstrated cows with BCS <3.0 had decreased relative P/AI independent of their DIM or fertility index.[9] EBI is heavily weighted for fertility, with the field trial concluding an EBI fertility subindex >€100 improved relative P/AI[9]. Drake et al (2020) concluded that both DIM >50 and parity 1-4 both had no impact on relative P/AI.[6] Using heifers followed by selected elite cows based on the criteria established in field trials should help alleviate risks of poor relative fertility with SS semen.[2]

4 **Bull variability**

Despite quality-control measures used by the laboratory, individual bulls can experience unpredictable poor field fertility following the sorting and freezing processes. Sperm can easily be damaged during the sorting process, and bulls can differ in susceptibility to these stressing procedures.[14] It has been established that bull fertility variation can be present with both fresh and frozen SS semen.[9] Maicas et al (2019) used split ejaculates to compare relative fertility of SS semen collected in Ireland and shipped to Cogent sex-sorting laboratory (UK) to that of SS semen collected from bulls resident at the sex-sorting laboratory. SS semen resulted in greater variation in P/AI in bulls whose ejaculates were shipped to the sorting center compared to resident bulls.[8] Selecting resident bulls from the sex-sorting laboratory can help reduce risks of poor bull field fertility using SS semen.

Teagasc 2018 and 2019 field trials also demonstrated that the bull had a significant effect on P/AI.[6,8,9] Bulls should still be selected for highest EBI and suit the herd's breeding objectives.[2] At this time, it is not possible to predict which bulls will have reduced fertility following sorting and how significant the reduction in fertility might be.[2] Further research is required to ascertain sperm function changes resulting from sex-sorting and promote the development of improved assessment techniques for SS semen. Currently, risk mitigation centers around accepting a proportion of bulls will experience suboptimal field fertility. For this reason, it is recommended to always select a minimum of five bulls used in equal proportions for SS semen use within a herd.[2]

5 **Critical semen handling**

The AI technician has an important role to play in the success of SS semen. Sperm are vulnerable to damage through the sorting process.[14] It is likely that straw handling procedures, thawing (including temperature and duration), and time from thawing to deposition of sperm in the uterus

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may be more critical for SS than CONV semen.[6]
Eighteen technicians were used in the 2019 Teagasc FTAI field trial with a wide variation in relative P/AI observed.[6] The majority of technicians with the poorest P/AI for SS semen conversely achieved the highest P/AI for CONV semen. This suggests their handling and technical skills favoured success of CONV semen. Similarly, the technician with 100% relative P/AI had a P/AI for CONV semen of 55%, suggesting his/her handling and technical skills favoured SS semen success.[6] At present, precise, preferential semen handling and technical skills for SS semen are unknown, and it is recommended to select a skilled and experience technician who strictly follows the current guidelines for handling SS semen to mitigate risks.[2]

SS semen handling guidelines[2]:

- Organise sexed straws within one goblet on the tank and minimise 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 35-37°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 min after loading).

6 Timing of AI

As a result of increased damage to sperm cells during the sorting process, viability of SS semen in the reproductive tract is shorter (<12 hours) than for CONV semen (>24 hours).[2] Consequently, the timing of AI is more critical when using SS semen straws. When heifers or cows are being inseminated with SS semen after an observed oestrus, AI should be conducted 14-20 hours after the onset of oestrus to best correspond to timing of ovulation.[2]
To accurately identify the onset of oestrus and achieve optimal oestrus detection rates with SS semen, observation must occur five times per day for a minimum recommended 20 minutes.[2] In the example herd in Figure 1, dams that are best suited for AI with SS semen are indicated based on the time since the onset of oestrus. To maximise submission of dams, AI must be conducted twice daily.[2] Although not all dams will still be suitable for SS semen, it is important to adhere to optimum oestrus detection and strict timing of AI 14-20 hours after onset of oestrus to achieve suitable fertility with SS semen.

First obs. oestrus	Hours since oestrus onset at 7 am	Suitable for SS semen	Suitable for CONV	Hours since oestrus onset at 3 pm	Suitable for SS semen	Suitable for CONV
06:00	1hr	-	++	9hr	+	++++
10.00	21hr	++++	++	5hr	-	+++
14.00	17hr	++++	++++	1hr	-	++
18.00	13hr	+++	++++	21hr	++++	++
22.00	9hr	+	++++	17hr	++++	++++

Figure 1: Example of oestrus observation and dam suitability for SS and CONV semen.[2]

USE OF SEX-SORTED SEMEN WITH FIXED-TIME AI

Fixed-time AI (FTAI) complements the use of SS semen by providing several advantages. The labour-intensive efforts of oestrus detection as well as the potential costly errors of missed observed oestrus are eliminated. Instead of twice-daily visits, AI technicians can be available at a prescheduled time to service the herd, further reducing costs and labour.
FTAI facilitates targeted use of SS semen on all selected dams, so desirable dams are not missed due to their timing of observed oestrus. This can be achieved on the MSD, advancing the submission of the targeted heifers/cows and reducing the risk of poor conception rates that ultimately cause a deterioration of the calving pattern.[2]
Teagasc has recommended FTAI protocols for SS semen based on their extensive field trials and international research (see Figure 2).[4,6] As with AI following oestrus detection, timing of FTAI is equally critical when used with SS semen. The 2019 Teagasc field trial using FTAI established that 16-22 hours following the second gonadotropin-releasing hormone (GnRH) injection is the optimal time to inseminate cows.[6] Heifers have other considerations such as their altered follicular wave patterns, optimised response to a shorter duration of progesterone, and practical concerns with reduction in the number of handlings. These factors are addressed with a shorter protocol and FTAI performed concurrently with GnRH for heifers.[4]

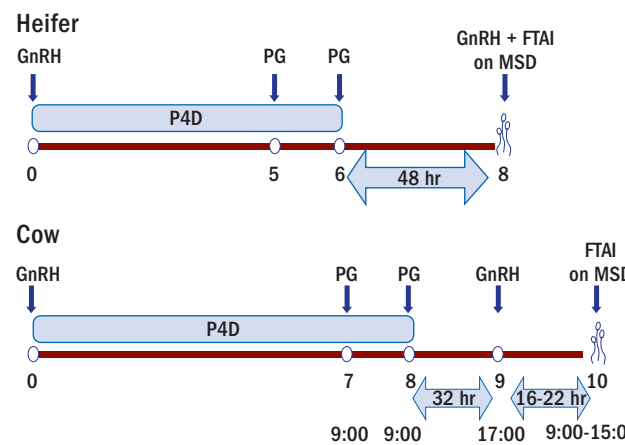


Figure 2: Fixed-time AI protocols for SS semen.[4,6] GnRH = gonadotropin-releasing hormone; PG = prostaglandin F2α; P4D = progesterone device; FTAI = fixed-time artificial insemination

Optimisation of the synchonisation protocol is necessary to maximise conception rates when dealing with an expensive product such as SS semen. Incomplete luteal regression is a well-documented obstacle impacting conception rates of synchronisation protocols in dairy cows and heifers.[11] Treatment with two prostaglandin F2α (PGF) injections 24 hours apart in an Ovsynch® protocol using progesterone-releasing intrauterine devices (PRIDs) increased the number of cows with luteal regression when compared to cows treated with a single PGF injection. Cows receiving a second PGF injection also documented a higher conception rate, 42.6 versus 35.7% for a single PGF treatment.[11]
It is important to use the recommended FTAI protocol to help

achieve luteal regression, thereby maximising subsequent ovulation and conception rates. Promising bioeconomic studies assessing herd profitability using SS semen with FTAI are currently under review. The use of FTAI can alleviate risks associated with SS use, minimise labour, and target SS semen use on all desired dams at the MSD to facilitate a profitable seasonal calving pattern.

HERD IMPLEMENTATION

When discussing SS semen use with a farmer, it is important to assess the herd's suitability for the product. Herds should already have strong fertility management and be achieving Teagasc recommended benchmarks including ≥60% conception rates with CONV semen, 90% calved in the first six weeks of the calving season, and a 365-day calving interval. If herds are not achieving these benchmarks, then there are management factors to address prior to considering SS semen as part of their breeding strategy.[4] Farmers must be informed of anticipated conception rates based on Teagasc field trials and international research (see Figure 3), obstacles influencing SS semen success and risk-mitigation steps that can be taken (see Figure 4).[2]

	CONV	SS (observed oestrus)	SS (FTAI)
Heifer P/AI	70	60	60
Cow P/AI	60	50	50

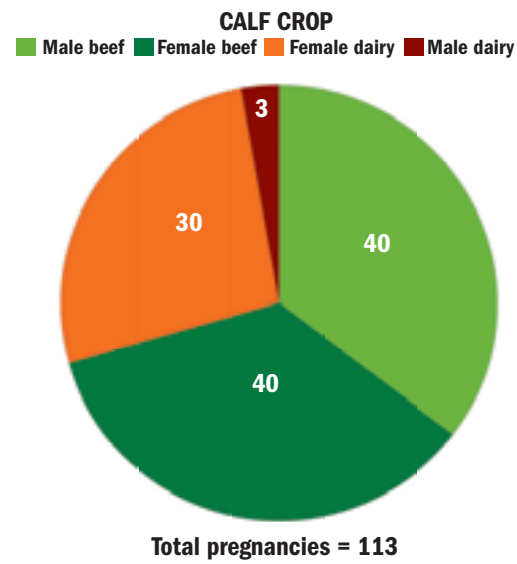
Figure 3: Expected P/AI achievable with CONV and SS semen.[2]

Obstacle influencing SS semen success	Risk mitigation step
Reduced P/AI can result in late calving, deterioration of seasonal calving pattern	· Use SS semen in first 3 weeks of breeding season, ideally first 10 days · Use FTAI to start SS semen AI on MSD
Herd variation of relative P/AI	· Select herds already achieving P/AI ≥ 60%, 90% calving in first 6 weeks of season and 365-day calving interval
Dam fertility	· Breed replacement heifers first · Breed elite cows selected for BCS≥3,DIM≥50, Parity 1-4 and highest EBI · Use FTAI to ensure submission of all desired dams
Variation in bull field fertility	· Use≥5 bulls in equal proportions · Avoid shipping prior to sex sorting · Select highest EBI buls
Critical semen handling	· Follow guidelines for SS semen handling · Select experienced and skilled AI technician
Timing of AI due to decreased duration of SS semen viability in female reprodcutive tract	· Use FTAI to control ovulation timing and reduce AI technician visits · Use FTAI to eliminate labour and errors with oestrus detection

Figure 4: Summary of risk-mitigation steps to consider when implementing SS semen in breeding strategy.

Once the herd has been established as suitable for SS semen usage, the desired calf crop should be determined. Accounting for the desired calf crop, expected conception rates, gender bias of SS semen, and anticipated final in-calf rates (FICR), the number of SS semen and beef straws can be calculated.[2] Teagasc has provided a calculation for an example herd (see Figure 5).[2]

The use of SS semen has the potential to achieve vast and profitable benefits for the dairy industry including expedited herd expansion, timely calving of replacement heifers to facilitate the seasonal calving pattern, reduction of low-value dairy bull calves while producing more profitable beef-cross calves and increasing annual genetic gain for the herd.[2] These benefits come with risks of reduced fertility. Careful planning and implementation of risk mitigation steps can help achieve a successful and profitable breeding program using sex-sorted semen



Herd of 100 cows and 25 replacement heifers

Desired calf crop:
- 30 day females (replacements)
- Remainder beef (male female)

Heifers
- AI all with dairy sexed semen (25 heifers)
  - 25 x 0.6 x 0.9 = 13.5 dairy replacements
  - 25 x 0.6 x 0.1 = 1.5 dairy calves
- Open repeats breed of beef AI
  - 95% FICR = 23.75 heifers
  - 23.75 - (13.5 + 1.5) = 8.75 beef calves

Cows
- How many to AI with dairy SS semen?
  - 30 - 13.5 = 16.5 needed
  - 16.5 (0.5 x 0.9) = 37 straws needed
  - 37 x 0.5 x 0.9 = 16.7 dairy replacements
  - 37 x 0.5 x 0.1 = 1.9 dairy calves

Open repeats breed of beef AI
- 90% FICR = 90 cows
- 90 - (16.7 + 1.9) = 71.4 beef calves

Figure 5: Semen straw calculations for example herd using SS semen.[2]

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READER QUESTIONS AND ANSWERS

1. BENEFITS OF SEX-SORTED SEMEN INCLUDE:
- **A.** Expedited herd expansion
- **B.** Increased annual genetic gain of herd
- **C.** Reduction of unwanted dairy bull calves
- **D.** Facilitates seasonal calving pattern
- **E.** All of the above

2. WHICH OF THE FOLLOWING IS NOT A FACTOR INFLUENCING SUCCESS OF A SEX-SORTED SEMEN BREEDING PROGRAMME?
- **A.** Bull variability
- **B.** Timing of AI from observed oestrus or within FTAI protocol
- **C.** Breed of dam
- **D.** AI technician

3. WHICH OF THE FOLLOWING IS A FEATURE OF RECOMMENDED FIXED-TIME AI PROTOCOLS FOR SEX-SORTED SEMEN?
- **A.** Heifers receive GnRH injection concurrently with timed insemination
- **B.** Single injection of prostaglandin F2α to achieve luteal regression
- **C.** Insemination 14 to 20 hours after observed oestrus
- **D.** 7-day progesterone device

4. WHICH OF THE FOLLOWING CRITERIA IS NOT CORRECT FOR SELECTING ELITE COWS FOR SS SEMEN USE?
- **A.** DIM > 50 at day of AI
- **B.** Free of reproductive disease
- **C.** Parity 1-4
- **D.** BCS ≥ 3.5

ANSWERS: 1E; 2C; 3A; 4D.