



# Artificial insemination increases the probability of a male calf in dairy and beef cattle

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## Abstract

The objective of this study was to determine if natural mating affected secondary sex ratio. Data consisting of 642,401 calving records from the Irish national database, during the years 2002–2005, were used in the analysis. Factors affecting the logit of the probability of a male calf being born were determined using multiple regression generalised estimating equations with sire of the calf included as a repeated effect. Month of the year at calving, sex of the previous calf born within dam, breed of service sire, parity of dam and type of mating (i.e., natural or artificial insemination) significantly ( $P < 0.05$ ) affected the likelihood of a male calf being born. Male calves were more likely to be born in the warmer months of the year, when the sex of the previous calf born to the same dam was male, in older cows and when the service sire was a beef breed. No significant interaction between the main effects existed. The odds of a male calf being born, following adjustment for confounding effects, varied from 1.04 to 1.08 ( $P < 0.01$ ) across the years of analysis when artificial insemination was used compared to natural mating. This equates to a 1% unit increase in the probability of a male calf being born following artificial insemination.

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## 1. Introduction

The ability to alter secondary sex ratio in cattle is financially attractive. In beef cattle, the increased growth rate and more efficient production of lean meat by males [1] has financial advantages. In contrast, dairy farmers generally seek female dairy breed calves to perpetuate their herd while male beef crossbred animals are desired for the reasons cited above. Furthermore, the requirement for a lower male to female sex ratio (i.e., lower sex ratio) in dairy cattle is augmented when replacement rates are high. Hence, the financial

attraction to manipulate sex ratio has led to increased interest in techniques such as semen sexing [2,3] and embryo sexing [3,4].

There is anecdotal evidence from Irish farmers that natural mating increases the probability of a female calf in dairy herds. However, this has never been scientifically proven or refuted. Several factors may affect any potential association between mating type and sex ratio. One contributing factor may be the effect of timing of mating on the resultant sex ratio with a higher proportion of female offspring following early insemination [5]; this may result in a lower sex ratio from natural mating which has to occur during estrus. Nonetheless, other studies in cattle have failed to identify a relationship between timing of service and sex ratio [5,6]. Another potential contributing factor to

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an association between mating type and sex ratio is the increased sex ratio from frozen semen compared to fresh semen [7].

Milk production in Ireland is predominantly based on compact spring calving, thereby requiring a compact breeding season to optimise profitability. In Ireland, farmers that use artificial insemination (AI), generally do so for the first 6 weeks of the breeding season and use natural mating thereafter. This alone may impact sex ratio because of the previously observed effects of time of the year and body condition score change from calving to conception on secondary sex ratio [8,9] as well as the effect of number of services on secondary sex ratio [10]. Additionally, natural mating with beef bulls tend to be more frequently used on dairy virgin heifers which may also confound any possible effect of mating type on sex ratio if age of dam or breed of service sire affects sex ratio [11,12]. Therefore, statistical approaches such as multiple regression should be used to adjust for confounding effects such as time of the year at service, age of dam and breed of service sire amongst others.

The objective of the present study was to determine whether natural mating or AI in dairy and beef cattle influences the sex of the resultant calf.

## 2. Materials and methods

### 2.1. Data

Data on calf, sire and dam identification number, as well as date of birth and sex of the calf, parity number of the dam and breed of the sire and dam were extracted from the Irish Cattle Breeding Federation database during years 2002–2005; 3,562,644 records were available for inclusion in the analysis. Calves with no identified sire ( $n = 1,972,240$ ) were removed as were records coded as abortions ( $n = 4134$ ). Records with no information on dam parity number ( $n = 6124$ ) were also removed from the dataset. No multiple births were included in the analysis. Only herd-years with between 20 and 200 records were retained. Only records from sires with at least 10 progeny in the remaining dataset were retained. Finally, only calves born from sires or dams where their main breed component (i.e.,  $>1/2$  their genes) were Holstein/Friesian, Simmental, Angus, Hereford, Charolais, Limousin, or Belgian Blue were retained. Furthermore, herds that only used either all AI or all natural mating within year were removed. In total, 642,401 records were available for inclusion in the analysis.

Month of birth was derived from birth dates for each calf. Sex of the previous calf within dam was also

determined. Dams with no prior information on calf sex (e.g., first parity animals) were coded separately to facilitate their inclusion in the multiple regression analysis. Parity number was recoded into five classes: 1, 2, 3, 4–6 and 7–9. Parity number of the dam in the present study was parity of the cow the day after she calved. Therefore, the calf of a first parity animal represents the calf born following a mating to a heifer. Breed of sire had seven levels representing the main breed component and were defined as Holstein–Friesian, Simmental, Angus, Hereford, Charolais, Limousin and Belgian Blue. Breed of cow was coded similarly. Calf breed was generated as a binary variable and received a value of 1 if the cow and sire were of the same breed, otherwise 0 indicating a crossbred calf. A dichotomous variable was generated where a record received a value 1 if the mating was AI, otherwise 0 indicating natural mating.

### 2.2. Analysis

The logit of the probability of a male calf being born was modelled using generalised estimating equations in PROC GENMOD [13]. Service sire was included as a repeated effect in the model with an exchangeable correlation structure assumed among records within sire. Initially, a series of univariate analyses were undertaken with explanatory variables individually included in the model of analysis. Subsequently, a stepwise forward backward algorithm was invoked to develop the most parsimonious multiple regression model; the threshold significance levels for entry and staying in the model were  $P < 0.15$  and 0.05, respectively. Significance was based on the GEE score statistic. Possible explanatory variables generated for inclusion in the multiple regression model were: natural mating or AI, sire breed, dam breed, whether the calf was a crossbred or not, parity of dam, sex of previous calf within dam and month of the year at birth. All variables were treated as class variables. Biologically plausible two-way interactions were also tested for significance in the model.

Computation restrictions did not facilitate analysis of the entire data set at once. Therefore, each of the 4 years, 2002 ( $n = 123,837$ ), 2003 ( $n = 147,862$ ), 2004 ( $n = 182,892$ ) and 2005 ( $n = 187,810$ ) were analysed separately. Furthermore, because of the large computation demands of estimating herd effects a preliminary analysis was undertaken on a subset of the data. A random sample of one-third of the herds within year was taken using PROC SURVEYSELECT (SAS, 2006). These datasets were used to determine if herd had a significant effect on sex ratio. Herd was not significant

( $P > 0.20$ ) in either analysis which is consistent with a previous study [14]. Therefore, all further analyses were undertaken on the entire annual datasets without herd in the model.

The probability of a male calf being born,  $P(X)$ , was estimated using the solutions from the multiple regression model as

$$P(X) = \left( 1 + e^{-(\hat{\alpha} + \sum_{i=1}^m \hat{\beta}_i X_i)} \right)^{-1}$$

where  $\hat{\alpha}$  is the predicted intercept of the model,  $\hat{\beta}$  the predicted regression coefficient for independent variable(s)  $i$  and  $X_i$  is the associated design matrix for variable(s)  $i$ . Odds ratios were calculated as the exponent of the model solutions. An odds ratio compares opposing probabilities to determine which is the more likely result for a given outcome; in this instance the outcome was the probability of a male calf. In the present study, if the odds ratio is 1.5, then animals exhibiting the level of the independent variable under investigation have a 50% greater likelihood of having a male calf. An odds ratio of 2 reflects double the likelihood of a male calf.

### 3. Results

Across the entire dataset, the ratio of males to females was 50:50; although it varied from 49:51 in 2000 to 50:50 in 2001 and 2002 to 51:49 in 2005. All variables significantly ( $P < 0.01$ ) affected the logit of the probability of a male calf in the univariate analyses with the exception of mating type. Table 1 outlines the proportion of calvings from natural matings in 2005 across different parities, months of the year and breeds with herds using either all AI or all natural mating also

Table 1  
Proportion of calvings from natural matings in 2005 across different parities, months of the year at birth and sire breed

Parity	Value	Month of the year	Value	Sire breed	Value
1	0.52	January	0.29	Angus	0.72
2	0.39	February	0.33	Belgian Blue	0.16
3	0.40	March	0.51	Hereford	0.81
4–6	0.42	April	0.65	Charolais	0.72
7–9	0.49	May	0.69	Simmental	0.73
		June	0.72	Limousin	0.70
		July	0.73	Holstein–Friesian	0.27
		August	0.49		
		September	0.30		
		October	0.33		
		November	0.36		
		December	0.38		

included in the dataset. Natural mating was more common in very young and very old cows. The majority of services to Belgian Blue sires that lead to a subsequent calf were through AI, which was similar for Holstein–Friesian sires. Based on the univariate analyses the odds of a male calf being born from natural mating compared to AI was 1.01, 1.02, 1.02 and 1.11 in 2002, 2003, 2004 and 2005 data, respectively. However, all confidence intervals included one implying that mating type was not significant.

#### 3.1. Multiple regression analysis

Factors affecting the logit of the probability of a male calf (in order of entry) in the multiple regression model were month of the year at birth, sex of previous calf born within dam, breed of sire, parity of dam and type of mating. Parity did not significantly affect sex ratio in the year 2004. Fig. 1 illustrates the effect of month of birth on the odds and predicted probability of a male calf being born. There was a generally higher probability of a male calf being born in the warmer months of February–June compared to the colder months; the exception being, the year 2002 when the probability of a male calf was lower for most months of the year. Nonetheless, across all months of 2005, the predicted probability of a male calf varied by only 2.4% units.

In 2005, the odds of a male calf were 1.01 times (95% CI: 1.00–1.03) greater when the previous calf born from that dam was male; the corresponding odds in 2002, 2003 and 2004 were 1.03, 1.04 and 1.07, respectively, and were significantly different from one in all years. The odds ratio of 1.01 in the year 2005 manifested itself as an increase in predicted probability of a male calf from 0.50 to 0.51 when the previous calf born from that dam was female or male, respectively, in a second parity cow, calving in March following mating to a Holstein–Friesian AI sire.

The odds of a male calf being born were significantly higher when the service sire was of a beef breed than when it was Holstein–Friesian (Fig. 2) although there was no effect on secondary sex ratio of the breed of the dam, or whether the mating was a crossbred mating or not. Generally, there was no significant difference in the likelihood of a male calf between the beef breeds represented with the exception of a significantly ( $P < 0.05$ ) greater likelihood of a male calf from Hereford than from Angus, Charolais or Limousin sires in the year 2002.

The probability of a male calf being born was significantly higher in older cows compared to younger cows (Fig. 3). No significant difference existed in the

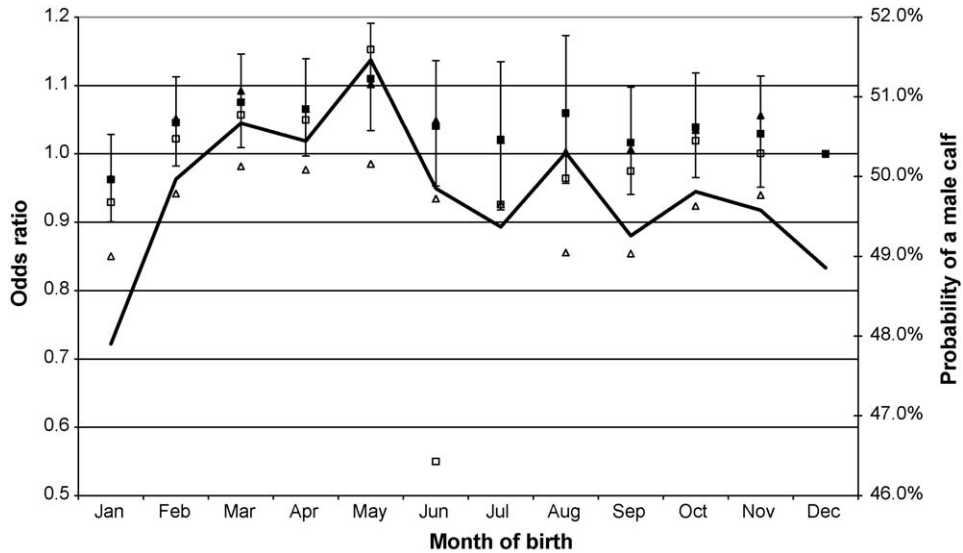


Fig. 1. Odds ratios (95% confidence intervals for the monthly odds ratios using the 2005 data included as vertical bars) for the effect of month of birth on the probability of a male calf using data from 2002 (open triangle), 2003 (shaded triangle), 2004 (open square) and 2005 (shaded square). December was taken as the reference class. Also included (continuous line) is the probability of a male calf being born for each month of the year by a second parity cow that previously had a male calf, mated to a Holstein–Friesian AI sire, using the 2005 multiple regression model solutions.

likelihood of a male calf being born from first parity (i.e., served as heifers) and second parity cows, the exception being 2002 when second parity animals had a greater ( $P < 0.05$ ) likelihood of a male calf compared to first parity animals. Older parity animals, however, had significantly greater odds of a male calf than first parity

cows. In the years 2003 and 2005, second parity cows had a significantly lower odds of a male calf than older cows.

Although not significant in the univariate analyses, mating type, following adjustment for confounding effects in the multiple regression model, significantly ( $P < 0.01$ ) affected the logit of the probability of a male

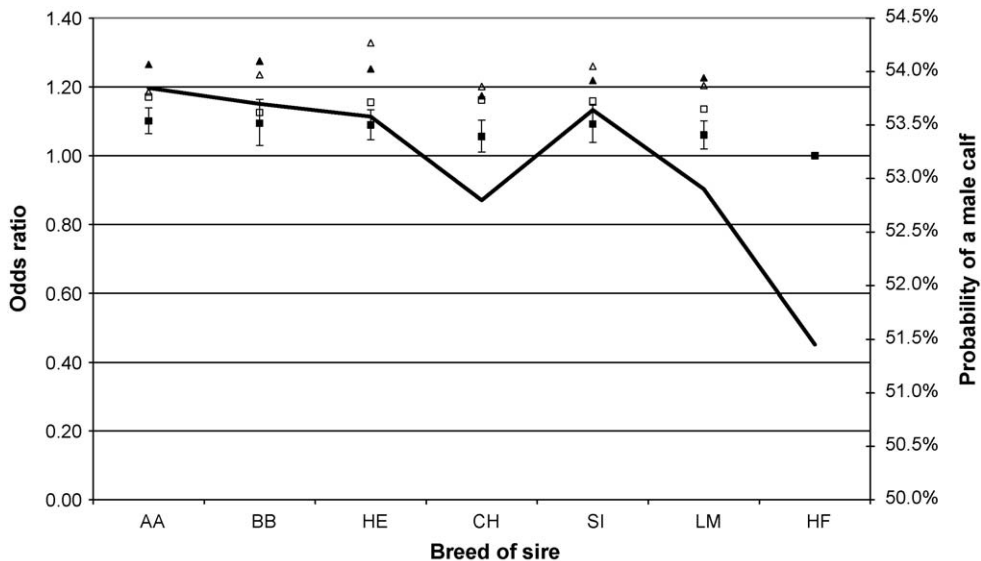


Fig. 2. Odds ratios (95% confidence intervals for the odds ratios using the 2005 data included as vertical bars) for the effect of breed of sire (AA, Angus; BB, Belgian Blue; HE, Hereford; CH, Charolais; SI, Simmental; LM, Limousin; HF, Holstein–Friesian) on the probability of a male calf using data from 2002 (open triangle), 2003 (shaded triangle), 2004 (open square) and 2005 (shaded square). Holstein–Friesian (HF) animals were taken as the reference class. Also included (continuous line) is the probability of a male calf being born to a second parity March calving cow that previously had a male calf and who was bred using AI to each breed of sire, using the 2005 multiple regression model solutions.

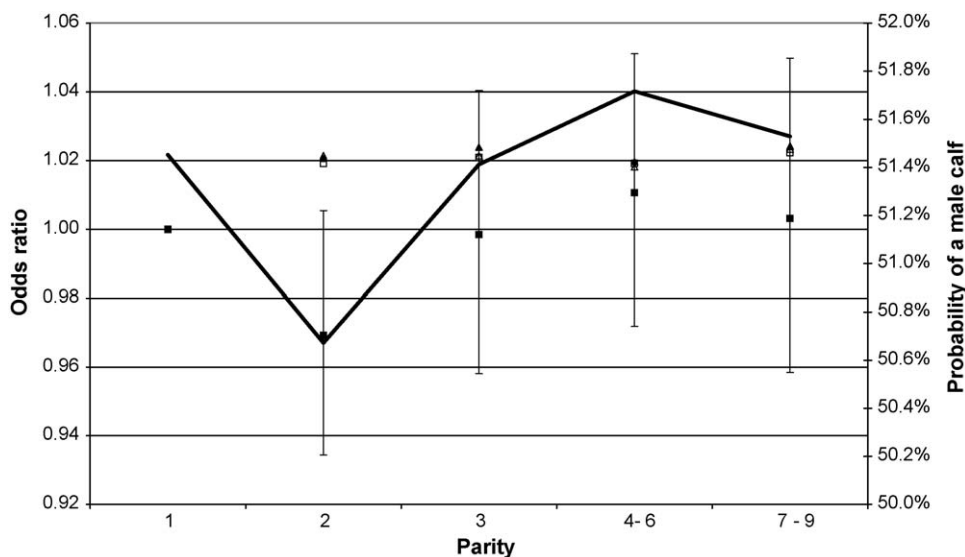


Fig. 3. Odds ratios (95% confidence intervals for the parity odds ratios using the 2005 data included as vertical bars) for the effect of parity on the probability of a male calf using data from 2002 (open triangle), 2003 (shaded triangle), 2004 (open square) and 2005 (shaded square). Parity one animals (i.e., animals inseminated as heifers) were taken as the reference class. Also included (continuous line) is the probability of a male calf being born by a March calving cow of different parity that previously had a male calf and was mated to a Holstein–Friesian AI sire, using the 2005 model multiple regression solutions.

calf. No significant interaction between mating type and any of the other effects in the model were evident. The odds of a male calf being born following AI in 2002, 2003, 2004 and 2005 was 1.04 (95% CI: 1.01–1.06), 1.04 (95% CI: 1.02–1.08), 1.06 (95% CI: 1.03–1.10) and 1.08 (95% CI: 1.03–1.12), respectively. Solutions of mating type from the year 2005 equates to an increased probability of a male calf being born of 1% unit from 0.50 to 0.51 in a March calving, second parity cow that previously had a male calf and was served to a Holstein–Friesian AI sire compared to a Holstein–Friesian natural mating.

#### 4. Discussion

The objective of the present study was to determine if AI in dairy or beef cattle increased the probability of a male calf compared to natural mating. To our knowledge no other study has compared both mating types. The results from this study clearly indicates that using AI will increase the likelihood of a male calf or in other words natural mating is more likely to result in a female calf. This is an important factor and should also be accounted for in the economic comparison of natural mating and AI. Additionally, several other factors such as time of the year at birth (or associated parameters such as time of conception), sex of the dam's previous calf, sire breed and parity of the dam also significantly affected secondary sex ratio.

The secondary sex ratio of 50% males observed in the present study disagrees with most previous international studies in dairy cattle which documented a higher incidence of males than females [7,8,10,11]. However, the sex ratio from Holstein–Friesian sires in the present study was 52:48 which is similar to other international estimates on Holstein–Friesians [7,8]. Lower sex ratios have been reported in beef cattle; Crews [12] reported as 50:50 sex ratio in purebred Charolais cattle while Berger et al. [15] reported a sex ratio of 50:50 in purebred Angus heifers although it was 51:49 in purebred Angus cows. The sex ratio from beef sires in the present study varied from 53:47 (Belgian Blue and Hereford) to 49:51 (Angus). One potential contributing factor to the lower overall sex ratio in the present dataset may be the greater use of natural mating in Ireland compared to most other international countries [16], which the present study indicates will decrease the sex ratio. There is no obvious reason for the significant effect of year on sex ratio. Parity number and calving pattern remained relatively constant over the 4 years of the study. Although the proportion of calves recorded with beef sires increased with time, which will increase the probability of a male calf, the level of AI decreased with time which will decrease the probability of a male calf. One contributing factor to the annual effect on sex ratio may be climate and its associated effects on sex ratio [8].

#### 4.1. Effect of confounding factors on sex ratio

In agreement with the present study Roche et al. [8], using 34 years data on New Zealand dairy cows, reported a significant effect of time of the year at conception (derived from subsequent calving dates less a fixed gestation length of 282 days) on secondary sex ratio. Nonetheless, others [10,17] failed to report any significant effect of time of the year on sex ratio in dairy cattle while Skjervold and James [11] reported a significant effect of calving season on sex ratio in parity 1 animals only. The sex of a calf is determined at fertilisation, although preferential embryo/foetal mortality may also impact the secondary sex ratio. Therefore, it is likely that factors acting at fertilisation or early in gestation play a large role in sex determination. Roche et al. [8,9] reported a significant effect of climate at conception as well as indicators of cow metabolic load (i.e., body condition score) at conception on secondary sex ratio. Climate in Ireland follows a seasonal pattern [18] thus providing one possible explanation for the effect of time of year on sex ratio if the documented effect of climate on sex ratio [8] exist in Ireland. Additionally, Ireland operates a seasonal calving system of milk production with the majority of cows calving in spring [19]. With a compact calving season, herd average stage of lactation is almost synonymous with time of year. Berry et al. [20] documented the body condition score lactation profile of Irish dairy cattle, indicating in spring calving systems of milk production, cows will be losing body condition in late spring to mid summer which has been shown to decrease the likelihood of a male calf [9]. Conception in late spring to mid summer corresponds to a subsequent birth of the progeny in late winter to mid spring when the probability of a male calf is lower. This may also impact the lower overall sex ratio in the present study.

The higher probability of a male calf being born when the immediately previous calf born within dam is male suggests some degree of repeatability of calf sex within cows. However, the proportion of this repeatability attributable to genetics is likely to be low. Xu et al. [7] reported a heritability of sex ratio of 2% suggesting that the permanent environmental effect of the dam is the main contributor to the repeatability of sex ratio. An example of such permanent environmental effect may be the position in the family into which the dam was born. For example, Vandenbergh and Huggett [21] in an experiment of rodents reported a greater probability of male offspring in dams born between two male siblings. They attributed this effect to a possibly higher level of testosterone in the dam acquired during development in the womb. Research in humans has

revealed a higher sex ratio in mothers with greater testosterone levels [22]. Nonetheless, not all studies have documented an effect of previous neonate sex on the sex of the subsequent birth [23,24].

Little is known on the effect of service sire breed on secondary sex ratio although heritability estimates of 2% for sex ratio have been reported in New Zealand dairy cattle [7]. Skjervold and James [11] reported a significant effect of breed of sire on sex ratio in Norway. Although not statistically tested in the study, chi-square analysis of the data provided by Foote [10] identified a significantly ( $P < 0.05$ ) lower proportion (50.5%) of males born from Holstein sires than from Jersey sires (52.8%). Similarly, a review of the literature [7,8,10,12,15], although confounded with other effects such as production system, model of analysis, confounding factors adjusted for in the model, suggests breed differences in sex ratios.

The effect of dam parity on calf sex is at odds with previous findings where no association between calf sex and dam age or parity was evident in dairy cows [8–11] or humans [25]. Nonetheless, maternal age was reported to positively influence secondary sex ratio in other studies across species [26–29], which is in line with trends observed in the present study. Although, not an objective of the study, Berger et al. [15] reported different secondary sex ratios in purebred Angus calves from heifer dams (ratio = 50:50) or cow dams (ratio = 51:49). Chi-square analysis of the reported values indicated a significantly ( $P < 0.001$ ) higher sex ratio in cows than in heifers which is in agreement with the present study. The positive effect of parity on sex ratio in the present study may reflect the Trivers and Willard [30] hypothesis which stated that mothers in poorer physiological condition or with limited resources would be advantaged by investing more heavily in the more reproductively stable sex (i.e., the female). A study on New Zealand Holstein–Friesian dairy cattle [9] revealed a higher probability of male calves in dams that lost less body condition score and body weight from calving to conception. Although primiparous animals do not lose as much body condition post-calving as multiparous cows [20], Irish Holstein–Friesian cows at first calving are less than 90% of their mature weight [31]. Therefore, animals that are still growing may incur a similar physiological status as cows in poor condition or with the anticipation of limited energy resources for lactating thereby reducing the probability of the more variable sex in first parity cows.

#### 4.2. Effect of mating type

No study to date has attempted to quantify the effect of AI versus natural mating on secondary sex ratio in

dairy or beef cattle. Results from the present study clearly indicate a significantly greater probability of a male calf being born when conceived through AI. Xu et al. [7] reported that frozen semen resulted in 1.24–1.66% units more male calves than fresh AI semen. Xu et al. [7] speculated that the difference may be due to the effect of freezing on the survival and function of the X- or Y-bearing sperm or on the subsequent effect on the survival of the embryos after fertilisation. Data on whether the service was fresh or frozen semen was not available for the present study.

Timing of service may also contribute to the difference in secondary sex ratio between mating type. Although a general consensus has not been reached in the scientific literature on the effect of timing of service on sex ratio some studies have reported a higher proportion of female offspring following early insemination [5], which would predominantly occur with natural mating.

In conclusion, a strong significant association was evident in the present study between mating type and sex ratio with AI resulting in a 1.04–1.08 times higher likelihood of a male calf across all both beef and dairy breeds and was consistent across the 4 years investigated in the present study. This manifested itself as approximately a 1% unit difference in sex ratio between natural mating and AI. Although of limited biological importance, the statistical significance of such a small effect is due to the large dataset used in the present study. Furthermore, the effect of natural mating may be artificially exaggerated within farm if a farmer uses natural mating on the heifers and/or on the early or late calving animals.

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