Veterinary and Animal Science 9 (2020) 100101

Contents lists available at ScienceDirect

Veterinary and Animal Science

journal homepage: www.elsevier.com/locate/vas

Effect of sexed semen on different production and functional traits in German Holsteins

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ARTICLE INFO

Keywords:
Sexed semen
Calf sex
Heifer
Stillbirth

ABSTRACT

The aim of this study was to analyze possible effects of semen type (conventional vs. female sexed) and calf sex on fertility and production traits. For this purpose, field data of German Holstein heifers in Lower Saxony were evaluated.

Sexed semen was mainly used for first insemination. 87.0% female calves were born from sexed semen, while 52.7% female calves were born from conventional semen. Heifers inseminated with sexed semen were on average 43 to 48 days younger at their first calving than heifers inseminated with conventional semen.

Calf sex had an influence on the average calving ease and the dystocia rates. Male calves showed higher calving ease scores and caused a higher risk for dystocia than female calves. The semen type had no influence on these characteristics.

Within the same calf sex, sexed semen had only minor effects on most traits, except for stillbirth rates: the stillbirth rate for male calves from female sexed semen was 30.6%, which was 2.86 times the stillbirth rate of male calves from conventional semen, possibly due to trisomies.

Sexed semen played only a minor role for production traits in first lactations.

The extrapolated 305-day milk yield was 200 kg lower for first calf heifers, which were inseminated with sexed semen compared to heifers inseminated with conventional semen. Fat and protein yield were 6 kg to 8 kg lower after use of sexed semen. Animals with female offspring from sexed semen showed higher survival rates than the other groups.

1. Introduction

Different total lengths of sex chromosomes in mammals lead to different DNA contents in X- and Y-chromosome bearing spermatozoa (Moruzzi, 1979). Attempts to precisely measure this difference succeeded in the 1980s (Gledhill, Pinkel, Garner & Van Della, 1982; Pinkel et al., 1982) and revealed a 4% higher DNA amount of the X-chromosome, which made it possible to sort X- and Y-sperm using flow cytometry (Garner et al., 1983).

In early flow cytometric approaches, the cell membranes had to be removed to use the membrane impermeant fluorescent dye DAPI (4′−6-diamindino-2-phenylindole), thus killing the sperm (Garner et al., 1983). The transition to the membrane permeant fluorescent dye Hoechst 33,342 enabled sorting with intact sperm (Johnson, Flook & Look, 1987).

The first live offspring were born after use of sex-sorted bull and rabbit semen in 1988 (Morrell, Keeler, Noakes, Mackenzie & Dresser, 1988).

With the implementation of the high-speed flow cytometry in 1996, the commercialized use of bovine sexed semen finally became feasible (Seidel et al., 1999).

Subsequent to staining, the sperm are arranged in a droplet stream and pass a laser beam. Two detectors are used, one to determine if the sperm head is oriented with the flat surface perpendicular to the laser and the other to measure fluorescence of those sperm oriented correctly. The relative fluorescence is measured by a photomultiplier tube

Abbreviations: (C_M), conventional semen × male calves; (C_F), conventional semen × female calves; (S_M), sexed semen × male calves; (S_F), sexed semen × female calves

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https://doi.org/10.1016/j.vas.2020.100101
Received 25 November 2019; Received in revised form 25 February 2020; Accepted 2 March 2020
Available online 04 March 2020

and analyzed by a computer. The droplets containing X- and Y-chromosome bearing spermatozoa are electrically oppositely charged and then sorted in an electrostatic field (Garner & Seidel, 2003). With that procedure, sex can be predetermined with 85 to 95% accuracy (Johnson, Welch & Rens, 1999).

Although alternative methods were tested in past years, the flow-cytometric technology is with some modifications still the method of choice for semen sex sorting. Fluorescent *in situ* hybridization techniques (Kawarasaki, Welch, Long, Yoshida & Johnson, 1998), gold nanoparticles binding to Y-chromosome-specific sequences (Rath et al., 2013) or semen separation by swim-up method with validation by real-time PCR (Asma-ul-Husna et al., 2017) do also provide opportunities for sperm sexing but are not of commercial interest.

Sex preselection of females is the most common treatment to achieve an adequate number of replacement heifers. Simultaneously, dystocia rates and the number of unwanted male dairy calves inferior for beef production are decreased (Hohenboken, 1999; Seidel, 2002). On the other hand, the time-consuming and costly sorting process makes semen more expensive. At the same time, the doses are sold with a reduced concentration, which may impair fertility and requires stringent insemination management. A sexed semen dose contains approximately 2 million spermatozoa, which is only a tenth of the conventional semen (20 million sperm/dose) (Mallory, Lock, Woods, Poock & Patterson, 2013; Seidel, 2014).

Furthermore, the sexing process encompasses different manipulations of the sperm which can cause damage (Garner & Seidel, 2003; Mallory et al., 2013). Moreover, conventional semen can cope better with cryopreservation (Schenk, Suh, Cran & Seidel, 1999).

Several studies examined different performance characteristics of dairy cattle after insemination with sexed semen and found some effects, especially lower conception rates (DeJarnette et al., 2008; Healy, House & Thomson, 2013; Norman, Hutchison & Miller, 2010; Schenk, Cran, Everett & Seidel, 2009).

The sperm sorting process seems to reduce the fertilization capability to 60 to 90% as compared to conventional sperm (Borchersen & Peacock, 2009; DeJarnette, Nebel & Marshall, 2009; Seidel et al., 1999). Lower conception rates in addition to the higher costs for sexed semen lead to the fact that sexed semen is primarily used for nulliparous heifers and first services, for which fertility is expected to be the highest (DeJarnette et al., 2008, 2009; Seidel, 2007). The reported conception rates widely differed which emphasizes the influence of farm-related factors such as estrus detection as well as cow-related factors such as age, body condition and parity (Seidel & Schenk, 2008).

The multitude of manipulations of spermatozoa during sex sorting might not only affect conception rate, but also the stillbirth rate or even calf viability. Several studies have already investigated this and DeJarnette et al. (2009) and Norman et al. (2010) e.g. reported strikingly higher stillbirth rates for male calves among Holstein heifers inseminated with female sexed semen.

Latest developments to minimize the negative influences of sorting steps resulted in the new SexedULTRA technology (STgenetics, USA) which is especially based on revised conditions of the media used during the various stages of the sexing process (Gonzalez-Marin et al., 2016; Lenz et al., 2016). Additionally, the new Genesis III sorting machine (STgenetics, USA) enables more rapid, accurate, and less damaging production (Thomas et al., 2017). Furthermore, a higher dose rate of 4 million sex-sorted sperm per straw was introduced to the market in 2017 (SexedULTRA-4 M, STgenetics, USA) in order to lessen the gap between conception rates of sexed and conventional semen (Lenz et al., 2016). First analyses showed greater conception rates compared to the usual sexing routine and non-return rates approaching conventional semen (Lenz et al., 2016).

The aim of the current study was thus to analyze possible effects of semen type (conventional vs. female sexed) and calf sex on fertility and production traits using field data of German Holstein heifers in Lower Saxony.

2. Materials and methods

Data of first lactation Holstein Friesian cows from the breeding value estimation of December 2015 were assessed (data cut-off: July 10, 2015). These data were provided by the breeding associations *Masterrind* and *Verein Ostfriesischer Stammviehzüchter (VOST)* via the data center *vit* (IT Solutions for Animal Production).

Different criteria had to be fulfilled for data selection:

(a) Only herd-years with at least 20 inseminations on heifers with sexed and unsexed semen were considered. The performance data of the first lactation were selected for these heifers.
(b) The interval from last insemination to calving was required to be between 265 and 295 days to assign the calves to the last insemination. If other inseminations were conducted within a range of ≤ 10 days around the last insemination, data were used only if the bull and the type of semen were the same both times.
(c) Selected herds had to show at least 10 such verified calvings from each, sexed and unsexed semen.

As the amount of male calves out of female sexed semen is relatively low and the effects therefore hard to find, the interaction levels *semen type* × *calf sex* were created. These levels were: conventional semen × male calves (**C_M**), conventional semen × female calves (**C_F**), sexed semen × male calves (**S_M**), sexed semen × female calves (**S_F**) (Table 1).

Initially, 105,565 heifers were included into the analysis to get an overview of the use of sexed semen. The ratio between the use of sexed semen and conventional semen for first and following services of nulliparous heifers was calculated based on these data.

The number of animals was higher for this partial analysis than for the following evaluations because exits till calving and the criterium c mentioned above were not considered.

55,554 calves were examined in total. 22,580 of them were born from sexed semen and 32,974 were born from conventional semen.

Calving ease: Only calvings with observed calving ease, categories 1 to 4 (ADR, 2017), were considered (n = 52,710).

Dystocia: Calving ease categories 3 and 4 were considered as dystocia, coded as '1', (n = 52,710).

Stillbirth: Stillbirths included all stillborn calves and those which died within 48 h after birth (ADR, 2017) (n = 55,554).

Calving to first service interval: Only heifers with less than 200 days from calving to first service were evaluated. Moreover, the time range between calving and cut-off date had to be more than 200 days to give the animals an equal chance for showing calving to first service interval (n = 37,629).

Non-return rate: For this evaluation, only heifers which had not exited the herd before day 56 (n = 37,173) or day 90 (n = 36,086) after first service were analyzed.

Number of services in first lactation: In addition to the mentioned selection criteria above, only heifers first inseminated with deep-frozen semen were considered (n = 35,727).

Lactation performance - milk, fat and protein yield: The lactation performance for milk, fat and protein yield was analyzed for all cows

Table 1
Description of the interaction levels *semen type* × *calf sex* (number of samples (n) = 55,554).

Abbreviation	Semen type	Calf sex	n	Proportion (%)
C_M	Conventional	Male	15,585	28.0
C_F	Conventional	female	17,389	31.3
S_M	Sexed	male	2930	5.3
S_F	Sexed	female	19,650	35.4

conventional semen × male calves (C_M), conventional semen × female calves (C_F), sexed semen × male calves (S_M), sexed semen × female calves (S_F).

with at least eight test days in first lactation ($n = 32{,}718$).

Survival rates within first lactation: Heifers with calvings at least 100 days before data cut-off were included in the analysis ($n = 51{,}436$).

2.1. Statistical analyses

Statistical analyses were performed with *R* (version 3.1.2; *R* Development Core Team, 2014).

Nominal variables are presented descriptively in cross tables.

The description of continuous variables against nominal variables are shown in mean value tables.

Logit models were used for binary dependent variables according to the general equation:

$$y_{ij}^{*} = sexed\ semen\ \times\ calf\ \ sex_{ij}$$

y_{ij}^{*} is a latent variable for individual *i* with interaction effect level *j* of the effect *sexed sperm* × *calf sex*, linked to observation y_{ij}^{*} (0/1) via the logit link function.

Odds ratios show the proportion between the probabilities of different effect levels in relation to a reference level with a given odds ratio of 1.

Survival rates for the interval *first calving to 450 days* were estimated using Kaplan-Meier estimators (Kaplan & Meier, 1958). The risk of an animal to be culled at a given timepoint $R(t)$ is estimated using the following equation:

$$R(t) = \frac{n_{animals\ culled\ at\ timepoint\ t}}{n_{animals\ being\ at\ risk\ for\ timepoint\ t,\ i.e.\ having\ survived\ t-1}}$$

The probability of an animal to survive from $t = 0$ up to timepoint t is then:

$$S(t) = \prod_{i=0}^{t} 1 - R(t)$$

$S(t)$ can also be considered as an estimator of the amount of all animals with a first calving which survived up to timepoint *t*.

The interval *first calving to 450 days* was also assumed as survived if the cow had a second calving.

In the following, the simplified term sexed semen is exploited instead of female sexed semen.

3. Results and discussion

The proportion of sexed semen was decreasing with the number of services. While 59.7% of nulliparous heifers were inseminated with sexed semen for first service, this proportion dropped to 6.6% for fourth service. Sexed semen is mainly used for first (and second) insemination of heifers. If the first and second insemination are not successful, the following inseminations will most likely be performed with conventional semen due to lower costs and higher conception rates (Schenk et al., 2009). Here, the percentage of first services with sexed semen is very high compared to older studies (DeJarnette et al., 2009; Norman et al., 2010). Although the studies were performed with data of another population, decreasing costs for sexed semen during the last years certainly contribute to this observation (McCullock et al., 2013).

87.0% female calves were born from sexed semen. In comparison to that, the percentage of female calves from unsexed semen was 52.7%. This distribution of calf sex after use of sorted semen correspond with literature: Tubman, Brink, Suh and Seidel (2004) and Healy et al. (2013) found rates of 87.8% and 86.0%, respectively. DeJarnette et al. (2009) and Norman et al. (2010) reported rates closer to the aimed 90.0%. These deviations might occur because of different accuracies of sorting or because of an incomplete or erroneous data recording (Healy et al., 2013; Seidel, 2003).

The sex ratio for conventional semen differed from expectations. Most studies achieved approximately 50.0% to 52.0% male offspring (DeJarnette et al., 2009; Tubman et al., 2004). In the present study, the ratio behaved in reverse proportion. 52.7% female calves were born after use of conventional semen. Norman et al. (2010) reported 51.5% female calves as well.

These various rates may be due to a data reporting bias. Female calves are of a higher value for the farmer and are therefore much more likely to be reported correctly.

The statistical models were kept simple, because many factors which affect the response variables might also be correlated to the effect of *sexed semen* × *calf sex*. For example, the different traits are heritable, but including a bull effect might lead to collinearity between the bull effect and the effect of *sexed semen* × *calf sex*.

Table 2
Age at first calving (days) for the interaction levels.

	N	M	SD	SE	MED
C_M	15,585	845.8[a]	103.1	0.8	824
C_F	17,389	840.1[b]	99.7	0.8	820
S_M	2930	798.2[c]	78.6	1.5	781
S_F	19,650	797.0[c]	77.5	0.6	780

conventional semen × male calves (C_M), conventional semen × female calves (C_F), sexed semen × male calves (S_M), sexed semen × female calves (S_F); Mean (M), standard deviation (SD), standard error (SE), median (MED).
[a-c] Means with different superscripts differ ($P < 0.001$).

3.1. Age at first calving

Heifers inseminated with sexed semen were on average 43 to 48 days younger at their first calving than heifers inseminated with conventional semen ($P < 0.001$) (Table 2).

The decreased age at first calving of heifers inseminated with sexed semen is in contrast to the results of other studies. Chebel, Guagnini, Santos, Fetrow and Lima (2010) reported a longer interval from first insemination to calving after use of sexed semen but no differences in age at calving compared to heifers inseminated with conventional semen. Joezy-Shekalgorabi, Shadparvar, de Vries and Gay (2014) even observed an increased age at first calving after an insemination with sexed semen while comparing different breeding strategies. The authors acknowledged a great influence of management strategies such as estrus detection rate or the percentage of sexed semen utilized in the herd.

The results of the current study coincide with the fact that sex-sorted semen is mainly used for first and second services (Schenk et al., 2009). Therefore, these heifers showed a lower age at first calving. The average age at first calving in the current data set (26 to 28 months) is relatively high compared to literature (Chebel et al., 2010; Ettema & Santos, 2004).

3.2. Calving ease and dystocia

The average calving ease showed differences between calf sexes ($P < 0.001$), but there was no significant differentiation of semen type used within calf sex (Table 3).

The same observation applies to dystocia rates (Tables 4 and 5). Male calves caused a higher risk for dystocia than female calves ($P < 0.001$). The semen type again had no influence on this characteristic.

Due to the larger size of bull calves at birth, they showed higher calving ease scores compared with female calves and had a larger incidence of difficult births (Gregory, Cundiff & Koch, 1991). The detected scores are in agreement with other studies for both traits. Tubman et al. (2004) reported least squares means of 1.15 for female calves and 1.30 for males regarding the calving ease.

Norman et al. (2010) showed dystocia rates of 8.0% to 9.0% for males in comparison to 4.0% for female calves.

Table 3
Calving ease for the interaction levels.

	n	M	SD	SE
C_M	14,680	1.41[a]	0.61	0.005
C_F	16,535	1.26[b]	0.50	0.004
S_M	2671	1.42[a]	0.61	0.012
S_F	18,824	1.25[b]	0.48	0.004

conventional semen × male calves (C_M), conventional semen × female calves (C_F), sexed semen × male calves (S_M), sexed semen × female calves (S_F).
[a-b] Means with different superscripts differ ($P < 0.001$).

Table 4
Incidence of dystocia for the interaction levels.

	Dystocia 0[1]	1[1]	Rate
C_M	13,786	894	0.061[a]
C_F	16,125	410	0.025[b]
S_M	2521	150	0.056[a]
S_F	18,419	405	0.022[bc]

conventional semen × male calves (C_M), conventional semen × female calves (C_F), sexed semen × male calves (S_M), sexed semen × female calves (S_F).
[1] 0: calving ease score 1 and 2 (no dystocia); 1: calving ease score 3 and 4 (dystocia).
[a-c] Rates with different superscripts differ ($P < 0.001$).

Table 5
Logit model of the dystocia rates.

	Odds Ratio	Lower bound 95% CI	Upper bound 95% CI
C_M	1.00	0.93	1.07
C_F	0.39	0.35	0.44
S_M	0.92	0.77	1.09
S_F	0.34	0.30	0.38

conventional semen × male calves (C_M), conventional semen × female calves (C_F), sexed semen × male calves (S_M), sexed semen × female calves (S_F). Confidence interval (CI).

3.3. Stillbirth rate

The stillbirth rates for female calves were 5.0%, independent of the semen preparation. 13.4% male calves out of conventional semen were born dead or died within 48 h after birth. However, the stillbirth rate for male calves from sexed semen was 30.6%, which is higher than for the other interaction levels ($P < 0.001$) (Table 6). The respective logit model (Table 7) showed an odds ratio of 2.86 for the probability of a stillbirth of male calves from sexed semen in comparison to male calves from conventional semen.

The incidence of female stillborn calves was relatively low for both semen types, namely only half the average described in other studies (Borchersen & Peacock, 2009; DeJarnette et al., 2009; Norman et al., 2010. DeJarnette et al. (2009) found an influence of herd, season of calving and sire within semen type on female stillbirth rate.

Table 6
Incidence of stillbirths for the interaction levels.

	Stillbirth 0[1]	1[1]	Rate
C_M	13,502	2083	0.134[b]
C_F	16,521	868	0.050[c]
S_M	2033	897	0.306[a]
S_F	18,675	975	0.050[c]

conventional semen × male calves (C_M), conventional semen × female calves (C_F), sexed semen × male calves (S_M), sexed semen × female calves (S_F).
[1] 0: born alive; 1: born dead or died within the first 48 h.
[a-c] Rates with different superscripts differ ($P < 0.001$).

Table 7
Logit model of the stillbirth rates.

	Odds ratio	Lower bound 95% CI	Upper bound 95% CI
C_M	1.00	0.96	1.05
C_F	0.34	0.31	0.37
S_M	2.86	2.61	3.13
S_F	0.34	0.31	0.37

conventional semen × male calves (C_M), conventional semen × female calves (C_F), sexed semen × male calves (S_M), sexed semen × female calves (S_F).

For male calves from conventional semen, the rate was comparable to literature (DeJarnette et al., 2009; Norman et al., 2010). However, the frequency of stillborn male calves from sexed semen was strikingly high. DeJarnette et al. (2009) and Norman et al. (2010) had already shown an obvious tendency for greater stillbirth rates of male calves born from sexed semen. They found rates of 15.6% (Norman et al., 2010) and 20.0% (DeJarnette et al., 2009) stillborn male calves, respectively. The current study presents an even higher incidence. The cause for this phenomenon was not identified so far. In 2009, DeJarnette et al. speculated about an aneuploidy among Y-bearing chromosomes. Non-disjunctions lead to a triple set of chromosomes. Because of these numerical chromosome aberrations, Y-bearing aneuploidic sperm will show a higher DNA content and therefore may be wrongly assigned during the sorting process. The XYY-trisomy and the Klinefelter's syndrome (XXY-trisomy) are the most known human gonosomal trisomies, but human individuals with these diseases are usually viable (Jacobs & Strong, 1959; Ross et al., 2012), whereas individuals with autosomal trisomies are not. These autosomal chromosomal disorders increase the risk of human neonatal mortality (Alberman & Creasy, 1977; Cereda & Carey, 2012). Consequently, autosomal trisomies are likely the cause of greater stillbirth rates of male calves born from sexed semen.

For cattle, there are not enough data available to prove this hypothesis.

Furthermore, the number of male calves from sexed semen is relatively low in general. With more than 2900 male calves born out of sexed semen, the current study achieved an adequate size of data set to demonstrate that this is a true effect.

Norman et al. (2010) did not only evaluate stillbirth rates among calves born from Holstein heifers, but also stillbirth incidences of calves born from cows. This analysis led to an opposite result. The male calves born from sexed semen were less frequently stillborn (2.6%) than the male calves from conventional semen (3.6%). The field study of Borchersen and Peacock (2009) showed a significantly increased incidence of stillbirths for male calves born from conventional semen (20.0%) in comparison to male calves from sexed semen (14.0%). Tubman et al. (2004) reported nonsignificant differences of male and female stillbirth rates.

Thus, differences in management and data recording should be considered to evaluate these partly divergent results. The monetary value of male dairy calves may also play a role for the reporting of stillbirths.

In future studies, attention should be paid to autopsies and cytogenetic investigations of stillborn male calves to examine the biological source of this phenomenon. Furthermore, a pedigree analysis of the sires used may be of interest.

3.4. Interval from calving to first service

The length of the interval from calving to first service differed slightly between the sexes of the calves: the interval was longer after birth of male calves (Table 8). A correlation between higher dystocia and stillbirth rates of male calves and lower reproductivity of the dam in first lactation might be possible. Again, there was no difference between semen types.

Table 8
Interval from calving to first service (days) for the interaction levels.

	n	M	SD	SE	MED
C_M	10,607	82.9[a]	32.7	0.3	76
C_F	11,828	81.0[b]	31.3	0.3	74
S_M	1907	82.1[ab]	31.1	0.7	76
S_F	13,287	80.6[b]	31.2	0.3	74

conventional semen × male calves (C_M), conventional semen × female calves (C_F), sexed semen × male calves (S_M), sexed semen × female calves (S_F).
[a-b] Means with different superscripts differ ($P < 0.001$).

Table 9
Incidence of non-return for the interaction levels within first lactation.

	Non-return 56 days			Non-return 90 days		
	0[1]	1[1]	Rate	0[1]	1[1]	Rate
C_M	4232	6297	0.598[b]	4882	5326	0.522[b]
C_F	4422	7263	0.622[ac]	5167	6170	0.544[ac]
S_M	706	1184	0.626[ab]	836	989	0.542[ab]
S_F	4812	8157	0.624[a]	5715	7001	0.551[a]

conventional semen × male calves (C_M), conventional semen × female calves (C_F), sexed semen × male calves (S_M), sexed semen × female calves (S_F).
[1] 0: Return; 1: Non-return.
[a-c] Rates with different superscripts differ ($P < 0.01$).

3.5. Non-return rate

The results for this characteristic were not distinct enough and applied for 56- and 90-day non-return rates. Heifers with calvings from sexed semen showed slightly increased non-return rates in first lactation, but differences did exist for the interaction levels c_m and s_f only ($P < 0.001$). Differences between calf sexes were found, if conventional semen was used ($P < 0.01$) (Table 9).

3.6. Number of services in first lactation

Same as for calving to first service interval applies for the number of services in first lactation. The higher averaged numbers of insemination after birth of male calves could be caused by problems during parturition. However, the differences between the numbers of services are negligible (Table 10).

3.7. Lactation performance, milk, fat and protein yield

There were no differences between calf sex within semen type. However, the lactation performance between semen types differed. The extrapolated 305-day milk yield was 200 kg lower for first calf heifers which were inseminated with sexed semen compared to heifers inseminated with conventional semen (Table 11). Fat and protein yield were 6 kg to 8 kg lower after use of sexed semen (Tables 12 and 13).

As mentioned above, the age at first calving was lower after insemination with sexed semen, which may affect lactation performance.

Table 10
Number of services for the interaction levels in first lactation.

	n	M	SD	SE
C_M	10,094	2.04[a]	1.45	0.01
C_F	11,217	1.95[b]	1.38	0.01
S_M	1797	1.98[ab]	1.47	0.04
S_F	12,619	1.91[c]	1.32	0.01

conventional semen × male calves (C_M), conventional semen × female calves (C_F), sexed semen × male calves (S_M), sexed semen × female calves (S_F).
[a-c] Means with different superscripts differ ($P < 0.05$).

Table 11
Milk yield (kg) for the interaction levels in first lactation.

	n	M	SD	SE	MED
C_M	9184	8658[a]	1408	15	8618
C_F	10,286	8656[a]	1382	14	8625
S_M	1685	8456[b]	1421	35	8418
S_F	11,563	8487[b]	1362	13	8434

conventional semen × male calves (C_M), conventional semen × female calves (C_F), sexed semen × male calves (S_M), sexed semen × female calves (S_F).
[a-b] Means with different superscripts differ ($P < 0.001$).

Table 12
Fat yield (kg) for the interaction levels in first lactation.

	n	M	SD	SE	MED
C_M	9184	338.2[a]	50.5	0.5	337.3
C_F	10,286	339.1[a]	50.6	0.5	338.1
S_M	1685	331.1[b]	51.9	1.2	329.5
S_F	11,563	331.6[b]	49.2	0.5	330.9

conventional semen × male calves (C_M), conventional semen × female calves (C_F), sexed semen × male calves (S_M), sexed semen × female calves (S_F).
[a-b] Means with different superscripts differ ($P < 0.001$).

Table 13
Protein yield (kg) for the interaction levels in first lactation.

	n	M	SD	SE	MED
C_M	9184	289.6[a]	45.0	0.5	289.4
C_F	10,286	289.9[a]	44.2	0.4	289.4
S_M	1685	282.5[b]	45.6	1.1	280.3
S_F	11,563	283.7[b]	43.4	0.4	283.3

conventional semen × male calves (C_M), conventional semen × female calves (C_F), sexed semen × male calves (S_M), sexed semen × female calves (S_F).
[a-b] Means with different superscripts differ ($P < 0.001$).

It should be mentioned that the records were not adjusted for age. Environmental and management influences should also be considered as possible effects on milk, fat and protein yield.

3.8. Survival rates within first lactation

The animals with female offspring from sexed semen showed higher survival rates than the other groups ($P < 0.001$). 88.0% of these heifers were still in production 400 days after calving (in comparison to c_m: 85.4%; c_f: 86.5%; s_m: 86.0%) (Fig. 1).

The higher survival rates of heifers with female calves from sexed semen can probably be attributed to better health characteristics and breeding values of these animals wherefore they had already been selected for sexed semen insemination.

4. Conclusions

Sexed semen within the same calf sex had only minor effects on most traits, except for stillbirth rates: the stillbirth rate for male calves from sexed semen was 2.86 times the stillbirth rate of male calves from conventional semen. Further research is required to disclose the reasons for this finding. As mentioned above, autopsies and cytogenetic investigations should follow.

For all other traits analyzed, we showed that the type of semen plays a minor role for performances in first lactation. In future studies, possible interactions with the calving season or the sires used should be taken into account.

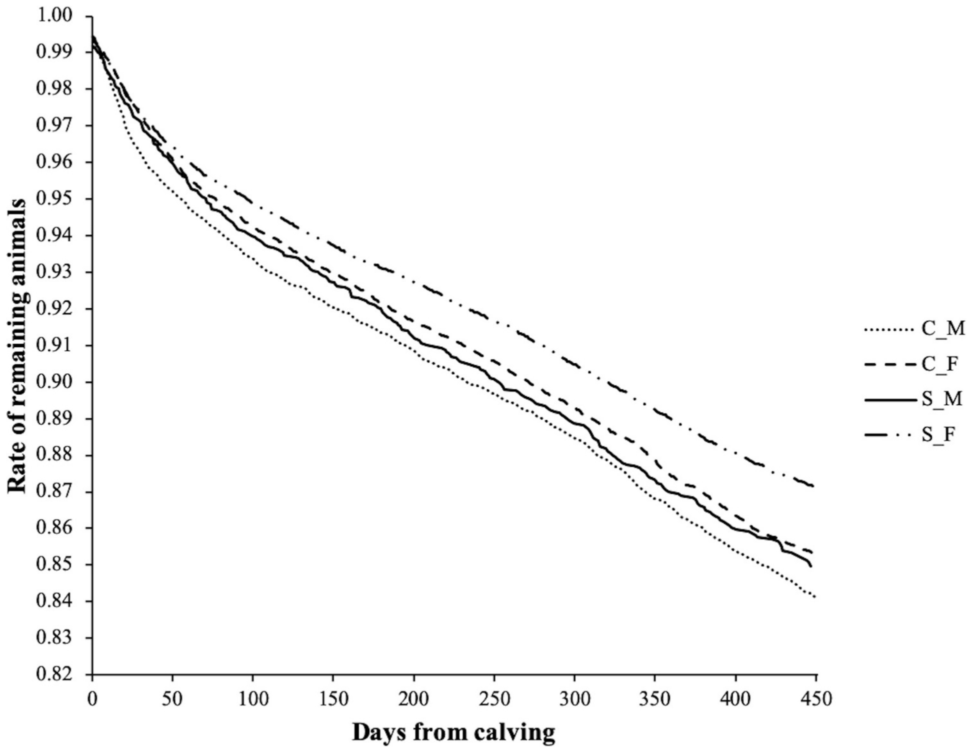

Fig. 1. Kaplan-Meier estimators of survival rates within first lactation.
conventional semen × male calves (C_M), conventional semen × female calves (C_F), sexed semen × male calves (S_M), sexed semen × female calves (S_F).

Ethical statement

The authors ensure that their manuscript has been carried out in accordance with the Uniform Requirements for manuscripts submitted to Biomedical journals.

The principles of the Committee on Publication Ethics (COPE), the Ethics in Publishing and the Ethical Guidelines for journal publication were considered.

Declaration of Competing Interest

The authors declare that there is no conflict of interest.

Acknowledgments

The authors kindly acknowledge *Masterrind* and *VOST* for the provision of data.

Funding

The authors would like to thank the H. Wilhelm Schaumann Foundation and the Open Access Publication Funds of the Göttingen University for financial support.

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