

What affects fertility of sexed bull semen more, low sperm dosage or the sorting process?

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Abstract

Until now it has been unclear to what extent the reduced fertility with sexed semen in the dairy industry is caused by too few sperm per AI dose, or by the effect of flow cytometric sorting, which is the established procedure for sexing semen. Therefore, we evaluated the effects of low sperm numbers per dose with and without sorting on non-return rates after 56 days (NRR56); in addition, we evaluated the effects of bulls, in order to further optimize use of sexed semen.

Based on results of using sexed semen from seven Holstein bulls, an overall numerical decline of 13.6% in NRR56 was observed ($P < 0.05$). About two-thirds of this decline (8.6%) was due to the low dose ($P < 0.05$), and a third (5.0%) due to the process of sorting ($P < 0.05$). The effect of low dosage and sorting differed among bulls. We observed a sex ratio of 91.6% females for sexed semen from the first 131 calves born.

Currently the best way to increase fertility of sexed semen is by closely monitoring fertility so that the highest fertility bulls are used, and by improving farm animal management. However, to make substantial progress, more in depth studies are needed on the sexing technology, especially on aspects such as sorting procedures and sperm dosage.

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Keywords: Reproduction; Fertility; Spermatozoa; Sex; Bovine

1. Introduction

To date, flow cytometric technology to separate X- and Y-chromosome-bearing sperm is the only reliable technique that has been used successfully for commercialization of sexed semen in the dairy industry [1]. However, compared to conventional semen, lower fertility has been reported [2,3]. Until now it is not

known to what degree this is an effect of low sperm numbers per AI dose or of sorting, as reports are not conclusive [2,3]. Due to the limited production efficiency of sorting sperm, a normal commercial dose of sexed bull semen contains only 2.1×10^6 sperm, whereas a conventional semen dose typically contains $15\text{--}20 \times 10^6$ sperm. There are a number of reports concerning fertility of sexed semen, but usually they are based on the results from just a few bulls. To further optimize the use of sexed semen, it is essential to know to what extent factors like low sperm dosage, the sorting process and bull differences affect fertility. We therefore evaluated effects of these factors on fertility of sexed

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and conventional semen from a number of bulls. In addition we determined the sex ratio of calves from sexed semen.

2. Materials and methods

2.1. Non-return model and statistics

In this study we used NRR56 as a measure for fertility. NRR56 were calculated using records of first and second inseminations as retrieved from farms, and determined per bull and per type of semen product used: conventional sperm (high or low dosage) or sexed sperm (low dosage and sorted). To estimate the effects on NRR56 as accurately as possible for the factors evaluated, corrections were made for several other factors that could bias the results. A regression model was used to correct for parity, herd, season, day of the week of AI service, first or second AI service, AI technician, and interval from AI to calving. To further correct differences in the AI protocol and discrete periods in which semen was used, we did not evaluate NRR56 per se, but evaluated numerical differences in NRR56 between two types of semen products per bull or overall, for each analysis as defined further on. Data were analyzed statistically for effects on NRR56 within and among bulls, or between weighted averages of bulls, by calculating one or two-sided 95% confidence intervals (CI).

2.2. Analysis of low dosage

Before the release of sexed semen produced in the Netherlands (ST Benelux BV, Deventer, The Netherlands) in September 2007, conventional semen was used in a field trial to measure effects of low dosage and bull on NRR56. Seven proven Holstein bulls were used with three ejaculates from each being processed to high (15.0×10^6 sperm), and three ejaculates at low dosages (2.0×10^6 sperm), within a period of 2 weeks. Collection and processing sperm, and its use in AI were done as described earlier, using Tris-concentrate (Invitrogen, Paisley, UK) as diluent and loading 0.25-cc straws (IMV, L'Aigle, France) at ambient temperature (20 °C) [4]. NRR56 were calculated for the statistical comparisons, using records of inseminations with semen from only these ejaculates.

2.3. Analysis of low dosage + sorting (sexing)

After the first field trial, the bulls continued in commercial production of high dosages of conventional

semen (six bulls at 15.0 and one bull at 20.0×10^6 sperm) and, approximately 6 months later in addition, low dosages of sexed semen (2.1×10^6 sperm). Sorting of X-chromosome-bearing sperm was done according procedures described previously, using MoFlo SXTM sperm sorters [1]. AI of both conventional and sexed semen was performed according guidelines for sexed semen (Sexing Technologies, Navasota, USA). NRR56 was used to determine the effects of low dosage + sorting and bull. We used all records of inseminations retrieved from these bulls over the course of one year after the introduction of sexed semen to obtain sufficient NRR56 data for statistical comparisons.

2.4. Analysis of sorting

To determine the effects of sorting alone and bull on NRR56, the differences in NRR56 per bull and overall, as determined according the above-mentioned two analyses were compared.

2.5. Analysis of sex ratio

To determine the sex ratio we evaluated the percentage of female calves born from the bulls that were evaluated in this study. We used all available calving records from farms over the course of one year after the introduction of sexed semen.

3. Results

For the analysis to determine the effect of low dosage and bull on NRR56, respectively, 8106 records ($n = 418$ to 2318 per bull) and 8378 records ($n = 627$ to 1558 per bull) of inseminations with 15.0 and 2.0×10^6 sperm per conventional dose were retrieved from seven bulls. NRR56 using 15.0×10^6 sperm was 69.2% overall (67.5–72.8% per bull). A significant ($P < 0.05$) decline in NRR56 was observed overall (8.6%), and for each bull when dosage decreased. However, these declines did not differ significantly among bulls (Table 1).

For the analysis to determine the effect of low dosage + sorting and bull on NRR56, respectively, 64,985 records ($n = 259$ to 23,341 per bull) and 2036 records ($n = 46$ to 585 per bull) of inseminations with 15×10^6 sperm or more per conventional dose, and 2.1×10^6 sperm per sexed dose were retrieved from the same bulls. NRR56 using 15.0×10^6 or more sperm was 66.4% overall (64.6–69.5% per bull). A significant ($P < 0.05$) decline in NRR56 was observed overall (13.6%), and for four bulls individually, when semen was both low dose and sorted. Some bulls differed

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