



Application of liquid semen technology improves conception rate of sex-sorted semen in lactating dairy cows

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ABSTRACT

The objective was to compare reproductive performance of liquid sex-sorted (SS) semen with that of conventional (CON) semen in lactating dairy cows. Between 2011 and 2013, commercial dairy herds ($n = 101, 203,$ and 253 for 2011, 2012, and 2013, respectively) with predominantly Holstein-Friesian cows were enrolled in a contract mating program to produce surplus heifers for export using liquid SS semen. During the spring mating period, each herd was allocated with liquid SS semen at 50% of its daily requirement and the remaining daily requirement was allocated with CON liquid semen. Sperm for producing SS semen was sorted by Sexing Technologies NZ Ltd. (Hamilton, New Zealand) and then packaged using the liquid semen technology of LIC (Hamilton, New Zealand) at a dose of 1×10^6 sperm. Artificial insemination (AI) with liquid SS semen was carried out between 43 and 46 h after collection. Conventional semen straws contained 1.25×10^6 , 1.75×10^6 , or 2×10^6 sperm for semen to be used on d 1, 2, or 3 after collection, respectively. Only CON inseminations on the same days as when SS semen was used were included in the comparison. Herd managers biased usage of SS semen toward cows with a longer postpartum interval before the mating start date (64.0 vs. 62.8 d), cows of higher genetic merit (NZ\$107.0 vs. NZ\$98.4), younger cows (5.1 vs. 5.2 yr), and cows in which they had more confidence of being genuinely in estrus as measured by a lower percentage of short returns between 1 and 17 d (5.3 vs. 7.5%). After adjusting for these factors, the estimated difference in non-return rate between AI with SS and CON semen over the 3 seasons was -3.8 percentage points (SS = 70.2% vs. CON = 74.0%; SS/CON = 94.9%). The estimated maximum difference in calving rate per AI between SS and CON semen was -3.1 percentage points for 2011 (SS = 51.2% vs. CON = 54.3%; SS/CON = 94.3%) and -3.0 percentage points for 2012 (SS = 49.7% vs. CON = 52.6%; SS/CON = 94.5%). Calving data for

2013 were not yet available. The percentage of heifer calves born to AI with SS semen was 87.0% for 2011 and 85.8% for 2012, both of which were lower than the expectation of 90% mainly due to misidentification of calf dams in seasonal dairy herds calving on pasture. In summary, results in this report showed that liquid SS semen only required half the dose rate of frozen SS semen to achieve a reproductive performance of over 94% of CON semen in lactating dairy cows. Careful planning and a robust distribution network are required to avoid semen wastage and to maximize the benefit of liquid SS semen.

Key words: liquid sex-sorted semen, reproductive performance, lactating dairy cow

INTRODUCTION

The ability to preselect the sex of the offspring at the time of breeding offers economic benefits to the dairy industry where commercial dairy farmers are primarily focused on breeding heifer replacements. Currently, the only commercially viable method for producing sex-sorted (SS) mammalian sperm is by flow cytometry to exploit the small difference in DNA content between X and Y chromosomes (Garner and Seidel, 2008; Seidel, 2012). Although this technology has undergone rapid development in the past decade, the current method of sperm sorting has a relatively slow throughput and, therefore, a suboptimal number of about 2.1×10^6 SS sperm per dose is commonly used as a compromise between cost and acceptable semen conception rate (Seidel, 2007). In addition, the sorting process causes damage to sperm (Seidel, 2007; Garner and Seidel, 2008). As a consequence of both a suboptimal dose rate and damage to sperm during the sorting process, conception rate from inseminations with frozen SS semen is typically between 70 and 80% of that from conventional (CON) semen at the normal dose rate (DeJarnette et al., 2009, 2011; Frijters et al., 2009). However, the reduction in conception rate with frozen SS semen cannot be fully overcome through increasing the sperm dose rate (Seidel and Schenk, 2008; DeJarnette et al., 2010, 2011; Lucena et al., 2014). The reduction in conception rate with frozen SS semen would negate some of the po-

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tential benefits from using frozen SS semen in seasonal dairy production systems where cows need to conceive within a short period of time during spring (Verkerk, 2003; Butler et al., 2014).

In New Zealand, most cows are bred with liquid semen and the typical dose rate for liquid semen is between 1×10^6 and 2×10^6 sperm compared with the typical dose rate of 15×10^6 sperm for frozen semen (Vishwanath, 2003). It is known that the freezing and thawing processes damage sperm and this effect may be greater for SS sperm because SS sperm have already been compromised during the sorting process (DeJarnette et al., 2011; Gosálvez et al., 2011a,b). Therefore, we hypothesized that the use of liquid SS semen to avoid damage during the freezing and thawing processes would overcome much of the decrease in conception rate with frozen SS semen. Following a successful pilot trial in 2010, a large number of inseminations with liquid SS semen was carried out between 2011 and 2013. Here, the results and experience are presented from using liquid SS semen at 1×10^6 sperm per dose in lactating dairy cows on commercial dairy farms.

MATERIALS AND METHODS

Cows and Trial Procedures

Data were collected over a 3-yr period between 2011 and 2013, involving lactating cows in 101, 203, and 253 dairy herds, respectively. These commercial dairy herds with predominantly Holstein-Friesian cows were enrolled in a contract mating program to produce surplus heifers for the export market using liquid SS semen. During the spring breeding period, each enrolled herd was allocated liquid SS semen at 50% of the estimated daily requirement. The remaining semen requirement was allocated with CON liquid semen. Artificial insemination was carried out by experienced technicians employed by LIC (Hamilton, New Zealand). Both the herd managers and AI technicians were aware which semen straws contained SS semen. The decision on which cows were to be bred with SS or CON semen was made by the herd managers.

Bulls and Semen Processing Procedures

Bulls ($n = 10, 11,$ and 12 for 2011, 2012, and 2013, respectively) that were for producing SS semen were genomically selected young (2 or 3 yr) Holstein-Friesian bulls and these were different from the Holstein-Friesian bulls ($n = 39, 44,$ and 34 for 2011, 2012, and 2013, respectively) used for producing CON semen, which were a mixture of mature bulls that had graduated from

progeny testing and genomically selected young bulls (1 to 3 yr).

Ejaculates for producing SS semen were collected using an artificial vagina per standard operation procedures between 1300 and 1400 h. Ejaculates that met the required quality standards were delivered to Sexing Technologies NZ Ltd. (Hamilton, New Zealand) to be sorted according to their standard operating procedures. The targeted purity for the X-sperm fraction was 90%. After centrifugation (undisclosed conditions) of the sorted sperm, the sperm pellet was rediluted using a Caprogen liquid semen extender (LIC) to 4×10^6 sperm/mL (Shannon, 1965). The diluted semen was delivered to the LIC semen production laboratory the following morning (about 0600 h). The SS semen was filled and sealed in liquid semen straws (0.25 mL) and dispatched to AI technicians for insemination in the following morning. The interval between semen collection and insemination was mostly between 43 and 46 h, depending on when insemination took place after the morning milking.

Ejaculates for producing CON semen were collected during early morning and diluted using Caprogen liquid semen extender to $5 \times 10^6, 7 \times 10^6,$ or 8×10^6 sperm/mL for semen to be used on d 1, 2, or 3 after collection, respectively. The CON semen was filled and sealed in liquid semen straws (0.25 mL) and dispatched to technicians every 3 d and used according to their target usage days.

Data and Statistical Analyses

Data on cows, AI, calving, and culling were extracted from the National Dairy Database maintained by LIC. Because reliable pregnancy diagnosis data were not available for most herds, reproductive performance outcome was measured using 24-d nonreturn rate (NRR), which was defined as the percentage of AI that were not followed by another AI between 18 and 24 d after the previous AI. An AI was counted as not returned if the following AI was >24 d after the previous AI. Artificial inseminations that were followed by another AI between 1 and 17 d were excluded from the NRR calculation because these short returns were either due to cows having short estrous cycles or due to estrus detection errors, but were not causally related to the performance of the first AI. Artificial inseminations within 24 d of the end of the AI breeding period were also excluded from NRR calculation because of the poor accuracy of mating records during the bull breeding period.

The average AI breeding period for herds in the trial was 42 d (between 27 and 88 d). The short AI breeding period precluded the use of an interval longer than 24

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