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Comparison between low-dose, high-sort and high-dose, low-sort semen on conception and calf sex ratio in Jersey heifers and cows

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ABSTRACT

The objective of this clinical trial was to compare conception and newborn calf sex ratios among Jersey heifers and lactating cows inseminated with either standard sex-sorted semen (low-dose, high-sort; LDHS) containing 2.1×10^6 sorted sperm at 90% purity or high-dose, low-sort (HDLS) semen containing 10×10^6 sorted sperm at 75% purity. After a specified voluntary waiting period (VWP), female subjects, consisting of nulliparous heifers (VWP 10 mo of age) and lactating cows (VWP 50 d in milk), received their first service and were systematically allocated to each treatment group in the order in which they presented for artificial insemination (AI). Females were bred to the same sire and type of sex-sorted semen for up to 2 additional services. Animals that were not pregnant after 3 breeding attempts were excluded. A total of 1,846 services were performed on 1,011 eligible females (LDHS; n = 494, HDLS; n = 517), which consisted of 516 nulliparous heifers and 495 lactating cows. Study groups were comparable with respect to the mean age at first AI for nulliparous heifers and the mean days in milk at first AI for parous cows. Insemination with HDLS semen did not result in a higher proportion of pregnancies per AI (P/AI) compared with LDHS semen for either nulliparous heifers (P/AI = 43 vs. 38%) or parous cows (P/AI = 47 vs. 43%). Insemination of nulliparous heifers using HDLS resulted in a lower proportion of newborn female calves compared with those bred to LDHS (76% vs. 87%). Similarly, lactating cows bred to HDLS gave birth to a lower proportion of newborn female calves compared with those bred to LDHS (79 vs. 90%). The odds ratio for a female calf to be born to an animal inseminated with HDLS compared with LDHS was 0.32 for nulliparous heifers and 0.19 for parous cows. Overall, the use of HDLS resulted in fewer females compared with LDHS, which may be explained by the lower concentration of X-bearing spermatozoa in HDLS compared with LDHS.
Key words: sex-sorted semen, sperm dose, pregnancy, sex ratio

INTRODUCTION

The commercial production of sex-sorted semen has been one of the major advances in dairy cattle reproduction in recent years. Based on the amount of DNA in each sperm, researchers in the 1980s were able to sort sperm bearing X and Y chromosomes by flow cytometry (Johnson et al., 1989; Seidel, 2007). Initially, sex sorting of semen was inefficient, with several difficulties including damaged sperm viability due to UV irradiation (Libbus et al., 1987; Johnson et al., 1989). The process was subsequently improved at various critical steps to allow for its commercialization. The characteristic flattened oval shape of the bovine spermatozoon required the development of a nozzle that orients the sperm heads (Rens et al., 1996), which is critical for increased accuracy of the sorting process (Johnson, 2000). Sorting speeds were increased, allowing higher sorting rates while maintaining high X-chromosome purity and reduced operating pressures of the flow cytometer, thus decreasing damage to the spermatozoa (Johnson and Welch, 1999; Suh et al., 2005).

Bovine X-bearing spermatozoa contain 3.8% more DNA than Y-bearing spermatozoa (Garner, 2006). The production of sex-sorted semen involves the staining of spermatozoa and their illumination by argon laser, allowing the greater fluorescence of the X chromosome to be distinguished and recognized by a detector (Seidel, 2007). Droplets exiting the nozzle contain an X-sperm, a Y-sperm, a combination of both, or neither. Only

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those droplets containing X-bearing spermatozoa are deflected by a charged plate, resulting in some 20% of the original sperm sample being sorted as purified X-bearing sperm (Seidel, 2007).

The dairy industry has an economic imperative to produce female offspring. The ability to alter sex ratios has allowed producers who utilize sex-sorted semen on nulliparous heifers to expand replacement inventories. The use of sex-sorted semen decreases the cost per female calf produced compared with nulliparous heifers bred to nonsorted semen and additionally increases the economic return during the first lactation (Chebel et al., 2010). To date, the use of sex-sorted semen in the dairy industry has been limited because of its poor reproductive efficiency and high cost (Seidel, 2003). The rate of sorting sperm is limited to 9 to 14 × 10^6 spermatozoa per hour (Schenk et al., 2009). Consequently, the rate of production of sex-sorted semen doses may not justify the capital investment in the required equipment (Moore and Thatcher, 2006). As a result of such expenses and the inefficiencies of the sorting process, the commercial production of sex-sorted semen has been at low insemination doses (Amann, 1999). Typically, a dose of sex-sorted semen contains 2.1 × 10^6 spermatozoa at 90% purity of X-chromosome spermatozoa, henceforth referred to as low-dose, high-sort (**LDHS**). Conception rates reported in virgin heifers using LDHS sex-sorted semen have consistently yielded about 75 to 80% of that obtained using non-sex-sorted semen in well-managed systems (Bodmer et al., 2005; DeJarnette et al., 2009, 2010). Similarly, conception rates reported for lactating cows have ranged from 23 to 29%, or 75 to 80% of the conception rate achieved with conventional semen in the same herds (Bodmer et al., 2005; DeJarnette et al., 2008). As a result, commercial use of LDHS has been mostly restricted to virgin heifers (Schenk and Seidel, 2007; DeJarnette et al., 2011).

Recent studies have focused on the effect of increasing the dosage of 90% X-chromosome sex-sorted semen above the 2.1 × 10^6 concentration; however, these studies did not find significant differences in conception rates between different doses in heifers or cows (DeJarnette et al., 2008, 2010). Data from a subsequent study showed that a semen dose of 10 × 10^6 sperm significantly improved conception rates compared with 2.1 × 10^6 in heifers (44 vs. 38%; DeJarnette et al., 2011). Although such an increase in dose resulted in higher conception rates, approximately 5 times the number of ejaculates per bull were necessary to produce the same number of these higher concentration LDHS semen doses. Other trials have examined the effect of dose as well as the effect of the sorting process on reproductive efficiency. A field trial comparing sex-sorted and nonsorted semen at a dose of 2 × 10^6 spermatozoa showed significantly improved pregnancy rates in heifers inseminated with nonsorted semen but not in cows (Bodmer et al., 2005).

In an effort to improve reproductive efficiency and reduce the cost of producing sex-sorted semen, a higher dose (10 × 10^6), lower sort (75% X-spermatozoa) product (termed high-dose, low-sort; **HDLS**) was formulated. During the production of HDLS, combined sperm droplets (i.e., droplets that contain both X- and Y-bearing spermatozoa) are retained in the sex-sorted semen, thus reducing the X-sperm purity to approximately 75%. The objectives of this clinical trial were to compare the pregnancy per AI (**P/AI**) and resulting calf sex ratios due to insemination with HDLS (10 × 10^6 spermatozoa per dose at 75% purity of X chromosome) or LDHS (2.1 × 10^6 spermatozoa per dose at 90% purity) semen in nulliparous heifers and lactating cows in a California Jersey herd.

MATERIALS AND METHODS

Heifer and Cow Housing and Diets

The trial was conducted in a 3,000-cow Jersey herd in the San Joaquin Valley of California. Enrolled nulliparous heifers were eligible to be bred at 10 mo of age, whereas lactating cows of 6 lactations or fewer were eligible after a 7-wk postcalving voluntary waiting period (**VWP**). Heifers and cows were housed in dry-lot pens and fed a TMR twice daily. Diets were formulated to meet the NRC (2001) nutritional requirements for growing heifers and for lactating dairy cattle. Lactating cows were milked twice daily and produced an average of 28 kg of 3.5% FCM per day.

Reproductive Management

Inseminations were performed by 3 farm technicians twice a day after visual estrus detection and evaluation of tail head chalk (All-Weather Paintstick, La-Co Industries, Chicago, IL) as a secondary sign of estrus expression. Eligible cattle detected in estrus in the morning were bred in the afternoon and vice versa. Lactating animals were presynchronized using 2 i.m. injections of $PGF_{2\alpha}$ (25 mg of dinoprost tromethamine; Lutalyse, Pfizer Animal Health, New York, NY) 14 d apart at 36 ± 3 and 50 ± 3 DIM. Cows that were not detected in estrus by 61 ± 3 DIM were enrolled into an ovulation-synchronization protocol (**OVS**). This consisted of an intramuscular injection of GnRH (100 μg of gonadorelin diacetate; Cystorelin, Merial Ltd., Duluth, GA) on d 0, 25 mg of $PGF_{2\alpha}$ by i.m. injection on d 7, a second i.m. injection of 100 μg of GnRH on d 9 and a timed AI 24 h later. Pregnancy status was determined weekly by rectal palpation of females who