

A Field Study on Fertility and Purity of Sex-Sorted Cattle Sperm

I. Cerchiaro, M. Cassandro, R. Dal Zotto, P. Carnier, and L. Gallo¹

Department of Animal Science, University of Padova, 35020 Legnaro, Italy

ABSTRACT

The study assessed the fertility and purity of sexed semen used for inseminating Holstein-Friesian heifers in commercial dairy herds. Sex-sorted semen from 4 proven Holstein-Friesian bulls and available under commercial conditions was used on nulliparous Holstein heifers reared on 61 dairy farms of northern Italy. Data from 536 artificial inseminations with pregnancy diagnosis and 258 calvings were analyzed using the logistic regression procedure. The effects of year and season of insemination or calving, age at insemination or calving, heifer inbreeding, and the sperm dose used for insemination on the probability of a positive pregnancy diagnosis or of the birth of a female calf, respectively, were studied. The overall pregnancy rate for sexed semen was 51% and was affected by year of insemination and bull. Heifers inseminated with sexed semen from 2 bulls had lower pregnancy rates than heifers inseminated with sexed semen from other bulls. Purity of the sexed sperm, based on the proportion of female calves, was 87% and this percentage was not affected by explanatory variables included in the logistic regression. The results demonstrate that bulls differ in terms of fertility of their sexed semen. Careful selection of the insemination sires used for sorted semen is advisable for avoiding low fertility inseminations.

Key words: sex-sorted sperm, fertility, purity, cattle

INTRODUCTION

Sperm sexing is an assisted reproductive technology that can increase the efficiency of breeding programs in dairy herds (Weigel, 2004). Several practical advantages have been suggested for sexed sperm, such as the reduction in the number of cows needed for progeny testing, the opportunity of using fewer and genetically better cows for producing replacement dairy heifers and a wider opportunity for crossbreeding of dairy cows with both dairy or beef bulls (Hohenboken, 1999; Seidel, 2002).

Several procedures have been proposed for sexing sperm, such as albumin gradient separation, sex-specific antibody binding, multitube swim-up, fractionation on a discontinuous Percoll gradient, and free-flow electrophoresis (Joerg et al., 2004). At present, the fastest, most reliable, and potentially most cost-effective procedure for sperm sexing is flow cytometry (Garner et al., 1983; Welch and Johnson, 1999; Seidel and Garner, 2002). This is the only routine procedure for sexing cattle sperm that is currently available for practical applications (Maxwell et al., 2004).

Live offspring from sperm sorted by flow cytometry were first obtained by surgically inseminating rabbit does into the oviduct (Johnson et al., 1989). Later, offspring of predetermined sex were produced in pigs (Rath et al., 1997), horses (Buchanan et al., 2000; Lindsey et al., 2002), and humans (Fugger et al., 1998). The production of calves by using sex-sorted sperm was reported by Cran et al. (1993, 1995), who combined this technology with in vitro fertilization and embryo transfer. Tubman et al. (2004) reported no difference between calves from sexed sperm and calves from control sperm for birth weight, calf vigor, weaning weight, and death rates (neonatal and through weaning) and no difference between cows inseminated with sorted sperm and cows inseminated with conventional sperm for gestation length, abortion rate, and calving ease.

Practical use of sexed sperm by commercial dairy herds depends on several factors, but fertility and accuracy of sex selection (i.e., purity) are probably the most important traits for evaluating the reliability of the technology. Lower pregnancy rates after insemination with sexed sperm have been reported for several species (Seidel and Garner, 2002) but data for the fertility of sexed sperm when used under field conditions are still scarce. Likewise, the purity of sexed sperm has been evaluated using laboratory procedures (Welch and Johnson, 1999) but studies on this outcome under practical conditions after insemination of dairy cows are limited. This field study assessed fertility and purity of sexed sperm used for inseminating Holstein-Friesian heifers reared in commercial dairy herds.

MATERIALS AND METHODS

Sources of Data

Heifer identity code and birth date, date of AI, sperm dose, and breeding bull identity code were recorded on

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¹Corresponding author: luigi.gallo@unipd.it

536 nulliparous Holstein heifers that were reared in 61 dairy herds of northern Italy and were inseminated between September 2002 and April 2004. Heifers were inseminated with sperm produced by 4 proven Holstein sires (A, B, C, and D), sexed by Cogent (Chester, UK) through flow cytometry, and available under commercial conditions. Inseminations were done with either full straw, containing approximately 2×10^6 sperm, or half straw, containing 1×10^6 sperm. Heifers returning to estrus were diagnosed as not pregnant, whereas heifers that did not return to estrus were diagnosed pregnant with ultrasonography or rectal palpation. Calving date and calf sex were recorded at parturition. Inbreeding coefficients were provided by the Italian Holstein Breeders Association (ANAFI). Overall, 536 records of AI with pregnancy diagnosis and 258 calvings provided with a calf sex registration were available for this study.

Statistical Analysis

Statistical analysis was performed using logistic regression to investigate the influence of a set of explanatory variables on pregnancy rate and sex ratio. The effect of the investigated set of explanatory variables on the outcome of the insemination has been evaluated using estimates and confidence intervals of odds ratio (OR), a multiplicative measure of probability that ranges from 0 to infinity. Values of OR >1 or OR <1 indicate an increased or decreased probability of a positive pregnancy diagnosis or of a female calf birth, respectively, in comparison with a "reference condition" summarized by the intercept of the logistic regression model.

To estimate OR, logistic regression analysis was performed using the LOGISTIC procedure of SAS (SAS Institute, 1999–2001) according to the following model:

$$\ln\left[\frac{\pi(x)}{1-\pi(x)}\right] = \mu + b_1\text{YEAR} + \sum_{i=1}^3 b_{2i}\text{SEASON}_i + \sum_{j=1}^2 b_{3j}\text{AGE}_j + \sum_{k=1}^2 b_{4k}\text{INBR}_k + b_5\text{DOSE} + \sum_{l=1}^3 b_{6l}\text{BULL}_l$$

where $\ln\left[\frac{\pi(x)}{1-\pi(x)}\right]$ is the logit of x ($x = 1$ for positive pregnancy diagnoses or occurrences of a female calf birth, $x = 0$ for negative pregnancy diagnoses or occurrences of a male calf birth), and $\pi(x)$ is the probability of a positive pregnancy diagnosis or of a female calf birth; μ is the intercept of the model expressing the reference animal; that is, a heifer of the highest class of inbreeding (inbreeding >5.0% or inbreeding >4.5% for analysis of fertility or purity, respectively) and age at insemination (age >590 d) or at delivery (age >850

d), inseminated with half a semen dose (half straw containing 1×10^6 sperm) of bull D in winter or calving in autumn of the first year of trial; YEAR is an indicator variable with a value of 1 when the insemination or calving occurred in the second year of trial for the analysis of pregnancy rate or the analysis of purity, respectively; SEASON_{*i*} is an indicator variable with a value of 1 when the insemination or calving occurred in season *i* (*i* = 1, from March to May; *i* = 2, from June to August; *i* = 3, from September to November); AGE_{*j*} is an indicator variable with a value of 1 when the insemination (analysis of fertility) or the calving (analysis of purity) occurred at age *j* (for analysis of fertility *j* = 1, age <450 d; *j* = 2, age from 450 to 590 d; for analysis of purity *j* = 1, age <730 d; *j* = 2, age from 730 to 850 d); INBR_{*k*} is an indicator variable with a value of 1 when the inbreeding of the heifer was of class *k* (for analysis of fertility *k* = 1, inbreeding <1.5%; *k* = 2, inbreeding from 1.5 to 5.0%; for analysis of purity *k* = 1, inbreeding <1.66%; *k* = 2, inbreeding from 1.66 to 4.50%); DOSE is an indicator variable with a value of 1 when a whole straw was used for insemination; BULL_{*l*} is an indicator variable with a value of 1 when the semen used for insemination was from bull *l* (*l* = 1, semen from bull A; *l* = 2, semen from bull B; *l* = 3, semen from bull C).

RESULTS AND DISCUSSION

The pregnancy rate obtained using sexed cattle sperm in field conditions was slightly greater than 50% (Table 1). The pregnancy rate of sexed sperm observed in this trial was similar to that obtained with sexed semen in several trials reviewed by Weigel (2004), which ranged from 48 to 57%. Fertility of sexed semen was considerably higher in this trial than in the study by Seidel and Schenk (2002), who reported a conception rate ranging from 31 to 46%.

Fertility of sexed semen is typically lower than that of conventional semen (Weigel, 2004). The lower fertility is caused by the lower insemination dose (2×10^6 vs. 10 to 20×10^6 spermatozoa) and also by physical and chemical stresses that occur during the sorting process. These stresses include high dilution of gametes, staining with the DNA binding dye Hoechst 33342, mechanical forces during sorting, light from the UV laser beam, and projection into the collection tube under high pressure and centrifugation (Garner, 2006). A direct comparison of fertility obtained using sexed or unsexed sperm was not possible in the present study because field data were used and unsexed sperm from the same bulls was not available. The reduction of fertility resulting from the use of sexed sperm, however, has been observed by Seidel et al. (1999), who reported

Table 1. Pregnancy rate and estimates of odds ratio for factors related to a positive pregnancy diagnosis obtained on heifers inseminated with sex-sorted semen

Variable	n	Percentage pregnant	P <	Odds ratio	95% CI ¹
Inbreeding			NS		
>5.00% ²	83	54.2		1	
<1.50%	79	48.1		0.948	0.499–1.800
1.50 to 5.00%	374	51.1		0.930	0.568–1.521
Insemination year			0.010		
2002 ²	146	56.8		1	
2003	390	48.9		0.507	0.302–0.851
Insemination season			NS		
Winter ²	219	50.7		1	
Spring	152	53.9		1.191	0.765–1.856
Summer	43	44.2		0.819	0.412–1.630
Autumn	122	50.8		0.680	0.398–1.159
Insemination age			NS		
>590 d ²	75	48.0		1	
<450 d	87	45.9		0.820	0.431–1.560
450 to 590 d	374	52.9		1.161	0.689–1.955
Sperm dose ³			NS		
Half straw ²	47	44.7		1	
Whole straw	489	51.7		1.357	0.727–2.534
Breeding bull			0.047		
D ²	161	59.0		1	
A	143	51.7		0.672	0.418–1.079
B	95	43.1		0.527	0.309–0.898
C	137	46.7		0.559	0.347–0.901
Overall	536	51.1			

¹CI = confidence interval.

²Reference condition = heifer with inbreeding >5.00%, age at insemination >590 d, inseminated with half sperm dose of bull D in winter of the first year of trial.

³Sperm dose: half straw = 1×10^6 sperm/dose; whole straw = 2×10^6 sperm/dose.

pregnancy rates with sexed semen from 70 to 90% of those obtained with unsexed control semen.

With respect to factors investigated as possible sources of variation of pregnancy rate, only year of insemination ($P = 0.010$) and bull ($P = 0.047$) were statistically significant (Table 1). Heifers inseminated in 2003 had a lower pregnancy rates than heifers inseminated in 2002. Data collection started in the autumn of 2002. Several studies pointed out the negative effects caused by high humidity and high temperature on cattle fertility (Wolfenson et al., 2000). The year effect, therefore, may have arisen because inseminations in spring and summer were done only in 2003.

Heifers inseminated with sexed sperm from 2 sires (B and C), had lower pregnancy rates when compared with heifers inseminated with sex-sorted semen from bull D (Table 1). Differences in pregnancy rates among bulls are well documented in the literature for both unsexed (Donovan et al., 2003) and sexed semen (Seidel et al., 1997; Bodmer et al., 2005). These results suggest the need for careful selection of bulls based on accurate analysis of semen fertility under field conditions.

The breeding season and the age of heifers at AI did not affect conception rate (Table 2). These results agree partly with those reported by Donovan et al. (2003),

who reported that pregnancy rate was not related to age at first service and was negatively influenced by the hot season in a field study on 601 nulliparous Holstein heifers. In our study, 44% of the heifers inseminated in the summer became pregnant and greater percentages of heifers inseminated in winter, spring, and autumn became pregnant. Perhaps the lack of statistical significance observed in this study for the season can be explained by the relatively low number of AI done in the summer (Table 1).

Inbreeding of heifers did not affect the conception rate (Table 1). This result does not agree with findings of Wall et al. (2005), who reported an unfavorable effect of inbreeding on several reproductive traits in a mixed population of Holstein and Friesian cattle. Conversely, Cassell et al. (2003) failed to identify a maternal inbreeding depression effect on 70-d nonreturn rate in a study of 50,613 Holstein cows.

Sperm dose did not affect pregnancy rate but there was a numeric advantage when whole straws were used (Table 1). Seidel et al. (1999) also reported a minimal difference in pregnancy rates after the use of 1.5×10^6 or 3.0×10^6 sex-sorted spermatozoa. The low number of services with the half sperm dose may have affected our ability to detect significant effects.

Table 2. Percentage of female calves and odds ratio for factors related to the birth of a female calf estimated on calves obtained using sexed sperm

Variable	n	Percentage of female calves	P <	Odds ratio	95% CI ¹
Inbreeding			NS		
>4.50% ²	64	89.1		1	
<1.66%	38	89.5		1.196	0.318–4.500
1.66 to 4.50%	156	85.9		0.810	0.318–2.064
Sperm dose ³			NS		
Half straw ²	21	90.5		1	
Whole straw	237	86.9		0.600	0.126–2.858
Breeding bull			NS		
D ²	88	89.8		1	
A	69	88.4		0.807	0.276–2.360
B	38	79.0		0.433	0.143–1.311
C	63	87.3		0.656	0.222–1.937
Calving year			NS		
2003 ²	176	88.1		1	
2004	82	85.4		0.851	0.240–3.020
Calving season			NS		
Autumn ²	103			1	
Winter	77	89.6		1.477	0.453–4.816
Spring	24	75.0		0.556	0.104–2.967
Summer	54	90.7		1.653	0.516–5.295
Calving age			NS		
>850 d ²	47	83.0		1	
<730 d	40	87.5		1.069	0.289–3.949
730 to 850 d	171	88.3		1.423	0.530–3.824
Overall	258	87.2			

¹CI = confidence interval.

²Reference condition = heifer with inbreeding >4.50%, age at calving >850 d, inseminated with half sperm dose of bull D and calving in autumn of the first year of trial.

³Sperm dose: half straw = 1×10^6 sperm/dose; whole straw = 2×10^6 sperm/dose.

The purity of sexed sperm was high and no effect included in the logistic regression model significantly affected the sex of newborn calves (Table 2). Results of sexed sperm purity obtained in the present study are in good agreement with the literature (Seidel and Garner, 2002) and provide a field demonstration that semen sorting is a reliable method for sex preselection in cattle.

CONCLUSIONS

The use of sperm sexed by flow cytometry to inseminate dairy heifers reared in a large group of commercial dairy farms yielded a pregnancy rate of nearly 50%, with 87% female calves. Pregnancy rates depended on year and insemination sire. Careful selection of the insemination sire used for sorted semen will be necessary so that low-fertility inseminations are avoided on commercial dairy farms.

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