Theriogenology xxx (2015) 1–5

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Transfer of cattle embryos produced with sex-sorted semen results in impaired pregnancy rate and increased male calf mortality

M. Mikkola*, M. Andersson, J. Taponen

Department of Production Animal Medicine, University of Helsinki, Saarentaus, Finland

ARTICLE INFO

Article history:
Received 8 March 2015
Received in revised form 15 June 2015
Accepted 17 June 2015

Keywords:
Cattle
Sex-sorted semen
Embryo transfer
Pregnancy rate

ABSTRACT

This study investigated the pregnancy rate and calf mortality after transfer of embryos produced using sex-sorted semen. Data for 12,438 embryo transfers performed on dairy farms were analyzed. Of these, 10,697 embryos were produced using conventional semen (CONV embryos) and 1741 using sex-sorted semen from 97 bulls (SEX embryos), predominantly of Ayrshire and Holstein breeds. Of the CONV embryos, 27.4% were transferred fresh, whereas of the SEX embryos, 55.7% were fresh. Recipient attributes (breed, parity, number of previous breeding attempts, and interval from calving to transfer) were comparable for both embryo types, heifers representing 57.8% of recipients in the CONV group and 54.8% in the SEX group. Recipients that were not artificially inseminated or did not undergo a new embryo transfer after the initial embryo transfer and had registered calving in fewer than 290 days after the transfer were considered pregnant. Pregnancy rate for recipients receiving CONV embryos was 44.1%, and for those receiving SEX embryos, it was 38.8%. The odds ratio for pregnancy in recipients receiving CONV embryos was 1.34 compared with SEX embryos ($P < 0.001$). The proportion of female calves was 49.6% and 92.3% in CONV and SEX groups, respectively. Overall, calf mortality was comparable in both groups. Mortality was similar in CONV and SEX groups (6.6% and 7.7%, respectively) for female calves. For male calves, mortality was 9.2% in the CONV group but significantly higher, 16.0% ($P < 0.05$), in the SEX group. This study showed that transfer of embryos produced with sex-sorted semen decreased the pregnancy rate by about 12% compared with embryos produced using conventional semen. Mortality of male calves born from SEX embryos was higher than for those born from CONV embryos.

1. Introduction

Flow cytometric sorting of bovine sperm has become a practicable approach to control the sex of offspring and enhance production of replacement heifers on dairy farms since the technique was commercialized in the early 2000s. In addition to the use of such semen in artificial insemination of single-ovulating cows and heifers, it can be used for superovulated animals in production of *in vivo* embryos and also for *in vitro* embryo production (IVP). Insemination of superovulated embryo donors with sex-sorted semen and *in vitro* production of embryos can multiply the number of high genomic female calves in a breeding program. However, pregnancy rates after insemination with sex-sorted semen do not reach those achieved using conventional semen (review by Seidel [1]) because of both a lower sperm dose and compromised viability of sexed sperm. When sexed semen is used for superovulated donors, the reduction in fertilization rate is even more apparent than for single-ovulating females, leading to a decline in the yield of transferable embryos [2–8]. Sex-sorted semen also

* Corresponding author. Tel.: +358 40 311 5031; fax: +358 40 381 2284.
E-mail address: marja.mikkola@vikinggenetics.com (M. Mikkola).

0093-691X/$ – see front matter © 2015 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2015.06.012

compromises *in vitro* production of embryos, deteriorating cleavage rate and blastocyst formation [9–14].

In addition to the increase in the proportion of unfertilized oocytes and degenerated embryos, evidence has emerged that sex sorting alters embryo quality. Deviations in the gene expression of embryos produced with sex-sorted semen were reported for ovine [15] and bovine embryos [10].

Sex ratio, embryo yield, and pregnancy and calving rate of embryos need to be considered to evaluate completely the economic impact of sexed semen on breeding schemes incorporating embryo transfer. The technique of sperm sorting has reached a state where typical accuracy of sperm sorting for commercial use is 90% X-sperm [1]. Transferable embryo yield is compromised, with a more discernible reduction in cows than in heifers [3,4,7]. In cows, the embryo yield has approximately halved from that achieved with conventional semen. However, in heifers, this reduction is not as extreme, and reasonable results can be obtained [7]. Authoritative data on pregnancy and calving rate after transfer of embryos produced with sexed semen are scarce. Whether decrease in the number of transferable embryos is the only outcome from the use of sexed semen, or embryo viability after transfer of Day-7 embryos is also affected, has not been determined on a large scale. Thus, the objective of this study was to compare the pregnancy rates after transfers of Day-7 *in vivo* embryos produced either with conventional or sex-sorted semen from numerous bulls commercially available and extensively used. In addition, calf mortality was studied for the two embryo types.

2. Materials and methods

Data from 13,982 embryo transfers, performed on dairy farms during a 5-year period (2008–2012), collected from the Finnish national database (Agricultural Data Processing Center Ltd., Vantaa, Finland), were analyzed. All embryos were derived from superovulated donors. Data on embryo transfers with ambiguous or inadequate information were eliminated from the original data set. The criteria for pregnancy were as follows: the animal did not undergo artificial insemination or have a new embryo transfer after the original embryo transfer and had registered calving data for fewer than 290 days after the transfer. This information was used when calculating the pregnancy rate, i.e., number of animals pregnant divided by the number of transfers. Consequently, 988 transfers were excluded because there were no data on calving and no service after the embryo transfer. Also, 203 transfers were removed because of conflicting calving date and transfer date. Additionally, data for animals with an aberrant history were removed. These comprised animals reinseminated 2 to 8 days after the transfer ($n = 9$), animals that had been inseminated more than four times before the embryo transfer ($n = 324$), and animals with an exceptionally short time period (<43 days) from calving to the embryo transfer ($n = 20$). The remaining data were for transfer of 12,438 embryos, both fresh and frozen thawed, of which 10,697 were produced with conventional semen (CONV embryos) and 1741 with sex-sorted semen of 97 bulls (SEX embryos).

Calf mortality was analyzed in addition to the pregnancy rate. Data for calf mortality were collected from the same database as the data for embryo transfers, and they comprised abortions at a stage of 180 or more days of gestation and neonatal death of calves less than 7 days of age.

2.1. Recipients

Embryos were transferred to heifers and primiparous and multiparous Ayrshire and Holstein cows. The numbers of breeding attempts, as well as the parity and breed of recipients, are shown in Table 1. The breed and parity of the recipients are expressed in similar proportions for both embryo types. Likewise, the reproductive history of heifers and cows after the previous calving was similar for both groups of recipients.

2.2. Embryos

Although SEX and CONV groups were similar regarding the recipients, there was, however, a bias in the proportions of fresh and frozen embryos. CONV embryos were transferred frozen more frequently, and only, 27.4% of them were transferred fresh. SEX embryos were biased toward fresh transfers (55.7%).

For the CONV group, the breed of embryo (breed of sire) was 55.2% Ayrshire, 43.2% Holstein, and 1.6% other breeds. For the SEX group, the breed was 32.5% Ayrshire, 62.6% Holstein, and 4.9% other breeds.

The quality codes for embryos transferred, as referred to the International Embryo Transfer Society classification of grade 1, 2, and 3 embryos [16], were similar in both groups. For the frozen embryos, the majority was grade 1. Grade 2 embryos represented 3.1% of the frozen CONV embryos and 4.1% of frozen SEX embryos. Of the fresh embryos, grade 1 embryos represented 52.9% and 54.4%, grade 2 embryos 32.7% and 29.7%, and grade 3 embryos 14.3% and 15.8% of transfers for CONV and SEX groups, respectively.

The developmental stages of embryos in CONV and SEX groups, respectively, were 27.6% and 31.4% compact morulas, 25.6% and 30.0% early blastocysts, 38.3% and 28.8% blastocysts, 7.4% and 8.8% expanded blastocysts, 1.0% and

Table 1
Distribution (%) of recipient breed, parity, number of breeding attempts, and interval from calving to embryo transfer (ET; for cows) when the transferred embryo was produced with conventional semen (CONV) or with sex-sorted semen (SEX).

Recipient attributes	CONV	SEX
Breed		
Ayrshire	63.7	59.2
Holstein	36.3	40.8
Parity		
Heifer	57.8	54.8
Primiparous	17.7	18.2
Multiparous	24.5	27.0
Breeding attempt		
1st	83.0	85.5
2nd	12.0	10.7
3rd	3.7	2.9
4th	1.2	0.8
Interval from calving to ET, days	120.2 ± 58.8	119.0 ± 52.8