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Quality and developmental rate of embryos produced with sex-sorted and conventional semen from superovulated dairy cattle

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ABSTRACT

This study investigated the effect of sex-sorted semen compared with conventional semen on the outcome of embryo recovery, placing special emphasis on the quality, and developmental stage of embryos. Data were analyzed for 443 embryo collections with sex-sorted semen (SEX group) and 1528 with conventional semen (CONV group) in superovulated dairy heifers and cows. The insemination protocol for conventional semen included two inseminations, comprising a total dose of 30 million sperm passing into the uterine body. For sex-sorted semen, two (30%) to three (70%) deep uterine inseminations were performed, the total dose ranging from eight to 12 million sperm. The data were analyzed separately for heifers and cows. The total number of recovered structures was similar among the groups. The number of viable embryos decreased in the SEX groups compared with the CONV (with 1.4 and 3.2 fewer embryos in heifers and cows, correspondingly, $P < 0.001$), and correspondingly the proportions of unfertilized ova and degenerated embryos increased in the SEX groups ($P < 0.001$). The proportion of unsuccessful collections, yielding no transferable embryos, increased in the SEX groups for both heifers (from 7.2% to 11.2%, $P = 0.025$) and cows (from 9.0% to 20.7%, $P < 0.001$). Regarding the quality of viable embryos, the quality grades were superior in the CONV group compared with the SEX group for heifers ($P < 0.001$) and cows ($P < 0.001$). The proportion of grade 1 embryos decreased by 6.5 percentage points in heifers and 11.9 percentage points in cows when sex-sorted semen was used. Correspondingly, the proportions of grade 2 and 3 embryos increased in heifers and cows when sexed semen was used. The mean developmental stages of embryo collections were numerically slightly lower in the SEX group. In heifers, the delay in developmental stage was statistically significant ($P = 0.001$), but in cows, there was only a tendency toward that ($P = 0.067$). In conclusion, sex-sorted sperm decreased the transferable embryo yield and increased the risk of a recovery yielding no transferable embryos. Furthermore, use of sex-sorted semen decreased the proportion of grade 1 embryos. In addition, it also seemed to delay embryonic development, although the delay in embryonic development was minimal and its biological relevance remains undefined. Despite the compromised embryo production, taken into account the optimization of recipient resources, the use of sex-sorted semen is advantageous, especially in superovulated heifers, which are of most importance in the modern breeding strategies using genomic selection.

1. Introduction

Production of offspring of the desired gender using sex-sorted semen has become an established, reliable technique in the dairy cattle breeding industry over the last

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decade. In dairy herds, the major application of sorted X-semen using flow cytometry is artificial insemination (AI) of virgin heifers selected to produce the next generation of replacement animals. Other applications include AI of superovulated embryo donors for *in vivo* embryo production and IVF of matured oocytes. *In vitro* embryo production is suitable for sex-sorted sperm because smaller numbers of spermatozoa are sufficient compared with *in vivo*, where the dose-related reduction in fertilization potential of the typical 2 million sperm dose is evident. Pregnancies can be successfully achieved even when frozen-thawed semen is sexed and used for *in vitro* production [1,2].

The technique of sorting sperm using flow cytometry has been improved in several aspects since it was implemented commercially about 15 years ago. One of the major improvements that resulted in an increase in fertility is lowering the pressure the sperm has to tolerate during the sorting procedure [3,4]. During recent years, sex-sorted semen has achieved pregnancy rates approaching those for conventional semen, currently being nearly 90% of the level for conventional semen, when used in well-managed dairy herds [5]. Despite the pregnancy rate of single-ovulating females being nearly comparable with that using conventional semen, the impaired fertilization rate in superovulated animals has hindered its use in multiple ovulation and embryo transfer (MOET) programs. Several studies concluded that the yield of transferable embryos is compromised markedly in cows, but this reduction is only moderate in heifers [6–11]. In addition, the proportion of unfertilized oocytes is elevated when sexed semen is used. After IVF, sexed semen results in decreased cleavage and/or blastocyst rates compared with conventional semen [1,2,12–17]. However, these observations are not consistent, apparently because of variability in sperm selection and *in vitro* production procedures, and some authors did not establish a difference in the IVF potential between the two sperm types [18,19]. There is evidence that sperm sorting carries effects into the developing embryo because differences in gene expression of *in vitro*-produced embryos were demonstrated [13]. In addition to the early developmental stages, there are data indicating that sex sorting also affects later embryonic development because pregnancy rates after transfer of Day 7 *in vivo* embryos are compromised in embryos produced with sorted semen [20]. However, similar pregnancy rates were achieved with *in vitro* embryos produced with sexed and conventional semen [2,21,22].

Despite many reports demonstrating a reduction in the number of transferable *in vivo* embryos when sexed semen is used, the quality grades of embryos are included in the analysis in only a few reports. Moreover, no comparison of the developmental stages of *in vivo* embryos produced with the two semen types has been published. A compromised fertilization rate in cows inseminated with sex-sorted semen is obvious, but with respect to the proportion of quality grade 1 embryos, either no difference [8] or a decrease has been reported [7,10,23].

A data set was analyzed to evaluate the embryo yield in superovulated donors inseminated with conventional or sex-sorted semen, with special emphasis on the quality and developmental stage of embryos.

2. Materials and methods

2.1. Donors and superovulation protocol

Data from 443 embryo collections with sex-sorted semen (SEX group) and 1528 with conventional semen (CONV group) were analyzed. Embryos were collected during an 8-year period (2008–2015) from superovulated donors on commercial dairy farms in Finland. Heifers represented 65.9% and 72.7% of the donors for CONV and SEX, respectively. Holstein and Ayrshire breeds were used, the proportion of Holstein being 66.2% in the CONV group and 74.7% in the SEX group.

Superovulation was induced by eight intramuscular injections of FSH, Folltropin (Vetoquinol S.A., Lure cedex, France) or Pluset (Laboratorios Calier, S.A., Barcelona, Spain), at 12-hour intervals over 4 days, beginning 9 to 12 days after the onset of standing estrus. Declining doses of 630 IU (Folltropin) or 850 IU (Pluset) FSH in total were administered to cows, and heifers received 420 to 490 IU (Folltropin) or 500 to 600 IU (Pluset). The donors were treated with a standard dose of prostaglandin $F_{2\alpha}$ or a synthetic agonist together with the sixth (cows) or seventh (heifers) FSH treatment.

2.2. Artificial inseminations and semen

In all superovulated females, inseminations were initiated 12 hours after the onset of standing estrus. The insemination protocol for the SEX group differed substantially from that for the CONV, for which two doses of 15 million sperm were deposited in the uterine body 9 to 15 hours apart. For sexed semen, deep uterine inseminations were performed two to three times 9 to 15 hours apart. One dose of 2 million sperm was deposited in each uterine horn at the first two inseminations, and at the third AI only a single straw was used, divided between the uterine horns. Of the donors in the SEX group, approximately 70% were inseminated thrice, the remaining 30% being inseminated only twice, as for the CONV group. The decision concerning the number of inseminations was based in each case on the onset and duration of estrous signs.

Semen doses were commercially produced by several AI centers. Conventional semen was collected from 615 bulls worldwide. The majority of the conventional semen straws contained 15 million spermatozoa. The total number of spermatozoa used per donor was 30 million, but in some cases, 45 million. Sex-sorted semen from 135 bulls was used. Most sexed straws contained 2 million spermatozoa. The total number of sex-sorted sperm used for each donor was generally 10 million, varying between 8 and 12 million.

2.3. Embryo collection and evaluation

Embryos were collected 7 days after inseminations by transcervical uterine flushing using a standard protocol and commercially available media. After collection, embryo morphology was assessed under a stereomicroscope ($\times$ 60 magnification), and embryos were classified according to the International Embryo Technology Society (IETS)