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Embryo production with sex-sorted semen in superovulated dairy heifers and cows

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ABSTRACT

The aim of this study was to examine the effect of sex-sorted semen on the number and quality of embryos recovered from superovulated heifers and cows on commercial dairy farm conditions in Finland. The data consist of 1487 commercial embryo collections performed on 633 and 854 animals of Holstein and Finnish Ayrshire breeds, respectively. Superovulation was induced by eight intramuscular injections of follicle-stimulating hormone, at 12-hour intervals over 4 days, involving declining doses beginning on 9 to 12 days after the onset of standing estrus. The donors were inseminated at 9 to 15–hour intervals beginning 12 hours after the onset of estrus with 2 + 2 (+1) doses of sex-sorted frozen-thawed semen (N = 218) into the uterine horns or with 1 + 1 (+1) doses of conventional frozen-thawed semen (N = 1269) into the uterine corpus. Most conventional semen (222 bulls) straws contained 15 million sperm (total number 30–45 million per donor). Sex-sorted semen (61 bulls) straws contained 2 million sperm (total number 8–14 million per donor). Mean number of transferable embryos in recoveries from cows bred with sex-sorted semen was 4.9, which is significantly lower than 9.1 transferable embryos recovered when using conventional semen ($P \leq 0.001$). In heifers, no significant difference was detected between mean number of transferable embryos in recoveries using sex-sorted semen and conventional semen (6.1 and 7.2, respectively). The number of unfertilized ova was higher when using sex-sorted semen than when using conventional semen in heifers ($P < 0.01$) and in cows ($P < 0.05$), and the number of degenerated embryos in cows ($P < 0.01$), but not in heifers. It was concluded that the insemination protocol used seemed to be adequate for heifers. In superovulated cows, an optimal protocol for using sex-sorted semen remains to be found.

1. Introduction

Since the introduction of commercially available sex-sorted bovine semen early in the 2000s, there have been numerous reports published on conception rates [1–7]. Generally, conception rates with sex-sorted semen are lower than with conventional semen, because of the adverse effects of the flow cytometry sorting procedure on sperm viability and the low number of sperm per dose of sex-sorted semen [8]. The decline in conception rates varies among these reports, depending on factors such as sperm dose, site of sperm deposition, parity, estrus synchronization, and management and generally ranged between 70% and 80% of that achieved with conventional semen.

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In the dairy industry, there is a strong interest toward applications that increase the production of female calves from genetically superior cows. The use of sex-sorted X-sperm in multiple ovulation and embryo transfer programs can be instrumental for the production of female progeny. Before sex-sorted semen was available, polymerase chain reaction-based embryo sex determination was the only approach to preselect the sex of calves and reduce the number of recipients needed. However, sex determination of an embryo demands skills and equipment for biopsy and polymerase chain reaction analysis, increases costs, and results in a decline in embryo vitality when freezing biopsied embryos [9,10]. The use of sex-sorted semen bypasses these disadvantages of embryo sexing.

Despite the advantages of sex-sorted semen, it has not been used widely in inseminations of superovulated donors. The compromised fertility because of low doses of sex-sorted semen, combined with the higher incidence of fertilization failure on superovulated versus single-ovulating cattle [11–13] counteracts the use of sex-sorted semen on embryo donors. Inevitably, all authors have reported a decline in the fertilization rate and in the proportion of transferable embryos using sex-sorted semen compared with conventional semen on donor cows [14–17]. In heifers, this decline is usually smaller [14,15,18,19]. However, comparison of the results is challenging, because of different insemination protocols, sperm dosages, site and timing of insemination, parity of donors, and other variables.

The dose of sex-sorted semen used for superovulated donors has varied from 2 to 20 $\times$ 10^6 sperm [14–19]. Most previous experiments were performed using conventional insemination techniques, with sex-sorted semen deposited into the uterine body, but some also involved deep uterine horn insemination [16,18,19]. The timing of inseminations using sex-sorted semen can have a more important role in superovulated than in single-ovulating animals because of the time interval between the first and last ovulations [2,19]. It has been suggested that sex-sorted sperm have a shorter functional life than conventional sperm [2]. In addition to the variation in these factors, most previous experiments were performed using a relative low number of animals (5–59 donors per treatment group). This, along with the high level of individual variation present in superovulatory responses and embryo yield, exacerbates the interpretation of the results. The optimal sperm dose and the site and timing of inseminations to optimize commercially satisfactory embryo production with sex-sorted semen remain to be determined.

The objective of this study was to examine the effect of sex-sorted semen on the number and quality of embryos recovered from a large number of superovulated heifers and cows on commercial dairy farms in Finland. In this study, a deep uterine insemination technique was used.

2. Materials and methods

2.1. Donors and experimental protocol

The data consist of 1487 commercial embryo collections performed in Finnish dairy herds between January 2008 and December 2011. The collections were performed in 633 and 854 animals of Holstein and Finnish Ayrshire breeds, respectively. The donors were inseminated with sex-sorted frozen-thawed semen (N = 218) or with conventional frozen-thawed semen (N = 1269, controls). Donors inseminated with sex-sorted semen consisted of 130 heifers and 88 cows (22 first parity and 66 older animals), whereas in the control group, there were 945 heifers and 324 cows.

2.2. Superovulation protocol and artificial inseminations

Superovulation and embryo recovery were performed by standard multiple ovulation and embryo transfer protocols. Superovulation was induced by eight intramuscular injections of follicle-stimulating hormone (FSH), Folltropin (Bioniche Teo, Inverin, Co., Galway, Ireland) or Pluset (Laboratorios Calier, S.A., Barcelona, Spain), at 12-hour intervals over 4 days, involving declining doses, 630 IU (Folltropin) or 800 to 900 IU (Pluset) FSH in total for cows, with heifers receiving 60% to 75% of cow doses beginning on 9 to 12 days after the onset of standing estrus. Our unpublished data showed no difference in the superovulatory response between these two FSH preparations. The donors were treated with a generally recommended dose of prostaglandin $F_{2\alpha}$ or a synthetic agonist along with the sixth (cows) or seventh (heifers) FSH treatment. Inseminations were started 12 hours after the onset of standing estrus. The donors were inseminated two or three times 9 to 15 hours apart. When using conventional semen, an artificial insemination (AI) technician or a farmer inseminated the animal with one dose of semen at a time into the uterine corpus. When using sex-sorted semen, an embryo transfer (ET) technician performed the inseminations. Instructions for the use of sex-sorted semen were to inseminate three times with 2 + 2 + 1 straws. When two straws were used at a time, each uterine horn received one straw, and when one straw was used, the content of the straw was divided between the two uterine horns. In 61 cases, the estrus was so short that the donor was inseminated only twice. In seven cases, the estrus was prolonged and the donor was inseminated four times. In the remaining 150 cases, the donor was inseminated three times. Embryo flushing was performed 7 days after AI. Recovered ova/embryos were evaluated according to the International Embryo Transfer Society classification system [20]. Flushing was performed by 10 experienced veterinarians and one ET technician.

2.3. Semen

Semen doses used for AI were commercially produced at several AI centers. Conventional semen was collected from 222 different bulls worldwide. Most conventional semen straws contained 15 million spermatozoa. The total number of conventional sperm used per donor was 30 to 45 million. Sex-sorted semen from 61 different bulls was used. Sex-sorted semen was produced using the XY sperm sorting protocol [21] at 10 AI centers in Canada, Denmark, Great Britain, Italy, Switzerland, and the United States. The straws contained 2 million spermatozoa. The total number of