

# Cytogenetic survey of Holstein bulls at a commercial artificial insemination company to determine prevalence of bulls with centric fusion and chimeric anomalies

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**Objective**—To determine prevalence of Holstein bulls with chromosomal anomalies, particularly the 1/21 centric fusion (CF), at a commercial artificial insemination (AI) company in the United States.

**Design**—Cross-sectional cytogenetic prevalence study.

**Animals**—All 606 Holstein bulls at a commercial AI company were cytogenetically screened to detect CF, chimerism, and other chromosomal abnormalities.

**Procedure**—Lymphocytes from heparinized blood samples were cultured by standard cytogenetic techniques, and chromosome spreads were prepared for microscopic examination. Chromosomal abnormalities were detected by examining 10 chromosome spreads per bull. Pedigree analysis was performed.

**Results**—None of the bulls had any type of CF. However, 6 bulls were identified as chimeras (ie, contained lymphocytes with male [XY] and female [XX] chromosomes). One bull was sire or maternal grandsire to 85 of the bulls tested, and 739 of 1,212 (61%) sire and maternal-grandsire possibilities were accounted for by just 18 bulls.

**Conclusions and Clinical Relevance**—Analysis of these results supports previous indications that CF is extremely rare in Holstein bloodlines available commercially via AI in the United States. However, chimeric bulls are more common, and they reportedly have decreased reproductive performance. Therefore, identification of chimeric sires in the AI facility reported here and the possibility of de novo onset of CF at any time indicates that early cytogenetic screening should be encouraged for prospective bulls intended for use in AI programs. (*J Am Vet Med Assoc* 2000;216:65–67)

**Centric fusion (CF)** is a heritable fertility-related anomaly in which 2 autosomes (ie, nonsex chromosomes) become permanently joined at their centromeric ends. This causes these chromosomes to segregate as an abnormal unit during the reduction division in the formation of ova and spermatozoa. Therefore, some ova and spermatozoa may contain too

many or too few chromosomes, and early embryonic death is likely when these abnormal gametes participate in fertilization.<sup>1,2</sup>

In 1973, Fechheimer<sup>3</sup> reported a cytogenetic survey of 743 bulls from 3 artificial insemination (AI) centers in the United States, including 538 Holsteins, but CF was not identified in any of these bulls. In Japan, there recently was a Holstein bull of US parentage that was identified with a 1/21 CF.<sup>4</sup> This anomaly subsequently was found in 10 of 23 of its offspring. Five other CF have been reported in Holstein and Holstein-Friesian cattle worldwide. A 2/4 CF was found in a British Holstein-Friesian bull and 40 of 89 (45%) of its offspring.<sup>5,6</sup> A 13/21 CF was diagnosed in a Canadian Red and White Holstein-Friesian bull.<sup>7</sup> A 1/29 CF was identified in a British-Friesian bull and 19 of 35 (54%) of its offspring.<sup>8</sup> In the United States, a 14/28 CF was diagnosed in a Holstein cow.<sup>9</sup> Recently, a 19/21 CF was reported in France in a Holstein-Friesian cow, its calf, and a related female sibling.<sup>10</sup>

More than 25 CF have been reported worldwide in approximately 40 cattle breeds.<sup>11</sup> The first report of CF in cattle was in 1964, when investigators found a 1/29 CF in Swedish Red and White cattle.<sup>12</sup> The CF reported for cattle in the United States have been found primarily in beef breeds (eg, the 1/29 CF in Charolais<sup>1,13</sup> and the 14/20 CF in Simmental<sup>2</sup> cattle). Subsequently, the mode of inheritance and fertility-decreasing effects of CF were described.<sup>14</sup> In other reports,<sup>15–19</sup> identification of CF in herds was associated with a reduction in herd fertility by 5 to 21%. The lower fertility of CF carriers is a consequence of genetic excess or deficiency in their germ cells, leading to early degeneration of embryos (8 to 15 days after insemination).<sup>18,22</sup>

Chimerism is the condition that exists when 2 genetically distinct types of cells are in the same animal. This condition can result when vascular fusion of the chorions of 2 embryos establishes common circulation before approximately day 40 of pregnancy.<sup>23</sup> When this happens in a cow pregnant with twin fetuses of opposite sexes, it is possible for signals from the male fetus to inhibit development of the reproductive system and cause varying degrees of masculinization of the female fetus. This causes approximately 90% of female calves born twin to a bull to be chimeric and infertile (ie, freemartins). Because bone marrow stem cells, including lymphocytic cells, of the twin fetuses also are exchanged via their fused placental circulation, a permanent condition of chimerism exists in both animals.<sup>23</sup> The existence of male (XY) and female (XX) sex

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Supported in part by Minnesota Agricultural Experiment Station funds.

The authors thank Glen Gilbert and Al Kuck for technical assistance.

chromosomes in the same animal is the basis of the cytogenetic test for chimerism in females and males, as well as for freemartinism in females.<sup>a</sup> Bulls born twin to heifers are almost always phenotypically normal, but a few reports indicate chimeric bulls are more likely to be culled for reproductive problems<sup>24,25</sup> or to have an altered sex ratio among their offspring.<sup>24</sup> Bulls born as single calves can be chimeras in situations in which a female fetus that was twin to the bull died undetected in utero.<sup>b</sup>

The objective of the study reported here was to conduct a cytogenetic survey of all Holstein bulls at an AI company. These bulls were believed to be relatively representative of all Holsteins in current AI use in the United States. The study is important, because 25 years have elapsed since the last reported cytogenetic survey; also, CF abnormalities have become quite common internationally in some cattle breeds, and such a survey has not been conducted since a new form of CF (ie, 1/21 CF) reportedly originated in Holstein bloodlines in the United States.

### Materials and Methods

The study included 606 Holstein bulls in an AI organization in the United States. Lymphocytes of blood samples were cultured, and chromosome spreads were prepared in accordance with standard methods.<sup>13</sup> Giemsa-stained slides of chromosomes in metaphase cells were examined microscopically for evidence of chromosomal anomalies, especially evidence of CF. At least 10 chromosome spreads were inspected to verify that a bull did not have CF or chimerism. This number was selected on the basis that it would allow accurate detection of all CF, because all cells in CF-carrier animals have the defect. It would also allow us to detect most chimeric bulls. Sire and maternal grandsire information was compiled from pedigree information to evaluate genetic diversity represented in the sample population.

### Results

Centric fusion chromosomal anomalies were not observed in any of the chromosome spreads of the 606 Holstein bulls examined. However, 6 chimeric bulls were identified. Five of these 6 chimeric bulls were recorded as being born as twins, with 1 of these 5 also recorded as resulting from establishment of pregnancy by use of **embryo transfer (ET)**. The sixth chimeric bull did not have a history of being a twin. Two additional bulls recorded as being born twins to another calf (sex unknown) were nonchimeric.

Analysis of sire and maternal grandsire information revealed considerable genetic duplication in our sample population. A total of 139 bulls accounted for the 1,212 possibilities of sire and maternal grandsire for the 606 Holstein bulls tested; 95 were sires, 78 were maternal grandsires, and 34 were sires and maternal grandsires. Furthermore, 1 bull was sire or maternal grandsire to 85 of the bulls tested, and 739 (61%) of all sire and maternal grandsire possibilities were accounted for by only 18 bulls.

### Discussion

In the study reported here, the fact that we did not find any bulls with CF in 606 Holstein bulls in major genetic lines in the United States is a reassuring indi-

cator that this dairy breed continues to be free of this potential problem; this is similar to the results of the original survey of Holstein bulls in the United States 25 years ago.<sup>3</sup> These findings were gratifying, considering that testing for CF is not required for bulls in use at commercial AI companies in the United States, and new CF can develop de novo in various beef and dairy cattle breeds throughout the world.<sup>26</sup>

Lack of CF in the sample population was considered primary evidence that the 1/21 CF found in a US-born bull in Japan was of recent de novo origin. Two other reasons led us to accept this scenario. First, we have not found any 1/21 CF-positive animals in > 2,000 Holstein cattle tested cytogenetically for freemartinism during a period of > 18 years.<sup>27</sup> Second, the minute cytogenetic fragment, a leftover product of the CF process, was seen only in early, but not later, generations of offspring from that bull.<sup>28</sup>

Reports from several countries<sup>4,29,32</sup> indicate that spread of CF can result from the introduction of carrier bulls; this discovery has led to vigilant cytogenetic efforts to prevent or eliminate the problem in some countries. This suggests the potential value of cytogenetic testing of cattle in the United States, particularly bulls, before they are selected for use as breeding stock or germplasm donors. In Europe and Canada, it is recommended that prepurchase cytogenetic testing be performed on cattle intended for use as breeding stock.<sup>1,2</sup>

Purebred cattle associations in the United States require that calves born as twins are identified as twins in their registration name. Unfortunately, identification of the sex of each twin is not required, so potential chimeric animals are not readily apparent. This modification would be an improvement over current registration procedures and would greatly assist the type of concerns reported in this study. Although being born a twin from a pregnancy established by use of ET seems to be an unlikely event, 1 instance of this circumstance was found in our sample population. This situation could be the result of a second embryo being unknowingly included with the embryo selected for transfer. Alternatively, twins could be the result of a single embryo that split after transfer. In the former situation, the twins would be fraternal and could include 1 of each sex. However, if a single embryo split after transfer, the calves would be identical and of the same sex. The chimeric bull that was a twin in an ET-produced pregnancy must have resulted from the first possibility, with someone unknowingly transferring 2 embryos of opposite sexes. Another less likely possibility is that the recipient was bred and conceived at the estrus prior to transfer of the ET embryo and was carrying its own embryo at the time it was used as an embryo recipient.

The chimeric bull we identified that was reportedly born as a single calf would appear to be the result of a mixed (1 male, 1 female) set of twins in which the female calf died in utero and was reabsorbed.<sup>b</sup> Rarely, the opposite of this situation has been observed in which female calves born as singletons have been identified as freemartins.<sup>33</sup>

Currently, dairy cattle breeding involves a high degree of genetic selection and concentration. Thus,

even though a large number of bulls was karyotyped, there was considerable genetic duplication in our sample population. Therefore, our sample population was not a complete representation of the Holstein breed in the United States. It must be recognized, however, that this genetic concentration nationally and internationally is a reflection of breeding tendencies currently used that are based on milk-production testing or selection programs and are made possible because of AI. For this reason, there is tremendous potential for rapid and wide dissemination of a bull proven to be genetically superior for production traits. Under current procedures, such a bull could easily be an undetected chimeric or CF carrier. Thus, we propose that all bulls entering AI progeny-proving programs undergo mandatory cytogenetic screening for CF defects and chimerism.

<sup>a</sup>Fechheimer NS, Herschler MS, Gilmore LO. Sex chromosome mosaicism in unlike sexed cattle twins (abstr). In: Geerts SJ, Abeelen JHFV, Anders GJPA, et al, eds. *Genetics today. Proceedings of the XI international congress of genetics*. New York: MacMillan, 1963;265.

<sup>b</sup>Kovacs A, Stukovszky J, Gippert E, et al. Single-born XX/XY chimeric bulls with normal phenotype (abstr). *Ann Genet Select Anim* 1977;9:533.

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