

NON-FREEMARTIN RATE IN HOLSTEIN HETEROSEXUAL TWINS

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INTRODUCTION

Bovine freemartinism is the sexual modification of a female twin by in utero exchange of blood from a male fetus. This condition was first described by Hunter in 1779¹ and was well known to result in sterility. The freemartin syndrome occurs in around 92% of bovine females born as a result of heterosexual twin pregnancies², which indicates 8% of female heterosexual twins will be normal. The diagnosis of freemartinism can be established by one of the following methods: 1) clinical genital abnormalities; 2) presence of sex chromatin bodies in circulating leukocytes in male co-twin; 3) presence of sex chromosome chimerism (XX/XY) of hemopoietic cells; 4) blood typing; and 5) skin grafting.

In order to promote the preservation of ideal genetic traits in newborn heterosexual co-twin heifer calves in the Holstein breed, the American Holstein Association authorized our laboratory to cytogenetically test calves for potential freemartinism. The purpose of this paper is to review the cytogenetical results of testing Holstein heifer heterosexual twins referred to our laboratory in the last 13 years.

MATERIALS AND METHODS

The program of cytogenetically testing Holstein calves for potential freemartinism began in 1978; since then 667 blood samples from heifer heterosexual twins were submitted and examined for cytogenetic abnormalities.

Chromosome preparations of lymphocytes were made from 10 drops of whole blood, which was cultured for 72 hours in RPMI-1640 medium containing 20% fetal calf serum and 3% Pokeweed mitogen. The cultured cells were harvested following 2 hours treatment with Colcemid (0.01 $\mu\text{g/ml}$), followed by 15 minutes of 0.075 M KCL hypotonic treatment, and fixed using 3:1 methanol acetic acid. Chromosome spreads were made, Giemsa-stained, and observed microscopically for the presence or absence of chromosomes of both sexes. The animals with both 60XY and 60XX cells were identified to be freemartins while the individual heterosexual twins with only 60XX cell were determined to be normal heifers; more than 100 metaphases were further examined in order to confirm the non-existence of the XY cells.

RESULTS AND DISCUSSION

In our laboratory, 667 cases of female heterosexual twins were chromosomally examined over the last 13 years, 1978-1991. Out of these, 553 (82.9%) cases

showed the sex-chromosomal chimerism (XX/XY) and were diagnosed tentatively as freemartins. The remaining 114 (17.1%) cases had only XX cells and thus were potentially fertile. To date no instances of inaccurate diagnosis have been brought to our attention.

The figure of 17.1% is substantially higher than 10% reported earlier by others. This higher percentage of potentially "normal" (non-freemartin) heifers occurred probably because the cases selected by veterinarians to be examined cytogenetically were nearly normal based on other clinical criteria, especially vaginal length.

According to Eldridge et al.³ although there are five methods available to make a diagnosis of freemartinism, each, including the cytogenetic method has some disadvantages. The clinical signs of the bovine freemartin such as abnormal development of the clitoris, the small vulva, the short length of the vagina, and the lack of estrus, are helpful in the diagnosis of a freemartin. However, many of these phenotypes do not manifest themselves at birth. Furthermore, variability in development of the freemartin reproductive tract would result in false diagnoses in many cases where only clinical signs are employed. The insertion of a lubricated test tube into the vagina of a suspected freemartin is a common early test for a normal vagina. Many freemartin calves have short vaginas where a 150 X 18 mm test tube can be inserted only 5 to 6 cm. The tube test is unreliable since some freemartins possess a vagina of approximately normal length but have anatomical abnormalities in the uterus, oviducts, or ovaries, resulting in sterility. Early diagnosis of the bovine freemartin by

clinical signs, therefore, is not completely reliable. Cattle erythrocyte typing involves a large number of antibodies and is time consuming and expensive, plus the necessity of interpreting the results of blood typing in cattle with caution for twins. This is not a practical routine diagnostic method for early recognition of the freemartin. Although skin grafting can be performed soon after birth, the time and effort for the technique renders the procedure impractical for routine diagnosis of the bovine freemartin condition. The use of sex chromatin in diagnosing freemartinism is not possible because drumsticks of neutrophils are unreliable, and non-blood cells do not cross the placental circulations. In comparison with the four methods described previously, an accuracy of 96% in the diagnosis of the non-freemartin condition can be achieved by the cytogenetic method for the females born co-twin to bulls. Based on the above we think that chromosome analysis of lymphocytes is a reliable aid and useful for routine early diagnosis of the bovine freemartin. However, to reduce examination costs, we suggest that dairy farmers send samples to a cytogenetics laboratory for a final diagnosis only from those co-twin heifers suspected to be non-freemartin animals by the clinical method performed at three to six weeks after birth.

In the U.S. about 200,000 dairy twin calves are born each year⁴. The incidence of dizygotic twins is 93 to 95 percent of all twins, and half of the dizygous twins will be heterosexual². This means that more than 93,000 female heterosexual twins are produced annually. Among them, 86,000 freemartins and 7,000 normal heifers are expected, respectively, according to the

incidence of freemartinism reported previously². If the above estimated figures are acceptable a total of about 1,118,000 freemartins and 91,000 normal heifers have been produced in the last 13 years; thus our cases account only for less than 0.06% of all the heterosexual dairy twins born in that period. Based on the information shown above, the degree of application of the cytogenetic test approach in diagnosis of freemartinism is very small, even though it is well known to be quite reliable and simple. These data also imply that a substantial number of freemartins were subjectively kept or clinically misdiagnosed to be normal heifers, which would result in uncalculated economical losses to dairy farmers.

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SUMMARY

Between 1978 and 1991 a total of 667 blood samples from Holstein heifer heterosexual twins were submitted and

examined for freemartinism. Out of these, 553 (82%) and 114 (17.1%) cases were diagnosed to be freemartins and normal heifers respectively. The normal heifer figure of 17.1% is substantially higher than 8% reported previously by others. This is probably due to the possibly normal cases being selected by veterinarians based on other clinical criteria. The data also show that less than 0.06% of all the heterosexual dairy twins were examined cytogenetically to rule out freemartinism, which implies a certain number of freemartins were subjectively kept or clinically misdiagnosed to be normal heifers. We think that chromosome analysis of lymphocytes is a reliable aid and useful for routine early diagnosis of the bovine freemartin, and encourage that dairy farmers send samples from possibly non-freemartin animals for a final diagnosis by cytogenetic evaluation.

ZUSAMMENFASSUNG

"Freemartinism" wurde zwischen 1978 und 1991 an 667 Blutproben von weiblichen Holstein Zwillingen getestet. Dabei wurden 553 (82%) als positiv und 114 (17.1%) als negativ beurteilt. Der 17.1% Anteil ist wesentlich höher als in früheren Arbeiten publiziert. Dies beruht vielleicht darauf, dass Veterinärpraktiker zur zytogenetischen Beurteilung auch Proben von normalen Zwillingen mitbenutzten. Unsere Ergebnisse betreffen ferner nur 0.06% aller weiblichen Zwillinge in USA, die zytogenetisch geprüft wurden. Möglicherweise sind daran klinisch-diagnostische Fehler oder Fehlbeurteilungen beteiligt. Wir glauben, dass der zytogenetische Test Vorteile bietet, und empfehlen ihn Viehbesitzern zur Anwendung.

RESUMEN

Entre 1978 y 1991, un total de 667 muestras de sangre de becerras Holstein provenientes de nacimiento gemelar heterosexual, fueron enviadas y examinadas para detectar freemartinismo. De todas ellas, 553 (82%) y 114 (17.1%) de los casos fueron diagnosticados becerras freemartines y normales respectivamente. La figura de las becerras normales de 17.1% es substancialmente mas alta que el 8% reportado por otros previamente. Esto es debido probablemente, a que los casos normales que son seleccionados por el veterinario están basados en otros criterios clínicos. La información muestra además que menos del 0.06% de todos los gemelos heterosexuales provenientes de vacas lecheras fueron examinados citogeneticamente para descartar freemartinismo, lo cual implica que un cierto numero de freemartines fueron subjetivamente dejados en los hatos o no fueron diagnosticados clinicamente y fueron diagnosticados como vacas normales. Nosotros pensamos que el análisis cromosomal de linfocitos es una ayuda confiable y útil para el diagnostico temprano de rutina de freemartinismo bovino y recomendamos a los ganaderos de vacas lecheras enviar muestras solo de animales no freemartines para un diagnostico final usando evaluación citogenetica.