FREQUENCIES AND CYTOMORPHOLOGICAL MANIFESTATION OF SEXUAL X-CHROMOSOME FRAGILITY (Fra Xq3.1) IN HOLSTEIN-FRIESIAN

MANIFESTACIÓN CITOMORFOLÓGICA Y FRECUENCIAS DE LA FRAGILIDAD DEL CROMOSOMA SEXUAL X (Fra Xq3.1) EN HOLSTEIN-FRIESIAN)

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Additional keywords

Dairy cattle. X chromosome. Chromosome fragility.

Palabras clave adicionales

Bovino lechero. Cromosoma X. Fragilidad cromosómica.

SUMMARY

The presence of fragility in the bovine X chromosome has been related to fertility problems, baldy calf syndrome, and diverse phenotypic alterations. Recently, previous cytogenetic studies in Holando-Uruguayo (Holstein-Friesian) have showed a fragile site in the Xq3.1 region.(G-negative band). In this work, spontaneous frequencies expression of Fra Xq3.1, in different bovine blood samples are presented. Lymphocyte standard culture with complete RPMI 1.640 was performed. Replication banding RBG was applied and a particular R positive band was expressed. Three samples were selected to this work: (A) contains 46 females of different ages with reproductive problems (repeat breeders, freemartins, anoestrus, abortions); (B) 15 females of different ages, with normal reproduction; and (C) 8 males (7 co-twins with females, 1 with clef palate). A total of 2758 metaphases plates were examined and 82 showed Fra Xq3. 1 (2.97 p. cent). Sample (A) showed 3.2 p. cent of Fra Xq3. 1; (B) 1.32 p. cent; (C) 5.2 p. cent. The cytomorphological manifestation corresponded 79.3 p. cent to chromatid breaks, 4.9 p. cent chromosome breaks, 11 p. cent chromatid gap and 4.9 p. cent iso-chromatid gap. Early replication band (RBG) expressed an interband in the R positive region of Fra Xq3.1, sugesting the presence of a short region of late replication.

RESUMEN

La presencia de fragilidad en el cromosoma X del bovino fue relacionada con problemas reproductivos, sindrome de baldy en terneros y diversas alteraciones fenotípicas. Recientemente, estudios previos realizados en Holando Uruguayo (Holstein-Friesian) mostraron la presencia de un sitio frágil en la región Xq 3.1 (banda G negativa). En este trabajo, se presenta la frecuencia de expresión espontánea del Fra Xq3.1 en diferentes muestras de bovinos. Las muestras sanguíneas se procesaron por la técnica estándar de cultivo linfocitario en medio completo RPMI 1640. La realización del bandeo de replicación RBG mostró una banda particular R positiva. La muestra se subdividió en: (A) 46 hembras de diferentes edades con problemas reproductivos (repetidoras de servicio, freemartins, anestro, aborto); (B) 15 hembras de

diferentes edades con reproducción normal; y (C) 8 machos (7 co-mellizos de hembras, 1 con paladar hendido). Se examinaron 2758 placas metafásicas de las cuales 82 presentaron Fra Xq3.1.(2,97 p. cien). En la muestra A, la freecuencia fue de 3,2 p. cien, en la B de 1,32 p. cien y en la C de 5,2 p. cien. En cuanto a la manifestación citomorfológica se encontró: 79,3 p. cien fracturas de cromátida, 11 p. cien de gap de cromátida, 4,9 p. cien fracturas cromosómicas y 4,9 p. cien de gap de iso-cromátida. La banda de replicación temprana (RBG) expresó una interbanda en la región R positiva del Fra Xq3.1, sugiriendo la presencia de una pequeña zona de replicación tardía.

INTRODUCTION

Fragile site in mammalian chromosomes are cytologically characterized as specific regions that exhibit constrictions, breaks, gaps when cells are cultured in a different condition (Sutherland and Hecht, 1985). Incomplete chromatin condensation caused by late replication, and incomplete replication, account for gaps and fragility (Laird *et al.*, 1987).

The presence of fragility in the bovine X chromosome has been related to fertility problems, baldy calf syndrome, and diverse phenotypic alterations (El-Nahass *et al.*,1974; Hanada *et al.*, 1980; Uchida *et al.*, 1986; Genest *et al.*, 1978). Uchida *et al.*, (1986) located a specific site in the Xq chromosome of Holstein cattle, near to the centromeric region and relate with a pale staining Q-band. Di Berardino *et al.*, (1983) described a map of BrdU-induced breaks in chromosomes of cattle, finding four different breakpoints of chromosome X, spreading among R-positive and R-negative bands.

Recently, Llambí and Postiglioni (1994) described in Holstein-Friesian a quantitative localization of a breakpoint in X chromosome long arms (X = 0.52, region 3.1) in a large negative G-band of metaphase chromosomes.

This paper describes cytomorphological manifestation of break points detected in X chromosome long arms of Holstein Friesian cattle and the possible relationship with dinamic R banding.

MATERIALS AND METHODS

Cytogenetic studies were carried out on 69 Holstein-Friesian cattle (Holando-Uruguayo). They were divided en three samples (A,B,C). A: it contains 46 females of different ages with reproductive problems (repeat breeders, freemartins, anoestrus, abortions); B: 15 females of different ages, with normal reproduction; C: 8 males (7 co-twins with females, 1 with cleft palate).

Lymphocytes were cultured in a standard complete medium RPMI 1640 (Sigma) supplemented with 20 p. cent fetal bovine serum (Gibco) and phytohemagglutinin. Cells were incubated for 72 h at 38°C in a Memmert bath.

Single synchronization of lymphocytes with fluorouracil (Sigma; 5×10^{-7} M) was applied after running 48 h and bromodeoxyuridine (Sigma; 1×10^{-4} M) was incorporated to the cells 6 h before harvesting. Air-dried chromosome slides were incubated with 4 mg/l Hoechst 33258 in 0.9 p. cent NaCl during 30 min. and 15 min. with Hoechst 33258 in a black-ray lamp (Transiluminator). After incubation in 2 x SSC for lh at 65°C the slides were washed and counterstained with Giemsa (pH 6.8) (Ronne,1984).

RESULTS AND DISCUSSION

Different induced substances have been applied to permit the expression of fragile sites (Jordan, 1991). In our case, the incidence of animals that presented fragility Xq3.1. was 34.8 p. cent and they had spontaneously expression, as it was cultured in a medium with a standard concentration of folic acid (1 mg/l).

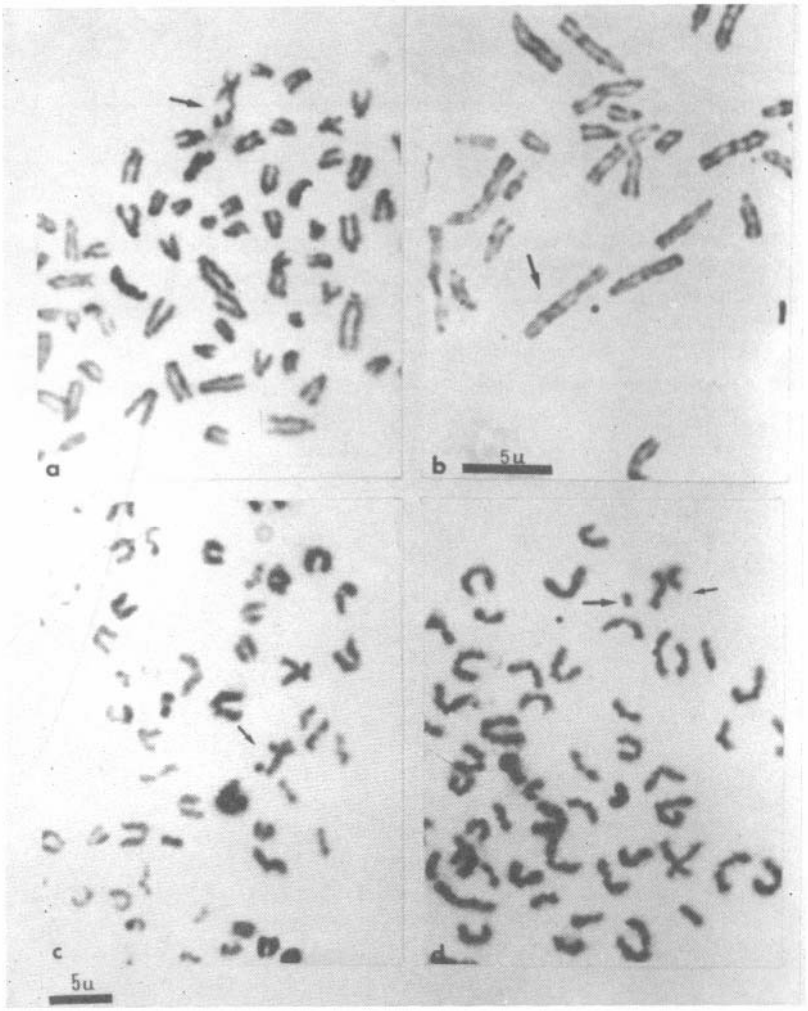

Figure 1. *Partial metaphases of Holstein Friesian. a, Chromatid breaks and gaps; b, positive band (RBG); c, Chromatid breaks with total separation; d, Chromatid breaks with displacement of the fragment.* (Metafases parciales de Holstein-Friesian. a, Fracturas y grietas en cromátidas; b, Banda positiva R (RBG); c, Fractura de la cromátida con separación total; d, Fractura de la cromátida con desplazamiento del fragmento).

Table I. *Frequency of cytomorphological expression.* (Frecuencia de expresión citomorfológica).

Number of animals	Number of metaphases	Chromatid break	Chromatid gap	Chromosome break	Chromosome gap	Total of aberrations
45	1469	*	*	*	*	-
24	1289	65	9	4	4	82
Percentage of aberrations		79.2	11.0	4.9	4.9	100.0

*No aberrations detected.

In human, heterozygous expression of Fra-X is reported, but neverless it was showed in the 100 p. cent of metaphases (Sutherland and Hecht, 1985). In this work, similar expression was found in the among of the sample. Otherwise, in

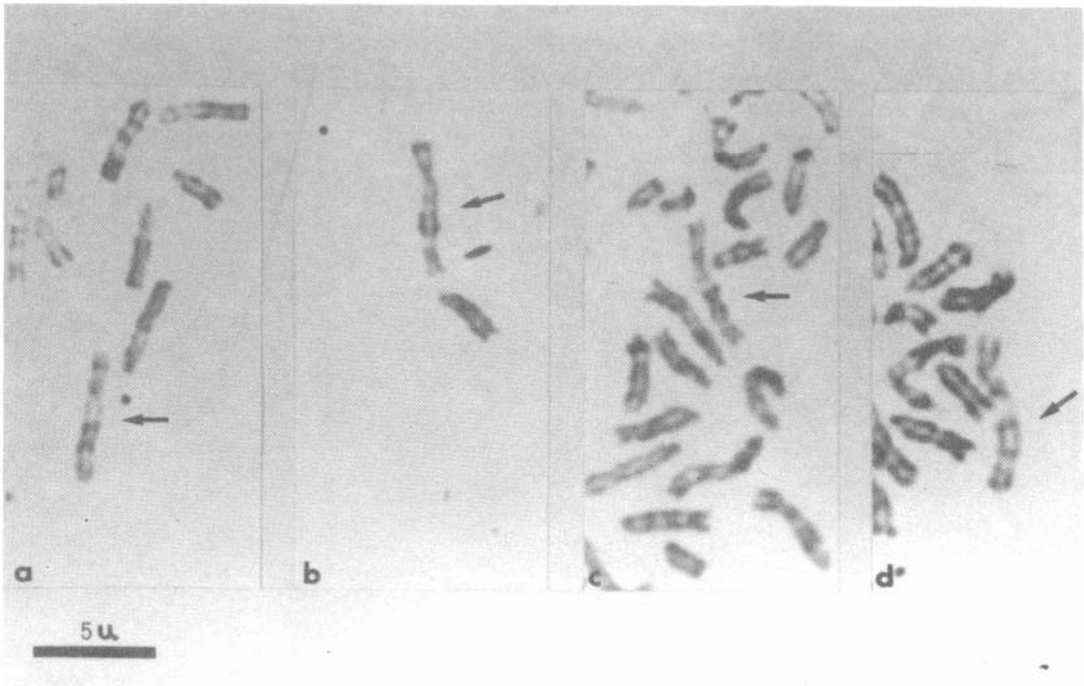

Figure 2. *Various elongations of R bands in the X chromosome.* (Varias elongaciones de las bandas R del cromosoma X).

Indian mole rat differencial X fragility expression associated to reproductive problems was reported (Tewari *et al.*, 1987).Furthermore, the cytomorphological expression of the fragile site Xq3.1 evidenced a prevalence of chromatid breaks and chromatid gaps (**figure1**and **table I**). In mammals similar cytomorphological results were reported, but they were obtained after inducing them with BrdU (Lin *et al.*, 1984; Morielle-Versute and Varella, 1994).

High resolution chromosome banding permitted to detect within the large R-band located in the 3.1 region a thin negative interband. This fact could be interpreted as a late replication region (**figures 1b**, and **2**).

According to the above results, we could conclude that: a) fragile site Xq3.1 are spontaneously expressed; b) its manifestation should be heterozygous; c) chromatid breaks and chromatin gaps had the most frequency manifestation; d) late replication band was observed in the middle of a high R positive band, region Xq3.1.

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