

Expression of X chromosome fragility in Holstein–Friesian cattle: a preliminary study

G Rincón, S Llambí, A Postiglioni *

*Laboratorio de Citogenética de Animales Domésticos, Area Genética,
Departamento de Biología Molecular y Celular, Facultad de Veterinaria,
Lasplaces 1560, Montevideo, Uruguay*

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Summary – Twenty Uruguayan Holstein–Friesian cows were cytogenetically examined, focusing on Xq 3.1 fragility manifestation (Fra Xq). Comparative culture media studies were carried out by simultaneously culturing blood samples in TC 199 and RPMI 1640 media. Taking into account that X chromosome aberrations may be interfering with fertility, the cattle population were also evaluated for their reproductive performance. One thousand metaphase spreads were studied in each culture media and showed normal chromosome constitution in most cells. Frequency of Xq fragility detected in TC 199 was 0.9% while in RPMI 1640 it was 1.8% ($0.05 > P > 0.01$). According to reproductive performance, repeat breeders manifested 92% of all Fra Xq detected. The differences in expression in both tissue culture media and the frequencies of the Xq fragility observed in cattle with reproductive problems are discussed.

fragile X / cattle / cytogenetics / fertility

Résumé – Étude préliminaire sur l'incidence de la fragilité du chromosome X en race bovine Holstein. Vingt vaches de race Holstein–Friesian Uruguayenne ont été examinées cytogénétiquement pour apprécier la fragilité Xq 3.1 (Fra Xq). Une étude comparative entre les milieux de culture a été menée par culture simultanée des échantillons sanguins dans le smilieux TC 199 et RPMI 1640. En raison de l'incidence possible des aberrations du chromosome X sur la fertilité, les animaux ont été aussi évalués pour leur performance reproductive. Mille métaphases ont été étudiées dans chaque milieu de culture et la majorité des cellules a montré une constitution chromosomique normale. La fragilité Xq exprimée dans le milieu TC 199 a été de 0,9 % tandis que dans le milieu RPMI 1640 elle a été de 1,8 % ($0,05 > p > 0,01$). En ce qui concerne la reproduction, les vaches infertiles ont exprimé 92 % de tous les Fra Xq détectés. Les différences d'expression dans les deux milieux de culture, ainsi que les fréquences observées chez les vaches présentant des problèmes de reproduction sont discutées.

X fragile / bovin / cytogénétique / fertilité

* Correspondence and reprints

INTRODUCTION

Cytogenetic studies of cattle herds are very important as they permit the establishment of associations between karyotype and phenotype in cases of hereditary defects including reproductive performance.

Recently, X chromosome fragility (Fra Xq) has been observed in cattle with reproductive problems and baldy calf syndrome (El Nahass et al, 1974; Genest and Guay, 1979; Hanada and Muramatsu, 1980; Uchida et al, 1986). In humans, on the other hand, an important fragile X site (Fra Xq 27.3) has been related to a particular kind of mental retardation, the Martin Bell syndrome (Sutherland, 1977; Laird, 1987; Oostra and Willems, 1995). Fragile sites may be distributed in the majority of domestic species in a similar way as for humans. In order to establish a relationship between cattle Fra Xq and those fragile sites described in humans, Uchida et al (1986) conducted a cytogenetic study on a Holstein cow and its calf, which had baldy calf syndrome. As an approach to cattle Fra Xq location, the authors reported an achromatic gap on the bovine X chromosome close to the centromere in a pale staining Q band. Further studies defined the Xq fragility in Holstein-Friesian cattle on a G negative band in region 3.1 on the long arm. This structurally abnormal X chromosome expression was observed in cell cultures performed in RPMI 1640 medium (Llambí and Postiglioni, 1994, 1996).

Nevertheless, the human Xq (27.3) fragility was observed only when lymphocytes were cultured in medium TC 199, deficient in folic acid and not in several other available culture media (Sutherland, 1977).

Generally, fragile sites are expressed in response to folate deficiency or to treatments with other chemicals such as aphidicolin, azacytidine, distamycin A and bromodeoxyuridine. Most of them are closely linked to folate metabolism (Sutherland and Hecht, 1985). As observed in previous investigations, Fra Xq 3.1 in cattle also occurs in cultures where the fragility has not been induced by chemicals (Llambí and Postiglioni, 1994, 1996; Postiglioni et al, 1996).

In search of better understanding of Fra Xq manifestation in Holstein-Friesian cattle, we performed a comparative culture media study. Cytogenetic examination was conducted by simultaneously culturing bovine blood samples in TC 199 and RPMI 1640 media.

Taking into account that X chromosome aberrations may be interfering with fertility (Tewari et al, 1987; Basrur, 1994; Rincón et al, 1995), cattle population in this study was evaluated according to its reproductive performance. Reproductive traits, such as calving interval, days open, and services per conception were considered. The differences in incidence of Fra Xq in both tissue culture media and the frequencies observed in cattle with reproductive problems were statistically evaluated.

MATERIALS AND METHODS

Twenty Holstein-Friesian cows from different dairy farms in Uruguay were submitted to karyotypic analyses, focusing on the expression of Xq fragility.

Each blood sample was drawn from the coccigeal vein into a heparinized syringe, and cultured *in vitro* for 72 h according to a modified protocol (Halnan, 1989).

Whole blood from each animal was cultivated in RPMI 1640 (Sigma) and TC 199 (Sigma) media, supplemented with 20% fetal bovine serum, penicillin (100 IU/mL), streptomycin (100 µg/mL), and phytohaemagglutinin (0.2 µg/mL). Colchicine (0.004 mg/mL) was added 2 h before harvesting the cultures. Folic acid concentration in TC 199 was 1×10^{-5} mg/mL, while in RPMI 1640, it was 1×10^{-3} mg/mL. Both media were thymidine free. All material was analyzed with standard Giemsa staining (pH 6.8).

A total of 2 000 cells were cytogenetically studied in order to compare Xq fragility expression. The karyotypic study was conducted by evaluating 50 metaphase spreads per animal for each culture medium.

The animals were divided into two groups, taking into account the reproductive performance of each animal. Twelve cows were culled as repeat breeders owing to repeated breeding, long calving intervals and abortions (group A) and eight cows were considered as controls owing to their normal reproductive performance (group B). In both cases reproductive traits, including calving interval, days open and services per conception were evaluated.

The Chi square test was used to establish the statistical significance between the level of Fra Xq occurrence in both culture media and in both groups A and B.

RESULTS

The results of cytogenetic investigations are shown in table I. Most cells presented normal chromosome constitution without any structural abnormality. Fra Xq was the main chromosome alteration in 27 metaphases, and in all cases a normal female complement $2n = 60, XX$ was detected. Chromatid breaks, simple gaps and chromosome breaks of Xq were found under both culture conditions (fig 1a, b). They were all detected in only one of the chromatids except for one metaphase plate where fragility was observed in both chromatids.

Table I. Fra Xq frequencies observed in RPMI 1640 and TC 199 culture media.

	<i>Metaphase spreads per medium</i>	<i>Fra Xq in RPMI 1640 (%)</i>	<i>SD^a</i>	<i>Fra Xq in TC 199 (%)</i>	<i>SD</i>
Group A	600	2.8	2.2	1.3	1.5
Group B	400	0.25	0.8	0.25	0.8

^a SD = standard deviation

The occurrence of Fra Xq in group B, for both tissue culture media, did not exceed the level of 2%. On the other hand, in group A, the occurrence of fragility ranged from 0% (detected in four animals) and 14%. In RPMI 1640 medium, six repeat breeders out of 12 expressed Fra Xq at a frequency of 2–14% of the cells with a mean value of 2.8%, SD = 2.2 (table I). In TC 199 medium, four out of 12 cows manifested Fra Xq at a frequency of 2–6% with a mean value of 1.3%, SD = 1.5 (table I). Reproductive trait data are shown in table II.

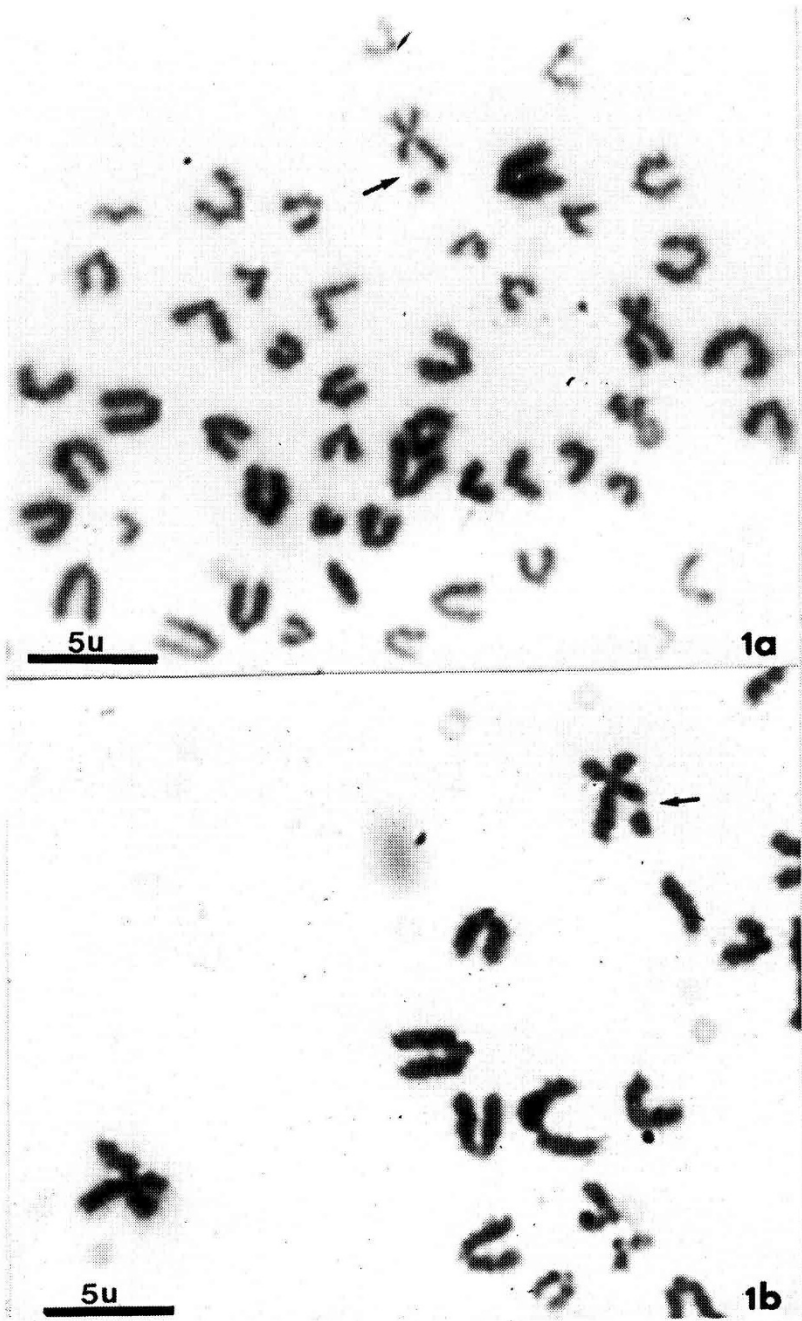


Fig 1. Partial metaphase spreads: arrows indicate Xq fragility. (a) Chromatid; (b) simple gap.

Table II. Reproductive traits.

	<i>Services/conception</i>	<i>Calving interval</i>	<i>Days open</i>
Group A	5.1 services	16.3 months	7.9 months
Group B	1.25 services	12.5 months	3.7 months

The Chi square test revealed statistical significance ($0.05 > P > 0.01$) when we considered the difference between Fra Xq expression in both culture media. Taking into account the reproductive performance, the Chi square test between groups A and B also showed a significant difference ($P < 0.001$).

DISCUSSION

Although the majority of fragile sites are revealed in response to external culture conditions, some occur independently. Fra Xq in humans is rarely expressed under normal culture conditions, and is dependent on external factors including the cultivation of cells in folate-deficient medium (Glover, 1981; Sutherland and Hecht, 1985). Manifestation of Fra Xq in cattle, as observed in this preliminary study, did not show a higher level of expression in TC 199 compared to RPMI 1640. On the contrary we observed an enhanced fragility expression in the latter tissue culture medium ($0.05 > P > 0.01$). The percentage of Fra Xq expression in RPMI 1640 was 1.8% while in TC199 it was 0.9%. Moreover, X fragility ranged among individuals in RPMI 1640 medium from 0 to 14% compared to 0–6% in TC 199. These differential findings support the conclusion that Fra Xq 3.1 does not represent a common folate-sensitive fragile site. The enhanced expression observed in RPMI 1640 is a feature shared by some aphidicolin inducible fragile sites (Sutherland and Hecht, 1985). Therefore a study of Fra Xq expression under this chemical inductor is also required to reach a definitive conclusion.

Chromatid breaks were observed as the main form of chromosome fragility. Gaps and chromosome breaks were also observed, but in a lower proportion, which is in agreement with our previous reports (Llambí and Postiglioni, 1996). Data gathered from cytogenetic investigations of domestic animals show that changes affecting one of the sex chromosomes (X chromosome) tend to affect the form and/or the function of the reproductive system. The animals are able to develop a normal phenotype but become subfertile or infertile depending upon the type of chromosome defect sustained. The adverse effect of a chromosome defect is expressed in monotocous animals as an increased interval between parturitions and reduced rates of non-return for services after artificial insemination (Basrur, 1994; Long, 1984, 1990). Xq fragility in repeat breeders represented 92% of all Fra Xq detected. In spite of that, we can not claim that Fra Xq is the direct cause of decreased reproductive efficiency in group A, but it must be considered as a possible cause of repeat breedings, long calving intervals and abortions in individual cases. These assessments both agree with and reinforce previous cases presented in the literature in which X chromosome fragility is directly related to reproductive problems (El Nahass et al, 1974; Guenet and Guay, 1979; Hanada and Muramatsu, 1980).

Undoubtedly, future studies of fragile X in cattle will be possible due to progress in the developing construction of the physical map and its functional analysis. Although mapping of the X chromosome has been limited to a small number of genes, DMD and DXS19 markers are involved in region 3.1 (Barendse et al, 1994; Eggen and Fries, 1995). The application of different molecular techniques would help us to better understand chromosome X fragility.

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