J. Appl. Genet. 42(2), 2001, pp. 205-209

A low-level X chromosome mosaicism in mares, detected by chromosome painting

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Abstract. Fluorescence *in situ* hybridization with the use of the equine X whole chromosome painting probe was carried out on chromosome spreads originating from three mares with poor reproductive performance (infertility, miscarriage or stillbirth). The numbers of analysed spreads were high (105, 300 and 480) and in all three mares a low frequency of mosaicism was identified. The mares had the following karyotypes: 64,XX/63,X/65,XXX (93.6%/5.7%/0.7%), 64,XX/63,X (98.9%/1.1%) and 64,XX/63,X (94.3%/5.7%). The incidence and importance of the low percentage X chromosome mosaicism are discussed.

Key-words: chromosome painting, horse, mosaicism, X chromosome paint, X monosomy.

Introduction

X chromosome monosomy is the main abnormality diagnosed among infertile mares and many of the cases are mosaics 64,XX/63,X (POWER 1990). This aberration was also found in the Polish horse population (PARADA et al. 1996, PAWLAK et al. 2000). The equine X chromosome is usually identified by the presence of an interstitial C-band on the Xq. Unfortunately C-banding results may not be conclusive in cases where chromosome spreads are not of good quality. Chromosome painting with the equine X chromosome-specific probe has been shown to be a much more sensitive approach than C-banding (BREEN et al. 1997).

Whole body mosaicism is caused by abnormal segregation of the sister chromatids of X chromosome during mitotic cleavages at an early stage of embryogenesis. However, it is not clear what was the primary status of the zygote: normal karyotype (64,XX) or monosomic karyotype (63,X). Interpretation of

Received: January 17, 2001. Accepted: March 8, 2001.
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the mosaic cases should consider the following factors: type of tissue, number of studied cells, sensitivity of the technique applied and clonal selection of cell lines (FERNANDEZ et al. 1996). It has been demonstrated in mosaic patients with Turner syndrome that detection of an aneuploid cell line with a high or low frequency in lymphocytes may not reflect the extent of mosaicism in the whole body (HELD et al. 1992, NAZARENKO et al. 1999). Thus, detection of aneuploid cells occurring with a low frequency seems to be very important for accurate diagnosis of causes of mare infertility.

In the present study we show that application of the equine X-chromosome specific paint facilitates the detection of a low-level X chromosome mosaicism in infertile and subfertile mares.

Material and methods

Chromosome preparations were obtained from lymphocyte cultures. Blood samples were collected from three mares (Armenia, Estebella and Husaria) among which one (Armenia) was earlier diagnosed by CBG banding as a carrier of 64,XX/63,X lymphocyte mosaicism (PAWLAK et al. 2000). The mares were subjected to cytogenetic evaluation due to poor reproductive performance: infertility (Armenia), stillbirth in the first season (Estebella) or miscarriage in the first season (Husaria).

The detection of X chromosome was carried out by FISH technique with the use of the equine X whole chromosome painting probe, derived from flow-sorted chromosomes (YANG et al. personal communication). The biotin-labelled probe was applied on lymphocyte chromosome preparations. Briefly, the slides were denaturated in 70% formamide in 2 × SSC for 150sec. at 70°C. The probe was denaturated at 70°C for 10 min. The hybridization was carried out in 37°C overnight. Post-hybridization washes were as follows: three times at 50% formamide in 2 × SSC and three times in 2 × SSC at 42°C. Hybridization signals were detected by the avidin-FITC and anti-avidin system on propidium iodide stained slides. Microscopic evaluation was performed under a fluorescence microscope, Nikon E 600 Eclipse, equipped with a cooled CCD digital camera and Lucia software.

Results and discussion

Microscopic evaluation of the preparations revealed the presence of three cell lines in one mare (Armenia) and two cell lines in the other two (Estebella and Husaria) – Table 1. Armenia was a carrier of 63,X/64,XX/65,XXX mosaicism. This status was identified by analysis of 300 chromosome spreads and the frequencies of the cell lines were 5.7, 93.6 and 0.7%, respectively (Figure 1).

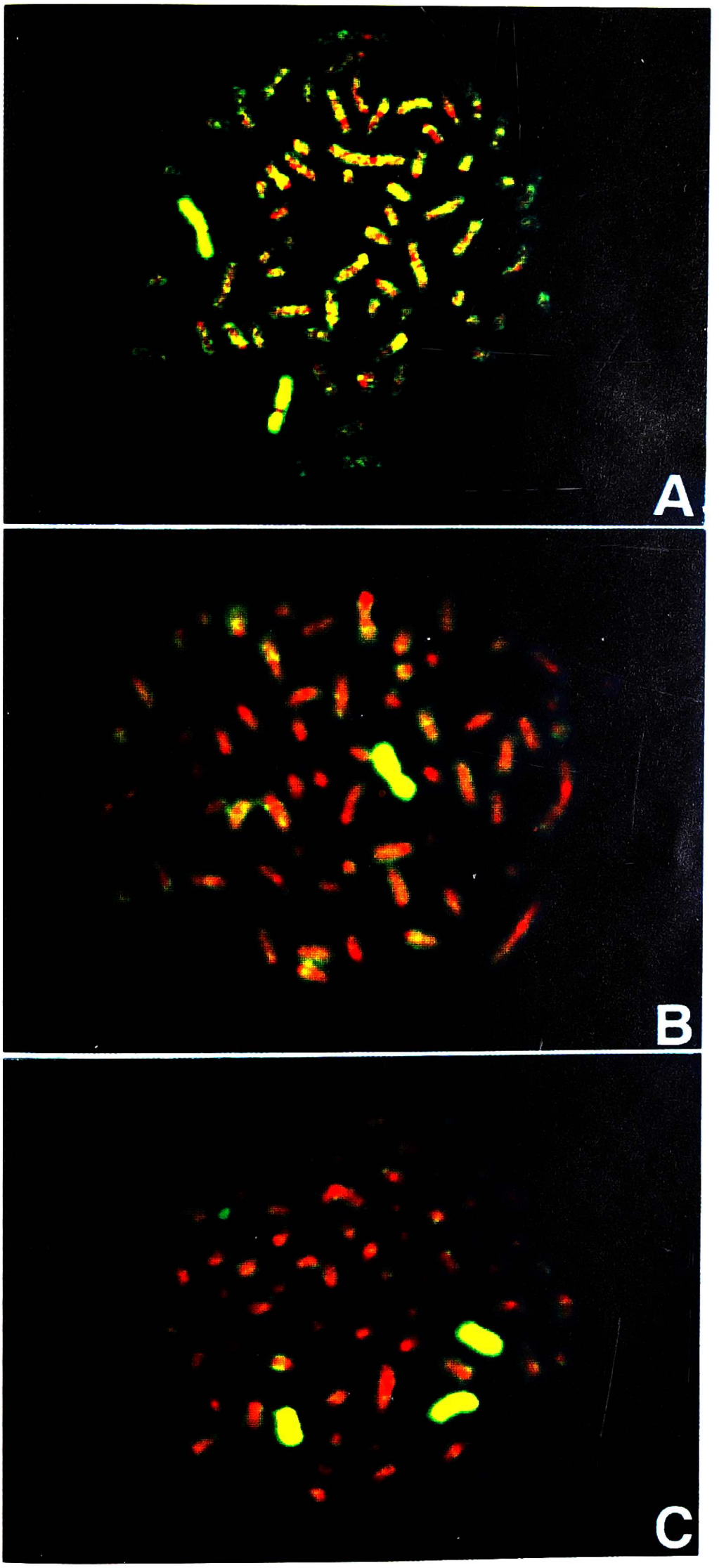

Figure 1. Metaphase spreads originating from the mosaic mare (Armenia) A = 64,XX; B = 63,X and C = 65,XXX. The X chromosomes are yellow.

Table 1. Reproductive performance and chromosome painting results of three mosaic mares

Mare name	Mare age (years)	Reproductive performance	Total number of spreads	Number and frequency of cells with a given karyotype		
				64,XX	63,X	65,XXX
Armenia	9	infertile (5 seasons)	300	280 (93.6%)	17 (5.7%)	3 (0.7%)
Estebella	4	stillbirth in first season	380	376 (98.9%)	4 (1.1%)	–
Husaria	10	miscarriage (twins) in first season, infertile in next three seasons	105	99 (94.3%)	6 (5.7%)	–

In the former study of this mare, based on CBG banding and conventional Giemsa staining, only two cell lines 63,X and 64,XX were found among 50 chromosome spreads and the frequency of the cell lines were 14% and 86%, respectively (PAWLAK et al. 2000). Estebella and Husaria appeared to be carriers of 63,X/64,XX mosaicism with a low frequency of the 63,X cell line: 1.1% and 5.7%, respectively (Table 1).

Our study reveals that the frequency of an aneuploid cell line may be very low which may result in its over- or underestimation if the total number of studied cells is not high enough. FERNANDEZ et al. (1996) showed that many human non-mosaic patients with Turner syndrome, diagnosed primarily by the use of conventional cytogenetic techniques, displayed a mosaic karyotype while studied with *in situ* hybridization or PCR techniques. In rare cases the FISH approach revealed false-positive and false-negative cases of human X monosomy mosaicism, earlier analysed by classical cytogenetic techniques (NAZARENKO et al. 1999). In such cases detection of mosaicism in two or more tissues improves substantially the analysis. In an extensive study of 87 women with Turner syndrome it was demonstrated that the percentage of aneuploid cell may vary between tissues in a very broad range (HELD et al. 1992). The authors described patients with a very low frequency (2% and 4%) of 45,X cells in lymphocytes and a very high frequency (98% and 99%, respectively) in fibroblasts. There were also cases with a high frequency of 45,X cells in lymphocytes and lack of such cells in fibroblasts, and *vice versa*. This clearly shows that detection of mosaicism in lymphocytes may not reflect accurately the situation in other tissues.

As reviewed by POWER (1990), among mares with abnormal complements of sex chromosomes a vast majority (142 cases) were carriers of the 63,X karyotype. Among mosaic mares the majority (62 cases) had the 64,XX/63,X karyotype, but in many cases (55) another type of mosaic or chimeric karyotypes with two or more cell lines, i.e. 64,XY/63,X; 64,XX/65,XXY; 64,XX/65,XXX, 63,X/64,XX/65,XXX; 63,X/64,XX/65,XXY etc., were identified. It is known that exclusion of the mosaicism with a high confidence (95% or 99%) depends on

the number of analysed cells. For example, to exclude 3% mosaicism with 0.95 confidence, evaluation of at least 100 cells is necessary, but to exclude 1% mosaicism with the same confidence, observation of at least 300 cells is recommended (HOOK 1977). Thus, it can be anticipated that application of molecular methods (facilitating evaluation of a high number of cells) for diagnosis of the above cases would reveal that the frequency of mosaics is much higher.

Results of the present study also show that X chromosome mosaicism may occasionally not cause infertility of the carrier. It is an open question whether the frequency of the aneuploid cell line may influence the reproductive performance of the carrier. In this study two mares with a low (Husaria) or very low (Estebella) incidence of 63,X cells were pregnant. There is only one report describing fertile mares carrying the 64,XX/63,X karyotype (HALNAN 1985). Recently, BREEN et al. (1997) described a 2-year-old Thoroughbred mosaic filly – 63,X/65,XXX (94%/6%) in which development of Graafian follicle and ovulation of an oocyte occurred. Unfortunately, insemination of this filly appeared to be unsuccessful. On the other hand there are known cases of fertile women-carriers of Turner syndrome with mosaic or non-mosaic karyotype (SWAPP et al. 1989). Among 21 pregnancies in non-mosaic women and 97 pregnancies in mosaic women only one-third resulted in a normal surviving child. Studies of preimplantation embryos, originating from X0 mice, were carried out by BANZAI et al. (1995). It was demonstrated that development of such embryos was slower, as compared with embryos originating from XX mice. In a consequence, the percentage of retarded or degenerated embryos was significantly increased in X0 mice. Additionally, a high incidence of abnormal karyotypes was observed in the offspring of X0 mice. The above data may indicate that unsuccessful reproduction (miscarriage and stillbirth) of the two studied mares (Estebella and Husaria) reflects a general phenomenon concerning developmental capacity of embryos obtained from the mosaic or non-mosaic females.

Our study supports earlier reports that among the mosaic mares some are subfertile. It can suggested that cytogenetic evaluation of mares suspected to be carriers of X monosomy, should be carried out on a high number of chromosome spreads, to increase the chance of identification of low frequency mosaicism. Fast and accurate detection of X-chromosome aneuploid cells by chromosome painting approach is recommended.

Acknowledgements. We wish to thank Grzegorz CHOLEWIŃSKI and Magdalena STAŃDO (Horse Genetic Markers Laboratory, Agricultural University of Poznań, Poland) for blood collection and providing breeding information.

REFERENCES

BANZAI M., OMOE K., ISHIKAWA H., ENDO A. (1995). Viability, development and incidence of chromosome anomalies of preimplantation embryos from X0 mice. Cytogenet. Cell Genet. 70: 273-277.

BREEN M., LANGFORD C.F., CARTER N.P., FISCHER P.E., MARTI E., GERSTENBERG C., ALLEN W.R., LEAR T.L., BINNS M.M. (1997). Detection of equine chromosome abnormalities in Equids using a horse X whole chromosome paint probe (WCPP). Vet J. 153: 235-238.

FERNANDEZ R., MENDEZ J., PASARO E. (1996). Turner syndrome: a study of chromosomal mosaicism. Hum. Genet. 98: 29-35.

HALNAN C.R.E. (1985). Sex chromosome mosaicism and infertility in mares. Vet. Rec. 116: 542-543.

HELD K.R., KERBER S., KAMINSKY E., SINGH S., GOETZ P., SEEMANOVA E., GOEDDE H.W. (1992). Mosaicism in 45,X Turner syndrome: does survival in early pregnancy depend on the presence of two sex chromosomes? Hum. Genet. 88: 288-294.

HOOK E.B. (1977). Exclusion of chromosomal mosaicism: tables of 90% and 95%, and 99% confidence limits and comments on use. Am. J. Hum. Genet. 29: 94-97.

NAZARENKO S.A., TIMOSHEVSKY V.A., SUKHANOVA N.N. (1999). High frequency of tissue-specific mosaicism in Turner syndrome patients. Clin. Genet. 56: 59-65.

PARADA R., JASZCZAK K., SYSA P., JASZCZAK J. (1996). Cytogenetic investigations of mares with fertility disturbances. Pr. Mater. Zootech. 48: 71-88 (in Polish, with English summary).

PAWLAK M., ROGALSKA-NIŻNIK N., CHOLEWIŃSKI G., ŚWITOŃSKI M. (2000). Study on the origin of 64,XX/63,X karyotype in four sterile mares. Vet. Med. 45: 299-301.

POWER M.M. (1990). Chromosomes of the horse. In: Domestic Animal Cytogenetics (R.A. McFeely ed.). Adv. Vet. Sci. Comp. Med. 34: 131-167.

SWAPP G.H., JOHNSTON A.W., WATT J.L., COUZIN D.A., STEPHEN G.S. (1989). A fertile woman with non-mosaic Turner's syndrome. Case report and review of the literature. Br. J. Obstet. Gynaecol. 96: 876-880.