STUDY ON THE ORIGIN OF 64,XX/63,X KARYOTYPE IN FOUR STERILE MARES

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ABSTRACT: Twenty four mares, demonstrating fertility disturbances, were cytogenetically investigated. In four mares (17%) the presence of two cell lines – 64,XX and 63,X – was found. Paternity testing for two of the above mares, based on blood typing and protein polymorphism, confirmed the status of mosaicism. No relationship between the occurrence of the X monosomy mares and the age of their parents was found.

X monosomy; chimerism; horse

INTRODUCTION

The most frequent chromosomal abnormality in the horse is the X monosomy which may occur in the pure – 63,X or mosaic – 64,XX/63,X forms (Power, 1990). Unambiguous distinguishing between chimerism and mosaicism, on the basis of leukocyte chromosome studies, can be difficult. In case of the presence of XX and XY cell lines, the chimeric classification is obvious. But in other cases – XX/X0, XY/X0 or XY/X0/XXY – the situation is not as clear. For instance, two cell lines XX and X0 can appear due to: (a) non-disjunction of chromatids of the X chromosome in the course of early embryonic development of XX or X0 zygotes, leading to mosaicism or (b) exchange through anastomoses of hematopoietic cells between homosexual twins XX and X0, leading to chimerism. The application of genetic markers polymorphism study is very helpful in the identification of blood cells chimerism, for instance in cattle (Glowatzki-Mullis et al., 1995; Rejduch et al., 1998).

From the horse breeding point of view, it is very important to reveal whether the age of parents may influence the incidence of aneuploidy, specially the most common X monosomy. There is very little data concerning this issue (Trommershausen-Smith et al., 1979).

In this study further data are provided on the origin of the X monosomy in mares.

MATERIAL AND METHODS

Altogether 24 mares were subjected to cytogenetic analysis due to various fertility problems: sterility, lack of oestrus, miscarriages, still birth and birth of malformed foals.

Blood samples were collected from mares of the Thoroughbred, half-bred, Arabian and Anglo-Arab mares from Polish studs. Standard lymphocyte culture technique was applied and the cytogenetic preparations were Giemsa stained and also C-banded.

At first, conventional Giemsa stained preparations were analysed. At least 50 metaphase spreads were observed and the diploid chromosome number was established. The C-banding technique facilitated the identification of sex chromosomes due to the presence of an interstitial C-band on the long arm of the bi-armed X chromosome and entire C-positive Y chromosome (Sumner 1982; Richer et al., 1990; Bowling et al., 1997).

RESULTS AND DISCUSSION

Among 24 mares, four (17%) demonstrated a mosaic karyotype 64,XX/63,X (Fig 1). Percentage of 63,X cells varied from 10 to 22%. All those mares were definitely sterile (Tab. I).

The presence of two cell lines (64,XX and 63,X) brings a question whether this status reflects mosaicism or chimerism. The XX/X syndrome in mares is commonly classified as mosaicism. However, the occurrence of chimerism may not be definitely excluded. Cytogenetic techniques are not sufficient to resolve this dilemma. To confirm that these cases were mosaic, blood typing and blood protein polymorphism studies (altogether 14–17 loci) were performed for two mares (Eroika and Martika) and their parents (Tab. II). Concordant results were obtained at all loci, thus we concluded that at least these two mares were mosaics. For another two mares data for both parents were not available.

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1. C-bandng of metaphase spreads originating from a mosaic 64,XX/63,X mare: (a) 64,XX; (b) 63,X. The X chromosomes are indicated by arrows.

I. List of four sterile mares with 64,XX/63,X karyotype

<table>
<thead>
<tr>
<th>Mare (age in years)</th>
<th>Age of mare's parents (years)</th>
<th>Blood type</th>
<th>Breed</th>
<th>% of cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dam (foaling) Sire</td>
<td></td>
<td></td>
<td>64,XX</td>
</tr>
<tr>
<td>Eroika (7)</td>
<td>5 (2) 10</td>
<td></td>
<td>Half-bred</td>
<td>78</td>
</tr>
<tr>
<td>Kamfora (14)</td>
<td>6 (2) 13</td>
<td></td>
<td>Thoroughbred</td>
<td>90</td>
</tr>
<tr>
<td>Marika (9)</td>
<td>8 (3) 6</td>
<td></td>
<td>Anglo-Arab</td>
<td>86</td>
</tr>
<tr>
<td>Armenia (9)</td>
<td>21 (12) 13</td>
<td></td>
<td>Thoroughbred</td>
<td>86</td>
</tr>
</tbody>
</table>

*Age of the parents at the time of foaling a mosaicomic offspring.

An abnormal number of sex chromosomes has very often been found in infertile mares with developmentally retarded reproductive organs (Huges and Trommershausen-Smith, 1977; Trommershausen-Smith et al., 1979; Mäkinen et al., 1986; Long, 1988; Power, 1990). In most cases of mares subjected for examination due to infertility and gonadal dysgenesis, sex chromosome abnormalities were found. Among 12 mares examined by Huges and Trommershausen (1977), nine mares with 63,X karyotype, one with 63,X/64,XX, one

II. Paternity testing for two 64,XX/63,X mares

<table>
<thead>
<tr>
<th>Name</th>
<th>Blood typing</th>
<th>Protein polymorphism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eroika</td>
<td>A: adf/b; C: -/-; D: bcm/eget; K: -/-; P: -/-; Q: abc/**</td>
<td>AL: -B; Es-I; Ge-FS; TF-DF1; Xk-K; PGD-; PGM-; PHI-</td>
</tr>
<tr>
<td>Mare's dam</td>
<td>A: adf/b; C: -/-; D: ecm/eget; K: -/-; P: adf/<strong>; Q: abc</strong></td>
<td>AL: AR; Es-I; Ge-FS; Hb-B1; Pl:RU; TF-F1; Xk-K; PGD-FS; PGM-FS; PHI-I</td>
</tr>
<tr>
<td>Mare's sire</td>
<td>A: adf/<strong>; C: a/</strong>; D: bcm/eget; K: -/-; P: acn/<strong>; Q: abc</strong></td>
<td>AL: B; Es-IM; Ge-F; Hb-BIBII; Pl-I; TF-DH2; Xk-K; PGD-FS; PGM-S; PHI-I</td>
</tr>
<tr>
<td>Martika</td>
<td>A: adf/<strong>; C: a/</strong>; D: cegmm/dkl; K: -/-; Q: abc/**</td>
<td>AL: B; Es-IS; Ge-F; Hb-BIBII; Pl-IL; TF-D; Xk-K; PGD-FS; PGM-S; PHI-I</td>
</tr>
<tr>
<td>Mare's dam</td>
<td>A: -/-; C: a/<strong>; D: del/dkl; K: -/-; Q: abc</strong></td>
<td>AL: B; Es-IS; GC-F; Hb-BII; Pl-JU; TF-DF2; Xk-K; PGD-FS; PGM-S; PHI-I</td>
</tr>
<tr>
<td>Mare's sire</td>
<td>A: adf/<strong>; C: a/</strong>; D: cegmm/eget; K: -/-; P: acn/<strong>; Q: abc</strong></td>
<td>AL: B; Es-I; Ge-F; Hb-BIBII; Pl-I; TF-D; Xk-K; PGD-F; CTA-S; PGM-S; PHI-I</td>
</tr>
</tbody>
</table>

*not examined
**genotype was not established (no data about parents available)
with 63,X/64,XY, and one with 64,XY karyotype were found. Long (1988) reported that three out of 25 examined fertile mares (12%) showed chromosomal anomalies: one was monosomic – 63,X, another one was mosaic – 64,XX/63,X and the last one was sex-reversed – 64,XY. It should however be mentioned that there are also rare reports showing fertile X monosity mares (Halnan, 1985; Long, 1988).

In the last decade, cytogenetic evaluation of mares in Poland received a lot of interest. Bielański et al. (1980) described a phenotypically female foal with 64,XX/65,XY chimism. The first mare with the X monosity was described by Syxa et al. (1989). A case of a trisomic horse 65,XXY was diagnosed by Kubień et al. (1993). Parada et al. (1996) analysed 244 mares with fertility disturbances. Among them, 78 animals were classified as sterile and nine (11.5%) of them showed an abnormal set of sex chromosomes: three of them displayed the X monosity, four were mosaic – 64,XX/63,X and two were chimeric – 64,XX/64,XY. Jaszczał and Parada (1999) conducted an analysis of eight heterosexual twins. Leukocyte chimism XX/XY was found in 4 pairs (7 horses). Two of these chimeric mares gave birth to foals. A similar case was described by Bugno et al. (1999). These results support earlier assumptions that chimeric XX/XY mares do not show severe fertility disturbances (Power, 1990).

The age of the mares’ parents was recorded, to find out whether it might influence the meiotic non-disjunction leading to establishing the X monosity syndrome in offspring (Tab. I). The average age of dams of the 64,XX/63,X mares was 10 years, but this was mainly influenced by the very old (21 years) dam of the mare Armenia. The average age of the mares’ sires was quite similar (10.5 years). A similar investigation was carried out by Trommershausen-Smith et al. (1979), who described 17 cases of pure X monosity, four mosaics – 63,X/64,XX, three 63,X/64,XY and two sex reversed 64,XY. The age of the dams ranged from 3 to 13 years (with the mean of 8 years). The above results show that there is a clear relation between the incidence of X monosity and the age of parents.

Our study shows that further investigation on the origin on X monosity in mares is needed. It seems that a more sensitive microsatellite polymorphism analysis, as it was shown for cattle (Głowatzki-Mullis et al., 1995) or dog (Switoski et al., in press), should be applied to distinguish between mosaic and possible chimeric cases. It is also postulated to collect more data on the effect of parents’ age on the incidence of the X monosity.

Acknowledgements

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REFERENCES


A CASE OF XX/XY CELL POPULATIONS IN SHEEP

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ABSTRACT: A group of 50 ewe lambs was selected for reproduction. Micturition and sexual behaviour anomalies were detected in one animal. Peripheral blood cell cultures showed this individual is a 2 n = 54 XX/XY chimera. Both kinds of cells appear in very similar proportions (51% XX; 49% XY).

XX/XY chimera; genitourinary abnormalities; sheep

INTRODUCTION

Ovine genetic improvement programs are presently being developed in Aragón (Spain). Our laboratory participates in these programs by performing chromosomal abnormalities detection, genetic identification and paternity testing by means of protein markers and microsatellites, and production controls.

In this work, we are presenting the case of an individual showing chromosomal and physical abnormalities.

A group of 50 ewe lambs (70 days old) was selected for reproduction. Six month later, micturition and sexual behaviour anomalies were detected in this animal. This was the start point for further anatomical, functional and genetic analysis.

MATERIAL AND METHODS

Standard peripheral blood lymphocytes cultures were performed. Metaphase plates were Giemsa stained. Images were obtained by means of a CCD camera.

OBSERVATIONS AND RESULTS

Behaviour

Farm staff detected abnormal sexual behaviour in this individual. Even if it was classified as a female, it started trying the covering of the rest of females, showing a male behaviour. It refused any mating attempt by the males and even confronted them. During

1. XX metaphase

2. XY metaphase
the oestrus period, Bibi took a clear dominant position in the ewes group. At present, its work as estrus detector is deeply appreciated by farm staff.

Genitourinary abnormalities

At the moment of the first selection, genitourinary observation was superficial. This shallow control revealed no abnormalities at all. However, after detecting the formerly described sexual behaviour, a deeper examination was performed. At this moment, a clear protuberance was detected at clitoris area, close to the urethra distal portion (see Colour Plate, Fig. 3). Due to this conformation, micturition is quite unusual, giving an horizontal liquid flow.

In the first deep inspection, a single testicle was detected in cryptorchid position. Some months later, two atrophic testes (around 25% of normal size) were found in the inner of atrophic mammary glands. Their position is clearly extra-abdominal.

Chromosomes

Chromosomal analysis was performed when Bibi was about one year old. Peripheral blood cell cultures showed this individual is a $2n = 54$ XX/XY chimera (Figs. 1 and 2).

Both kinds of cells appear in very similar proportions (51% XX; 49% XY).

DISCUSSION

Several morphological and hormonal abnormalities have been reported by Bruere and McNab (1968), Smith et al. (1998), Vallenzasca and Galli (1992). In this papers, intersex, chimerism and sexual reverse are identified with different levels of abnormality in affected individuals.

Our results are widely concordant with those of Bosu and Basur (1984) who detected an ovine intersex registered as a female at birth. That animal developed male-like appearance and behaviour and showed XX and XY cell populations too.

Further analysis are now under development, including hormones quantification, testicle biopsies and abdominal endoscopy to clarify the exact sexual organs morphology.

REFERENCES


