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Short Communication

First evidence of sex chromosome mosaicism in the endangered Sorraia Horse breed

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ABSTRACT

The Sorraia Horse is a critically endangered Portuguese breed with an extremely reduced effective size, having reached unusual high levels of inbreeding. Fertility is of crucial importance for the long-term survival of the extant population and it has been shown that chromosomal abnormalities, especially on sex chromosomes, in horses are associated with infertility or subfertility. To date, no cytogenetic studies were performed in the Sorraia breed to assess the extent of chromosome abnormalities in animals with fertility problems. We now report the results of the first studied case — a subfertile mare with a stallion-like behaviour. A mosaic 63,X0/64,XX karyotype (with 10.45% and 89.55% frequency, respectively) was found. Further cytogenetic screenings in Sorraia horses with low breeding performance and ambiguous sexual phenotypes are needed, to determine the extent of chromosomal abnormalities, since early detection of these animals is of paramount importance for the management and conservation of the breed.

1. Introduction

The Sorraia horse is one of the three native Portuguese horse breeds and it is believed to represent a primitive equine type with a continuous presence in the Iberian Peninsula since early Pleistocene (Luís et al., 2006; Oom et al., 2004). Recovered in 1937 from only 12 founders in the Sorraia river valley, the breed has been managed as a closed population since (e.g. Oom et al., 1991). As an important animal genetic resource (AnGR) with less than 150 extant breeding mares, this breed was considered in "critical maintained risk status" by FAO (FAO/UNEP, 2000), and is the only equine breed recognized as "rare/particularly endangered" by national authorities (MADRP, 2007).

The small number of founders, the reduced effective population size (N_e), the complete genetic isolation and the breeding management adopted, led inbreeding to steadily increase, reaching extremely high levels (average $F = 0.37$), and mating of closely related individuals is now unavoidable (e.g. Oom et al., 2004). Complete pedigrees are available and were crucial to correctly estimate inbreeding coefficients. All these data strongly indicate that the Sorraia breed is a unique biological model to investigate the effects of inbreeding on different traits in horses.

Previous studies provided general information about the Sorraia breed through genetic, pedigree, ethological and morphological analyses. Molecular markers evidenced a decreased genetic variation, indicating that the genome has been largely affected by founder effect, genetic drift and inbreeding (Luís et al., 2007a,b).

Since reproductive success is of paramount importance for the conservation and management of this endangered breed, a comprehensive knowledge of the likely causes of reduced fertility is required. Inbreeding is unavoidable in the Sorraia breed and is commonly associated with decreased fitness, such as reproductive characteristics and offspring viability. The inbreeding effects vary between populations and traits and phenotypes often lack accuracy and give little information to evaluate the extent of the resulting events. In some preliminary studies (Kjöllerström, 2005; Oom et al., 1991), inbreeding was significantly negatively correlated with

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juvenile survival and some suggestions exist that it may be related with both male and female fertility problems, though more data are required for an accurate evaluation. This is of particular concern as a small N_e makes the population even more sensitive to demographic stochasticity, thus special attention must be given to study and improve reproductive performance.

Extensive cytogenetic investigations in horses showed that chromosomal abnormalities, especially of sex chromosomes, are commonly associated to horse infertility or subfertility and to repeated early embryonic death, abortion and stillbirth, although occasional pregnancies may occur (e.g. Breen et al., 1997; Bugno et al., 2008, 2009; Chowdhary and Raudsepp, 2000; Lear and Bailey, 2008). Cytogenetic analyses were never performed in the Sorraia breed, despite several known cases of infertility and subfertility.

Most sex chromosomes abnormalities correspond to normal phenotypes in mares and karyotyping is needed for definitive diagnosis. As it is possible, although not common, that mares with some chromosomal abnormalities produce live foals, those with a small number of life-time foals are potential carriers of chromosomal aberrations and should be checked (Lear et al., 2008; Vanderwall, 2008).

The nuclear genome of the horse comprises 64 chromosomes (31 pairs of autosomes plus the sex chromosomes, X and Y) which are well characterized (ISCNH, 1997). The most commonly reported sex chromosome abnormality is X monosomy (63,X0), first described by Payne et al. (1968), representing more then 50% of all identified chromosome abnormalities in horses (Bugno et al., 2001). Also reported are the XY sex reversal (second most common anomaly found in infertile mares), the XX sex reversal, different types of mosaicism and chromosomal rearrangements and, more rarely, the XXX trisomy. Chromosomal mosaicism (around 30% of horse chromosomal abnormalities according to Lear and Bailey, 2008) is the presence of two or more chromosomally distinct cell lines in an individual.

In the frame of an ongoing cytogenetic survey of the extant Sorraia horses' population, particularly focused in animals with reduced breeding performance and ambiguous sexual phenotypes, we now report the results of the first studied case – a subfertile mare with a stallion-like behaviour – that evidenced a karyotype with a mosaic pattern.

2. Material and methods

2.1. Case study

A subfertile mare, born in 1993, with a phenotypic normal external genitalia but exhibiting stallion-like behaviour and a masculinised body conformation (Fig. 1), was cytogenetically analysed. Ultrasonography showed normal developed ovaries and uterus, although no natural cyclic pattern of oestrus behaviour was detected (P. Bravo, personal communication).

With a high value of inbreeding coefficient ($F = 0.33$), the mare has a low fertility value (0.18) when compared to the average fertility achieved at the same breeding farm (0.38) (Fig. 2): only two foals were produced during her lifetime, one male in 1998 and one female in 2007 (the former by natural covering in the pasture, the latter after an intensive hormonal treatment and artificial insemination, AI). From March to July of 2009 she was submitted to another intensive program of assisted reproduction at the ESAC Animal Reproduction Laboratory Dr. França Martins (Coimbra, Portugal) for induced oestrus and ovulation with Dinolytic® (Pfizer) and Chorulon® (Intervet Schering–Plough). Although developed follicles and corpus luteum were successively detected by ultrasonography, negative gestation diagnosis was confirmed after AI with fresh diluted semen (P. Bravo, personal communication). Sperm concentration, motility, vitality and morphology from the selected stallion were considered above average as regards the Sorraia breed semen characteristics (S. Gamboa, personal communication).

2.2. Cytogenetic analysis

We used standard methods for obtaining chromosome preparations from peripheral blood lymphocytes cultures (Raudsepp and Chowdhary, 2008). Slides were first stained with Giemsa and metaphase spreads assessed by karyotyping following ISCNH (1997) (data not shown). As X chromosomes have a conspicuous interstitial heterochromatic band and the Y chromosome is also well recognized by the length of a heterochromatic block, both in the q arm, we primordially used CBG-banding technique, adjusting the classic protocol (Sumner, 1972): 25 min HCl (0.2 N) at room temperature, 5 min $Ba(OH)_2$ (2.5%) at 60 °C, 10 min SSC 2X

Fig. 1. Phenotype of the studied animal showing male-like characteristics unusual in normal mares (thicker and crested neck, higher bone mass and muscle condition).