IS SUBFERTILITY OR INFERTILITY RELATED TO CHROMOSOME ABNORMALITIES IN THE SORRAIA HORSE? - PRELIMINARY RESULTS -

KJÖLLERSTRÖM, H.J., COLLARES-PEREIRA, M.J. & **OOM, M.M.**

Universidade de Lisboa, Faculdade de Ciências,
Centro de Biologia Animal / Departamento de Biologia Animal, Lisboa, Portugal

mmoom@fc.ul.pt

Objectives

CARRY OUT ROUTINE KARYOTYPE ANALYSIS IN THE SORRAIA HORSE BREED to detect potential chromosome aberrations as a likely cause of observed reduced fertility in both sexes.

Introduction

✓ The **Sorraia Horse** has been managed as a closed population since its recovering, in 1937. It is believed to represent a **primitive equine breed** with a continuous presence in the Iberian Peninsula since early Pleistocene, being an important Portuguese AnGR to preserve.

✓ With less than 150 breeding mares, it is listed as **"critical maintained risk status"** by FAO.

✓ **Inbreeding** is extremely high (see poster S13_5437), and negatively correlated with juvenile survival. It may also be related with both male and female infertility. These problems are of particular concern as the small N_e makes the population very sensitive to demographic stochasticity.

✓ **Chromosomal abnormalities**, especially those of sex chromosomes, are known to be associated with horse infertility or subfertility, early embryonic death and abortion. Most sex chromosomes abnormalities correspond to normal phenotypes in mares and karyotyping is needed for definitive diagnosis.

✓ Horse Karyotype comprises **64 chromosomes** (31 pairs of autosomes and the sex chromosomes, X and Y). The most commonly reported sex chromosome abnormality is X monosomy (63,X) followed by XY male-to-female sex reversal, XXX trisomy, and different types of mosaicism and chromosomal rearrangements, **all associated with infertility** (although occasional pregnancy can occur) **or reduced fertility.**

Methodology

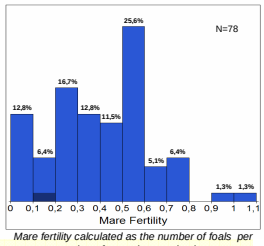
✓ We use standard methods for obtaining chromosome preparations from peripheral blood **lymphocytes cultures**. Because conventional chromosome banding techniques allow a more detailed and reliable karyotypic comparison, **C-, G- and R-banding** will be applied to diagnose specific chromosome structural changes. Whenever necessary, **FISH** based cytogenomic analysis will be performed.

✓ A minimum of **25 metaphase spreads** ***per specimen*** will be screened to detect possible mosaicisms. To start with, special attention will be given to animals with **reported fertility problems** and normal to subnormal phenotypes.

Case Study

A mare, MIMOSA, born in 1993, with phenotypic normal external genitalia but exhibiting stallion-like behaviour and a masculinised body conformation, was subjected to cytogenetic analysis.
Very inbred (F=0.33), she exhibits a reduced fertility (0.182): only two foals produced, one in 1998 and one in 2007 (this one after an intensive hormonal treatment and AI with fresh semen).

Mare MIMOSA

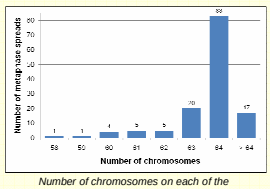


Mare fertility calculated as the number of foals per number of years in reproduction.
The shaded area corresponds to MIMOSA

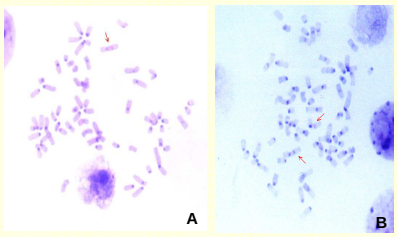
From March to July of 2009 she was submitted to another intensive program of assisted reproduction at the ESAC (Coimbra, Portugal): although oestrus and ovulation was successively achieved, no gestation was confirmed after IA with fresh semen.

Results

A total of 136 metaphase spreads were analyzed, using standard Giemsa staining and C-banding techniques. The latter facilitates the detection of sex chromosomes, and was used to identify the missing chromosome on the 63's metaphases: 60% of those were identified as X monossomy, the others representing hypomodal values.

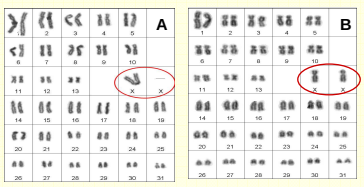


Number of chromosomes on each of the 136 metaphase spreads analyzed



C-banding metaphase spreads from the mosaic obtained . Red arrows indicate X chromosomes. ***A****: 63, X* ***B****: 64/XX*

Preliminary results show the presence of two cell lines : 63,X/64,XX , with frequencies of 8,8 % and 61,0%, respectively.



Standard Giemsa Karyotypes of the two cell lines present on the mosaic obtained.:
A: 63,X B: 64,XX

The fact that the mosaic karyotype of 63,X0/64,XX found in this study, although already reported in horses, was never associated with such a phenotype, corroborates the importance of carrying out a systematic cytogenetic analysis of all possible specimens.

Final remarks

Cytogenetic analyses have **NEVER BEEN PERFORMED** in the SORRAIA breed, despite several known cases of infertility and subfertility. Since breeding success is of capital importance, early detection of the animals that will later show none or poor breeding performance, due to chromosomal abnormalities, **can substantially save breeders efforts and resources.**