Chromosomal abnormalities and DNA genotyping in horses

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This article was modified from a printed version that appeared on the Fedequinas Journal, 57:34-40, July-August 2009. Permission to reprint granted by Fedequinas.

The genetic material of animals is found in structures called chromosomes, which are contained in the nucleus of a cell. Different species of animals have different numbers and structures of chromosomes. The horse has a total of 64 chromosomes or 32 pairs (one from each parent). Two of the 64 chromosomes (one pair) are called sex chromosomes because they contain genes that determine the sex of the individual. A normal mare has 64 with two X chromosomes (64,XX) and stallions also have 64 but have one X and one Y (64,XY) (Figure 1).

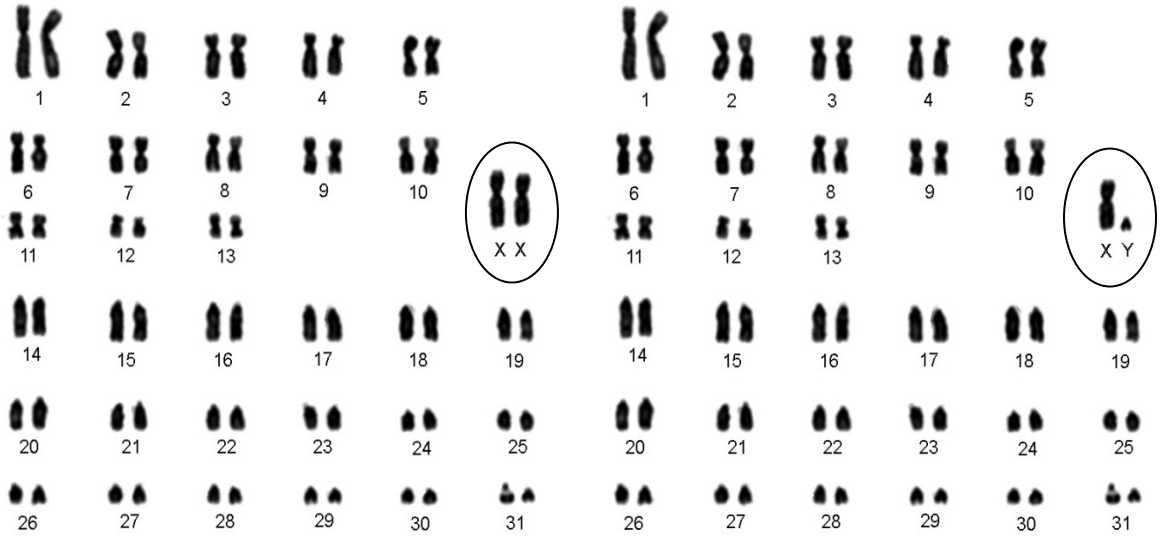

Figure 1. The female and male karyotypes where the only difference is the composition of the sex chromosomes – females are XX and males are XY.

Each parent contributes half of the genetic material to the offspring via egg or sperm. The dam's egg contributes 31 chromosomes and one X while the sire's sperm also contributes 31 and either an X or Y chromosome. The fusion of egg and sperm results in a normal zygote with 64 chromosomes that develops into an embryo and ultimately a foal. When an egg fuses with a sperm that bears an X the resulting foal will be female – 64,XX. If the egg fuses with a sperm that bears a Y then the foal will be a male 64,XY.

Occasionally chromosomes are lost or rearranged during egg or sperm formation. Such changes are usually very harmful to normal development and the affected egg or sperm will fail to mature, fail to form an embryo, or stop the development of an embryo to full term, which is then lost by miscarriage or resorption. On rare instances, the chromosomal changes are minor enough that the embryo goes to term but will produce a foal that may have defects in growth, behavior, and reproduction.

Karyotyping is a procedure where the chromosomes of an animal are examined and analyzed. This is typically performed when chromosomal abnormalities are suspected. For example, good candidates for karyotyping are animals that show abnormal development or growth, particularly of the reproductive tract, that have very small ovaries and follicles, and with poor heat or heat patterns. Karyotyping is routinely performed using whole blood samples. Typically, white blood cells are isolated from fresh (less than 5 days old) whole blood samples that are collected in appropriate anticoagulant, such as sodium heparin or acid citrate dextrose (ACD). The white blood cells are cultured for at least 3 days and then treated with reagents that stop the cell division at a stage where chromosomes are easily visualized and examined. The cells are prepared on microscope slides, stained and examined with a light microscope at a magnification of 1000×. The number and structure of the chromosomes are examined to determine whether all chromosomes are present and normal or whether there are abnormalities, and if so, of which type.

The most common abnormality that we encounter in horses is the 63,X karyotype where one of sex chromosomes is missing. These animals develop and appear to be a normal female. However, such horses are very much compromised and fail to develop normal reproductive tracts. This congenital condition basically renders these horses infertile. The origin of the abnormality is most likely a spontaneous event and is not inherited. The incidence of this condition in horses is estimated to be around 0.06%.

The second most common abnormality seen in horses is the 64,XY sex-reversal or testicular feminization. The animal appears to be female yet has the karyotype of a male (Figure 2). These horses have malformed reproductive systems. They have also been reported to have behavioral problems and often exhibit stallion-like behavior when they are around other mares. There are other rare chromosomal abnormalities that have been reported, such as 65,XXX; 65,XXY or even instances where whole or pieces of chromosomes are rearranged and still manage to produce a viable foal. Such abnormalities are often associated with pronounced physical defects, and thus, these horses are often removed from breeding considerations.

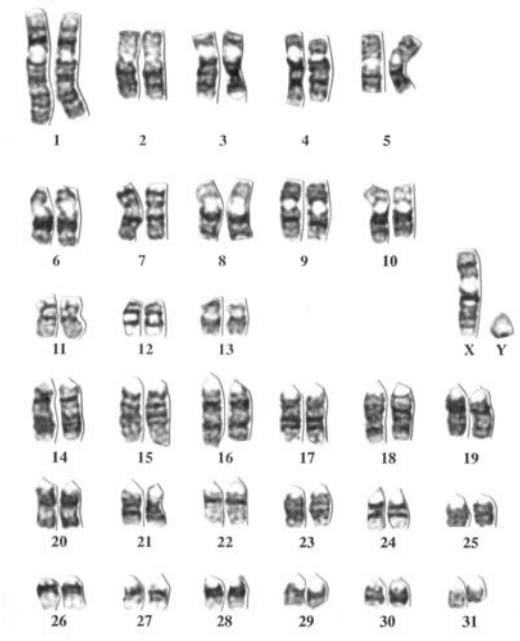

Figure 2. Banded karyotype of a 64,XY female (sex-reversal). The chromosomes are treated to show banding patterns that help identity the matching pairs of chromosomes and the sex chromosomes.

DNA genotyping for pedigree and parentage verification has also proved to be useful in identifying animals that are suspected of possessing chromosomal abnormalities. Abnormal profiles of genetic markers located on sex chromosomes can help identify animals that are likely to have chromosomal defects. The panel used for horse DNA testing by the Veterinary Genetics Laboratory at the University of California, Davis includes two markers linked to sex chromosomes. Additional sex-linked markers are also available at the laboratory for extended analyses of suspect cases.

A select set of genetic markers on the X and Y chromosomes will occasionally yield results that are contrary to the expected sex of the animal. Amelogenin, a gene with distinct X and Y alleles, readily identifies sex chromosome composition. Screening of this gene is used in DNA testing to verify the genetic sex of samples. However, animals that are identified as females sometimes have genotypes that are consistent with the opposite sex, and vice-versa. For example, we are occasionally presented with females that have DNA types that are typical of males. Such animals contain the Y form of amelogenin, and other X-linked markers indicate that only one X chromosome is present. Various examples exist where the genotyping results may indicate other abnormalities – females that have only one X (63,X), XXY (65,XXY), XX/XY (64,XX/64,XY -- a mixture of female and male cells) or XX males. The examples shown in Tables 1 and 2 are from horses with abnormal results for sex chromosome markers. These cases were uncovered in the process of DNA testing the horses for parentage verification and registration.

Table 1. Filly suspected of having a 63,X karyotype. The filly appears to have one X chromosome inherited from her dam (underlined letters) but did not receive an X chromosome from the stallion (note absence of markers from paternal X – F, M, Q, O).

Animals	Reported Sex	Amelogenin (molecular sexing)	Markers on the X chromosome			
			LEX3	LEX22	LEX27	TKY598
Foal	Female	X only (female)	P	N	P	N
Dam	Female	X only (female)	MP	NO	PQ	KN
Sire	Male	XY (male)	F	M	Q	O

Table 2. Mare with presumed abnormal karyotype. The mare displays X-linked markers in common with her dam yet she also inherited the Y chromosome from the sire. This horse was confirmed to have a karyotype of 64,XX / 64,XY (a mixture of male and female cells).

Animals	Reported Sex	Amelogenin (molecular sexing)	Markers on the X chromosome			
			LEX3	LEX22	LEX27	TKY598
Foal	Female	XY (male)	FL	LO	PQ	LM
Dam	Female	X only (female)	FL	LO	PQ	LM
Sire	Male	XY (male)	F	N	M	P

Through the routine DNA genotyping of animals, it is possible to identify profiles that are indicative of chromosome abnormalities. With the approval of AMHA, the Veterinary Genetics Laboratory implemented a procedure to report to the registry cases of animals that have DNA types for sex chromosome markers inconsistent with the declared sex of the animal. While a few of these discrepancies are explained by a simple error in sex identification, most of such cases correspond to defects in the sex chromosomes. The animals that present anomalous genotyping results and yet qualify to their parents should be karyotyped to have a more thorough and definitive diagnosis of abnormalities that could adversely affect their fertility or breeding status.

Horses that are good candidates for karyotyping are animals that show poor development or growth, with small ovaries and follicles, with abnormal genitalia, or with poor heat or heat patterns. Often we are able to make a diagnosis before more resources are used for treatment. Early diagnosis of chromosomal abnormalities saves owner's time and money by identifying horses with high risk for poor performance and breeding. The analysis of sex chromosomes during routine DNA testing for parentage

verification represents a first line of clinical applications that help monitor and improve the reproductive health of the Morgan horse.