Case Report

Infertile mares with XO and XY sex chromosome deviations

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Introduction

In horses, small ovaries and failure to cycle during the breeding season suggest abnormalities in the sex chromosomes (Chandley *et al.* 1975; Long 1988). Horses with sex chromosome deviations often reach maturity, because foals with sex chromosome defects have less severe abnormalities than those with autosomal deviations (Bowling and Millon 1990). Therefore, the connection between infertility and sex chromosome alterations in horses is possible to detect.

Mares with XO and XY sex chromosomes usually do not show normal oestrous behaviour. However, mares showing irregular oestrous signs have been reported, and many affected mares have been able to be bred because a lack of progesterone production leads to a passive acceptance of the stallion (Miyake *et al.* 1979; Long 1988; Bowling and Hughes 1993; Mäkinen *et al.* 1999, 2001). Their external reproductive organs are normal, and on transrectal examination these mares are impossible to distinguish (Chandley *et al.* 1975). In most cases, owners of such mares are not aware of the condition until puberty, when the mares with XO or XY sex chromosomes fail to cycle.

In this report, we describe the clinical and genetic examinations and necropsy findings in 2 infertile mares, referred for further studies by veterinary surgeons experienced in equine practice.

Case details

Case 1: XO mare

A 10-year-old Finnhorse mare was presented having failed to cycle. The mare was small in size with normal external genitalia, and was the first foal of a 9-year-old dam who had subsequently foaled 2 other female offspring. The 16-year-old sire had sired 49 female and 41 male offspring. The sire and dam were not alive at the time of diagnosis.

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In all lymphocyte metaphases studied, the mare had only 63 chromosomes. The missing chromosome in the karyotype was identified as an X chromosome by fluorescence *in situ* hybridisation using the Cambio (STAR*FISH)[1] biotinylated human X chromosome paint probe (Mäkinen *et al.* 2001) as well as the standard C- and G-banding methods for chromosome identification.

After the diagnosis of the XO sex chromosome condition, the mare was subjected to euthanasia and a necropsy performed. The uterus was found to be small but normally shaped and symmetrical. The *portio vaginalis* was oedematous and soft. Uterine mucosa showed prominent folding and a thickness of 4 mm. The total length of the uterine body and cervix was 26 cm, and the uterine horn measured 8.5 cm. The left ovary was 2.5 x 1.5 cm in size and bean-shaped, with a 1 x 1 cm parovarial cyst attached to the mesovarium. The right ovary was round to ovoid and 2 x 4 cm in size. Histologically, both ovaries consisted of well-vascularised spinocellular connective tissue with mild multifocal haemosiderosis. Primordial follicles or follicles at any stage of development were not seen.

Additional autopsy findings not related to reproduction were also reported. The mare had a deviation between the lumbar spine and sacral bone, approximately 15° to the right, as well as a focal rim of dysplastic ossification at the left atrioventricular border of the heart. The liver capsule showed a multifocal filamentous perihepatitis due to migrating parasites (*Strongylus* spp.).

Case 2: XY mare

This Warmblood mare was 7-years-old, of female phenotype and weighed 460 kg. The external genitalia and vagina appeared normal on visual inspection. On rectal examination, the cervix and uterus were both short and thin-walled.

The mare was the third offspring of a 9-year-old dam, who had further 6 male and 2 female offspring. The 7-year-old sire had 4 male and 3 female offspring. The mare had

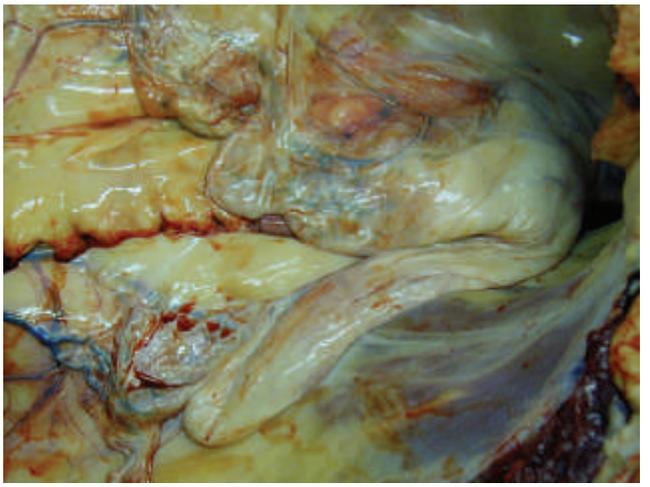

Fig 1: Hypoplastic ovaries and the flaccid uterus of* Case 2 *(XY mare)* in situ*; the left ovary measured 2 x 4 cm, the right 2 x 5 cm.

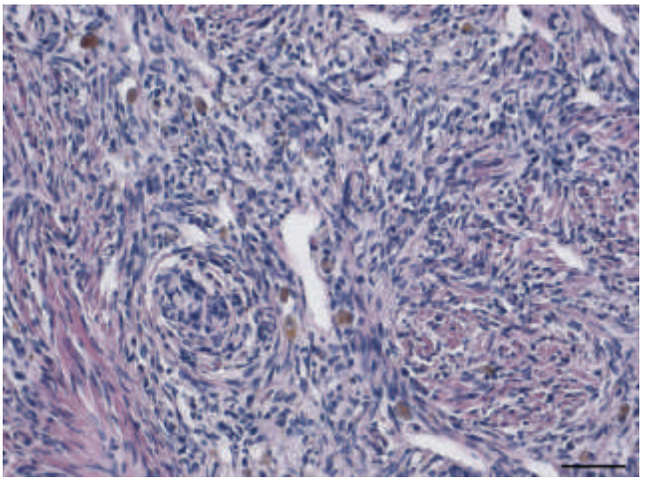

Fig 2: Histological section of the ovary of* Case 2 *(XY mare), consisting of spinocellular connective tissue lacking follicular structures, with multifocal haemosiderosis. H&E stain; magnification x20; bar = 50 µm.

one full brother. Despite an excellent pedigree, the horse was not suitable for trotting competition, causing problems when driven in the sulky because of sensitivity in the hindquarters; the intention was therefore for the mare to become a broodmare. The animal was aggressive towards other horses, sometimes reacting to oestrous mares by trying to mount them.

The mare had 64 chromosomes in the lymphocyte metaphases. The C- and G-banding methods for chromosome identification revealed a male sex chromosome complement XY (sex reversal, 2n = 64, XY). SRY (sex reversed gene in Y chromosome), AMELY (amelogenin gene located in the Y chromosome) and AMELX (amelogenin in the X chromosome) gene fragments were simultaneously amplified (Hasegawa *et al.* 2000). The horse had altered gene structure in the Y chromosome. The SRY gene region (Hasegawa *et al.* 1999) was missing from the Y chromosome, indicating that the female phenotype was affected by loss of active SRY gene. However, AMELY and AMELX genes were present, in accordance with the existence of XY sex chromosomes in the metaphases.

Necropsy findings included a normally shaped, symmetrical, but clearly hypoplastic uterus. The total length of the uterine body and cervix was 13 cm, and the uterine horn measured 6 cm. The left ovary was ovoid and 2 x 4 cm in size, while the right ovary was round to ovoid and 2 x 5 cm in size (**Fig 1**). The *portio vaginalis* was dry and pale. The uterus was flaccid and the mucosa thin, with a thickness of 1 mm. Additional autopsy findings consisted of mild multifocal endocardial fibrosis in the heart.

Histologically, both ovaries consisted of centrally well-vascularised, spinocellular connective tissue with a peripheral dense fibrous capsule. Mild multifocal haemosiderosis was seen in the stroma and primordial follicles or follicles at any stage of development were absent (**Fig 2**). The cervical mucosa showed a mild to moderate proprial and intra-epithelial infiltration of neutrophilic granulocytes and low numbers of lymphocytes and plasma cells.

Discussion

Turner syndrome is the only complete horse monosomy that allows development to adulthood. Many cases are mosaics 64,XX/63,X (Chandley *et al.* 1975; Wieczorek *et al.* 2001). Reported instances of mares with Turner's syndrome which have foaled have been mosaics (Halnan 1985). The 10% of women with Turner's syndrome who experience menstrual cycles are mosaics or have partial deletions of the X chromosome (Barad 1998). Mares with monosomy X have been reported to be smaller than average for their breed (Bowling and Hughes 1993), but the life expectancy of XO mares who survive infancy is not reduced. Short stature is present in almost all women with Turner's syndrome. They also have characteristic dysmorphic features in their appearance and approximately half have kidney and cardiac anomalies (Barad 1998); however, abnormalities have not been reported in XO mares.

Only one X chromosome is required for development of the cortical sex cords and ovarian structure, but 2 are essential for normal ovarian development and maturation. Ovaries of 45,X human embryos appear normal at 14–18 weeks of gestation, but degenerate after formation of the medullary cord. The loss of oocytes is completed between the neonatal period and the first few years of life (Barad 1998). On histological examination in XO mares, the ovaries have been found to consist of undifferentiated ovarian stroma (Miyake *et al.* 1979). In this study, the parental origin of the X chromosome could not be tested because the sire and dam of the mare in *Case 1* were no longer alive. However, in the study by Mäkinen *et al.* (2001), nondisjunction was seen to have occurred in a stallion because the sex chromosome was absent in the 2n = 63, XO mare in the report.

In phenotypically female horses with XY chromosomal complement, the existence of both AMELX and AMELY genes indicates the presence of XY sex chromosome complement. Absence or structural errors of the SRY gene on the

Y chromosome result in the pluripotent gonads differentiating towards the female sex (Abe *et al.* 1999; Mäkinen *et al.* 2001; Bugno *et al.* 2003).

The XY female condition in horses is usually associated with complete gonadal dysgenesis: normal female outer genitalia, but underdeveloped internal genitalia and abnormal endocrinological profiles (Chandley *et al.* 1975; Long 1988). Follicular activity has been reported, but is rare (Long 1988). Affected mares are often somewhat taller in size than normal female horses, and some have had successful show and performance careers prior to being sent to stud (Bowling and Hughes 1993; Mäkinen *et al.* 1999).

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Manufacturer's address

[1]Cambio Ltd, Cambridge, Cambridgeshire, UK.

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