ABSTRACT

A 19-month-old Standardbred colt was donated to the University of Pennsylvania School of Veterinary Medicine with a suspicion of intersexuality. The anal–genital distance and penis were normal, and there was no evidence of intersexuality, but the colt was bilaterally cryptorchid. Several aspects of the colt’s behavior appeared unusual, including general temperament and behavior described as sympathetically dull and affable. With herd mates, the colt appeared slow to perceive or to learn the usual intraspecies social cues. An atypical gait characterized by intermittent unnatural shuffle of the hind limbs, sliding them along in short rhythmic strides for 3 to 10 seconds at a time was noted at times when a horse might normally transition from a slow walk to a fast walk or a slow trot. Occasionally the colt exhibited slight protrusion of the tongue through the teeth and lips with jaw movements and smacking of the tongue against the teeth as if struggling to retract the tongue to the normal position. Evaluation of the karyotype combined with fluorescent in situ hybridization (FISH) revealed an abnormal male karyotype showing trisomy of chromosome 27 (65, XY + 27). The colt was euthanized at 24 months of age, and a necropsy revealed no significant abnormalities. This case of trisomy was not associated with developmental abnormalities described in other rare reports of trisomy in horses; however, some features were strikingly similar to that of humans with trisomy 21. FISH was demonstrated to be an excellent method for correct identification of equine chromosomes.

Keywords: Equine trisomy; Equine aneuploidy; Equine karyotype; Equine behavior

INTRODUCTION

Aneuploidy includes numerical chromosome abnormalities that involve a missing (monosomy) or extra single chromosome (trisomy) resulting from chromosome nondisjunction or abnormal chromatid separation. This might occur either during meiosis I or II during gametogenesis, or during mitosis in early embryonic development.1-3 Numerical chromosomal abnormalities are often lethal and are the most common cause of pregnancy loss in humans, accounting for almost 50% of spontaneous abortions before 15 weeks of gestation.1,3 The incidence of numerical and structural chromosome abnormalities in human live births is approximately 1 to 2:1,000 and usually results in severe physical and mental problems in the affected children.1,3 Trisomies of all chromosomes with the exception of chromosome 1 have been reported in spontaneous abortions in humans; however, the only numerical autosomal anomalies surviving to birth are trisomies 13, 18, and 21.1,3 There are only six reported cases of autosomal trisomies in live horses (Equus caballus [ECA] 23, 26, 27, 28, 30, and 31),4-8 suggesting that these are rare in this species. Similar to that observed in humans, trisomies in horses predominantly involve small chromosomes.1-3

CASE REPORT

A 19-month-old Standardbred colt was donated to the Georgia and Philip Hofmann Research Center for Animal Reproduction of the University of Pennsylvania School of Veterinary Medicine with a suspicion of intersexuality. The colt was reportedly always small for its age group, but during training as a pacer could go at 20 mph, an average speed for the age group. Both the colt’s sire and dam were 5 years old when the colt was conceived, and no abnormalities of gestation or parturition were reported. Both the colt’s sire and dam were 5 years old when the colt was conceived, and no abnormalities of gestation or parturition were reported. The colt’s sire is a successful stud and has produced numerous normal foals. The colt’s dam has produced at least two normal foals, including a full brother of the colt described in the current report that became a successful competitive race horse.

On examination on arrival, the colt was observed to be in good general condition and health. No abnormalities of the musculoskeletal system were observed, but the colt was small for its age and had an unusual stiff hind limb gait and a rough coat. Reproductive examination revealed
that the anal—genital distance was normal and that a scrotal pouch was present, but the colt was bilaterally cryptorchid. An ultrasonographic examination showed that both testes were retained within the inguinal canal. Testosterone concentrations before and 1 and 2 hours after a human chorionic gonadotropin (hCG) stimulation test (10,000 IU, intravenously) were considered normal for the age and time of year (5.0, 46.5 and 59.6 pg/mL, respectively). The penis was normal, but the prepuce was enlarged; an ultrasonographic examination showed enlarged vessels that were a few millimeters up to 2 cm wide, with characteristic valves and positive Doppler signal. There was no evidence of intersexuality. In an attempt to collect semen and evaluate sexual behavior, the colt was repeatedly exposed to estrous mares in a standard breeding shed situation, as well as at liberty in a 2-acre pasture with an estrous mare. The colt’s sexual behavior was similar to that of a slow-starting novice breeder, which was clearly within the range of normal for a colt of his age. One exception to normal was that when investigating a mare, the colt repeatedly nudged the mare with small circular motions of his muzzle in a pattern that we had not seen in horses before. This colt was especially awkward at mounting, repeatedly lodging a flexed foreleg in the perineal area of the mare and then sliding off. Although not common, we have seen this before in slow-starting novice breeders. With assistance in positioning the foreleg, the stallion accomplished reasonably good coupling. Pelvic thrusting occurred in response to application of an artificial vagina. Although no sperm were found to confirm ejaculation, on three occasions it appeared that the colt experienced weak ejaculatory pulses.

Over the course of 6 months, several 8- to 24-hour videotaped samples of the colt in a stall were evaluated. Patterns of behavior were within normal limits for a colt of his age, including typical patterns and frequencies and durations of eating hay, drinking, resting, recumbency, urination, defecation, and spontaneous erection and penile movements (masturbation). In the daily care and interaction with this animal, experienced horse handling caretakers voluntarily and independently described the colt’s general temperament and behavior as sympathetically “dull” and friendly to humans and herd mates. The colt appeared to be attracted to people and to any herd mate, and appeared always “in the way,” in that he preferred to stand as close as possible to people or other horses. Both with human handlers and with other horses, the colt’s social interactions were described as slow to usual correction. When in a pasture with other males (geldings and colts), this animal appeared slow to perceive or to learn the usual interspecies social cues. Initially, the colt was observed as a frequent recipient of aggressive correction from herd mates, which appeared to be the result of his failure to follow or to learn “personal space” rules of herd mates. His facial expressions when being corrected were not typical, having a persistent look of “innocent confusion,” with no sign of aggressive response or strong sign of fear. In his pasture group, his social failures eventually appeared to be tolerated, and several of the pasture mates appeared to guard and protect the colt as stallions and geldings often protect a young foal. Other than this apparently impaired social interaction and learning, the colt appeared cognitively normal, efficiently acquiring the conditioned responses inherent to domestic handling and training.

In addition to the stiff hind-limb gait noted on his initial physical examination, other atypical gaits were noted. At a slow walk, the colt was observed to exhibit an unnatural shuffle of the hind limbs, sliding them along in short rhythmric strides for 3 to 10 seconds at a time. This occurred intermittently at a slow walk, both when the animal was being led by a human handler and when at liberty in a pasture. It was the handlers’ impressions that this shuffling gait was associated with moments of exuberance or urgency to move forward, for example, to rejoin a herd mate, when being led toward an estrous mare, or when offered a food treat from a distance of at least a few strides. These would be occasions when a horse might normally transition from a slow walk to a fast walk or a slow trot. Occasionally the colt exhibited odd mouth and tongue movements, including slight protrusion of the tongue through the teeth and lips with jaw movements and then smacking of the tongue against the teeth as if struggling to retract the tongue to the normal position. While the sound was similar to a common equine tongue sucking stereotypy that appears more like nursing behavior, this tongue behavior was clearly different in that the smacking was slower and less rhythmic and the tongue was pushed forward and pulled back with each smack.

Pokeweed-stimulated lymphocyte cultures were established from peripheral blood and chromosome preparations were prepared using standard cytogenetic procedures. The slides were CBG-banded\(^9\) to identify the sex chromosomes. GTG-banding\(^10\) was performed to identify the autosomes. Banded chromosomes were arranged according to the International System for Cytogenetic Nomenclature of the Domestic Horse.\(^11\) In addition, fluorescent in situ hybridization (FISH) was performed using markers previously mapped to horse chromosomes (ECA) 25, 26, 27, and 28. These markers included DBH (025N1, 25q18-q19; CHORI-241 address and cytogenetic location, respectively), TIAM1 (258L22, 26q15), LOC84549 (217O21, 27q15-16), and IGF1 (16L15, 28q15).\(^12\) Bacterial artificial chromosome (BAC) clones from the Texas A&M CHORI-241 equine BAC library were selected and individually cultured to isolate DNA using the Qiagen midiprep kit (Qiagen, Chatsworth, CA) according to the manufacturer’s instructions. Isolated DNA was separately labeled with either biotin-14-dUPT or digoxigenin using the BioNick Labeling System (Invitrogen, Carlsbad, CA).
and DIG-Nick Translation Mix (Roche, Indianapolis, IN), respectively. Labeling of the DNA, in situ hybridization, signal detection, and image analysis were performed as previously reported.\textsuperscript{13,14} CBG-banding showed the karyotypic constitution of the proband to be 65,XY. A closer look at the karyotype showed that although the number of metacentric chromosomes was normal, that is, 26, there was an extra small acrocentric chromosome in all cells examined. This was further confirmed by GTG-banding, which suggested the extra chromosome to be ECA27 (Fig. 1). FISH with BAC clones from ECA25, 26, 27, and 28 clearly showed three hybridization signals with ECA27 marker LOC84549 on all metaphase chromosomes (Fig. 2), confirming that the colt was a trisomic autosomal 27.

The colt was euthanized at 24 months of age, and no gross abnormalities were observed on necropsy. Histopathological examinations revealed no lesions on the brain, heart, lungs, liver, kidney, spleen, thymus, pituitary gland, or adrenal gland. Left and right testes weighed 25.1 and 36.6 g, respectively. Dimensions (length, width, height) of left and right testes were $5.8 \times 3.2 \times 3.3$ cm and $6.4 \times 4.0 \times 4.1$ cm, respectively. Left and right epididymides weighed 13.8 and 14.1 g, respectively. Histopathological evaluation of the testes revealed Leydig cells of normal appearance in the interstitial tissue. There was extensive vacuolation of the seminiferous epithelium, and the vast majority of seminiferous tubules were lined only by Sertoli cells and spermatogonia. Primary spermatocytes were rarely observed in a few tubules, but no spermatids were present. No sperm were observed in samples obtained after flushing the epididymides.

**DISCUSSION**

The only case of autosomal 27 trisomy in a horse was described in a Quarter Horse male foal\textsuperscript{6}; however, as pointed out by Lear et al,\textsuperscript{8} based on the presented GTG-banded karyotype, this classification did not appear to conform to the corresponding chromosome in the standard karyotype of the horse.\textsuperscript{11,12} Some of the small acrocentric
chromosomes in the equine karyotype (ECA25–ECA31) are relatively difficult to identify with available banding approaches. The availability of a high-resolution horse gene map (T. Raudsepp, personal communication) and a variety of markers provide an alternative that facilitates accurate identification of the chromosomes. Equine BAC clones for individual mapped markers serve as molecular probes for FISH that permit unambiguous identification of the chromosome. The horse–human comparative gene mapping data shows that ECA27 shares homology with the terminal part of the long arm of human chromosome 4 (HSA4) and part of the short arm of HSA8.12,14 Because only lymphocytes were karyotyped, the possibility that the colt was a mosaic cannot be completely ruled out. However, most cases of mosaicism show a normal karyotype when the lymphocytes are examined, and the abnormal cell line is generally only apparent when different tissues such as the skin are examined. Hsu et al16 evaluated the relationship of karyotype results after amniocentesis and follow-up results of karyotype of different tissues. The authors found only one case in which lymphocytes displayed a pure trisomic cell line with other tissues (fibroblast) showing a different karyotype. In that study, the authors describe blood as a poor tissue for confirming mosaicism seen in other tissue types, because lymphocytes tend to show only the normal chromosomal complement. Given these observations, it seems that the probability of mosaicism of the colt described in the report is low.

In humans, there is great variation among chromosomes in the origin of autosomal trisomies. Maternal origin predominates in all cases of trisomy 13, 18, and 21, but there is great variation in the proportion of nondisjunction during MI or MII accounting for the aneuploidy. In humans, increasing maternal age, but not paternal age, is the only factor unequivocally associated with chromosomal abnormalities.2,17 Increased maternal age (14–26 years of age) is a constant in all cases of autosomal trisomy reported in horses, except in the case involving centric fusion of two chromosomes 26.4–8 The dam of the presumed trisomic 27 foal described in the literature was 26 years old, but the dam in the current case was only 5 years old. Therefore, it seems that the possibility of paternal origin of trisomy in the current case cannot be dismissed. Trisomies 13, 18, and 21 of paternal origin are observed in 4% to 13% of the cases in humans.1,3 Unfortunately, samples from the sire and the dam were not available for identification of the source of the extra chromosome in the current case.

Although trisomies 13 and 18 are observed in live babies, only trisomy 21 (Down syndrome) may allow survival into puberty and adulthood.1,3 In horses, intensive care was required for a presumptive trisomic 27 colt to survive,6 and health concerns led to euthanasia of a trisomic 31 colt at 6 months of age.8 Trisomies 23, 28, and 30 were reported in horses 1 or 2 years old, and trisomy 26 was reported in a mare that lived to be at least 5 years old.4,5,7 In the current case, the colt was electively euthanized at approximately 2 years of age, but there were no health problems to suggest that the colt would not survive to adulthood. Although trisomies in humans have been associated with cardiac and gastrointestinal anomalies,3 no abnormalities of internal organs were observed after

**Figure 2.** Fluorescent in situ hybridization confirming the identity of chromosomes (A) ECA25 (DBH marker; green), ECA26 (TIAM1 marker; red), (B) ECA27 (LOC84549 marker; red), and ECA28 (IGF1 marker; green) in a Standardbred colt with abnormal male karyotype showing trisomy of chromosome 27 (65, XY + 27).
necropsy of the trisomic colt described in the current report. Neurologic deficits also have been reported in cases of trisomy in horses. In one case the head and tail were held to the side and the colt had a dysmetric and uncoordinated gait, whereas in another case there was a left-sided head tilt and when blindfolded, the foal was unable to maintain its balance and fell to the left. No neurologic deficits were observed in the current case.

Trisomies reported in live horses have been frequently associated with musculoskeletal abnormalities including carpal flexural deformities, angular forelimb deformities, mild polydactyly (articulated splint bones), facial asymmetry, or brachygnathia inferior. Contrary to these previous reports, the trisomic colt described in the current report did not have any musculoskeletal abnormalities, which was similar to the presentation of a Thoroughbred colt with trisomy 28. However, the colt was smaller than expected for its age and had a stiff gait that appeared to be caused by some degree of joint rigidity, which are characteristics that have been described in other cases of trisomy in horses. This colt also had an intermittent hind limb shuffling gait in certain situations when transitioning from a slow walk to a faster gait. A variety of gait abnormalities are common among Down syndrome, including a shuffling, scurrying walk when excited. In children, various gait abnormalities, including the tendency for a shuffling fast walk when excited, are often attributed to limb and feet abnormalities and obesity. This colt had no limb or feet abnormalities and was thin. Study of a mouse model of Down syndrome, Ts65DN, indicated an altered stride at the fast walk, including shorter stride length and greater stride frequency than in control mice. This is similar to one variation of gait abnormality seen in Down syndrome children, and apparently similar to the abnormal gait in the trisomic colt described in the current report. Other abnormal gait dynamics of these trisomic mice, including breaking duration for the hind limbs, suggest specific motor dysfunction as the major contributor to abnormal gait.

Malformation of the prepuce with only a slit-like fold through which the foal urinated was described in a trisomic 31 colt, but the dilated preputial vessels observed in this case were quite unique and had not been observed in horses by us before. Cryptorchidism in stallions is relatively common, with a prevalence of 2% to 8%, but only 7% of the cases are bilateral. No cryptorchidism or abnormalities of the reproductive tract were reported in male colts with trisomy 23 and 27, but bilateral cryptorchidism like that described in the current report was previously reported in a trisomic 28 colt, and unilateral cryptorchidism was reported in a trisomic 31 colt. Increased testosterone secretion in response to hCG treatment similar to that observed in the current case was also reported in the trisomic 28 colt. The incidence of cryptorchidism in humans with Down syndrome is greater than in the general population, but approximately 70% of the cases are attributed to ascending testes, that is, testes that descend to the scrotum early in life, but ascend out of the scrotum later on. Failure of normal growth of the spermat cord has been suggested as the cause of ascending cryptorchidism, but as far as we are aware this condition has not been observed in horses.

Evaluation of spermatogenesis in trisomic males reveals arrested spermatogenesis at meiosis, although spermatids and sometimes sperm may be observed in some seminiferous tubules. Consequently, trisomic males are usually azooospermic or oligospermic. Although fertility is rare, a recent report describes the birth of a healthy, normal baby fathered by a man with Down syndrome. In the current case, spermatogenesis did not proceed beyond primary spermatocytes, and no sperm were observed in the seminiferous tubules or epididymides. Unfortunately, it was not possible to attribute the testicular histopathologic changes observed in the current study to trisomy, because these changes also resemble those observed in cryptorchid testes recovered from a random population of horses. Similar to that observed in the current case, normal sexual behavior was reported, but one attempt to collect semen at 2 years of age from a bilateral cryptorchidic, trisomic 28 colt resulted in one clear ejaculate with no sperm. Reported behaviors in trisomic horses did not resemble that observed in the current case. Behavior was considered to be abnormal in a trisomic 28 colt as it constantly walked in small circles even when in an open field, whereas a mare with trisomy 26 was reportedly intolerant to pain and physical restraint and appeared mentally dull. On the contrary, a yearling mare with trisomy 30 was mentally alert, very energetic and active, and had no reported behavioral problems. Observations of the colt’s behavior in the current case (before a diagnosis of trisomy) indicated certain features that were strikingly similar to that of humans with Down syndrome. This colt was remarkably friendly with humans and herd mates, and it was gentle and free of the aggressive tendencies typical of pubertal colts. A hallmark of Down syndrome is a “natural spontaneity, genuine warmth, gentleness, patience, and tolerance.” The colt also appeared socially slow and awkward in intraspecies interactions and with “personal space” correction from human handlers. Down syndrome persons have varying degrees and types of mild to moderate cognitive impairment, typically with some delay in emergence of expressive language and slower than normal learning of social skills. The colt had an unusual tongue protrusion that the authors had not seen before in horses. This behavior appeared similar to what is described as tongue thrusting and smacking seen in almost all Down syndrome children. In Down syndrome, this tongue protrusion and thrust is attributed to tongue hypotonia along with the often abnormally small oral cavity...
with a normal size tongue, a typically misshapen palate, as well as the tendency for open-mouth breathing caused by abnormal nasal passages. This colt’s mouth was not examined closely to assess relative oral cavity and tongue size and tone, but there were no conspicuous abnormalities. Horses do not breathe through the mouth, and this colt kept its mouth normally closed most of the time.

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