

INTERSEXUALITY IN THE HORSE

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ABSTRACT

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A cytogenetic and clinical study of an intersexual Arabian horse revealed it to be a genetic female but a gonadal male intersex. This corresponds in general to previously reported cases, which have been genetic females despite the presence of male gonads (or ovotestes). The reliability and ease of polymorph sexing in the horse as a method of determining the genetic sex has been confirmed. Once this is known an easy decision can be made as to the feasibility of castration or not. In the case described castration resulted in an improved feminine appearance.

INTRODUCTION

Chromosome studies have given a new incentive to studies on intersexuality. Formerly these studies were mainly morphological (Levens, 1911; Howell, 1938; Krediet, 1939) but now, since the advent of cytogenetics, they can actually be correlated with the genetic sex (Bornstein, 1967; Frans & Widmaier, 1960). According to Gerneke (1967) the latter may be determined in two ways:

(a) by direct chromosomal visualization in which the male or female sex chromosomes can be identified microscopically [Plate 1 (4)], or

(b) by nuclear sexing in which either the Barr body, representing one condensed x-chromosome, is determined in nervous tissue [Plate 2 (6 and 7)] or other cells, or the presence or absence of drumsticks in polymorphonuclear leucocytes [Plate 1 (5)] in blood smears is ascertained; their presence indicates the female sex. Although polymorph sexing, as the latter method is called, is unreliable in ruminants and pigs it is unquestionably applicable to the horse (Zaoralek, 1959) and the dog (Gerneke, de Boom & Heinichen, 1968).

The intersex state in the horse is very rare indeed. Between 1909 and 1938 Krediet (cited by Bornstein, 1967) only encountered 17 cases some of which were incompletely described. All had a penis-like clitoris [Plate 2 (8 and 9)], a pair of hypoplastic testes [Plate 1 (1)] and revealed male libido. Some regularly came on heat every 3 weeks. The penis-like phallus, directed caudally, erected when in the presence of a mare on heat. Internal sexual organs were female. Krediet (1939), however, described one horse having an ovotestis. Levens (1911) examined 15 foals with defectively developed sexual organs; they had been sired by the same stallion bred to different mares. He found spermatozoa in the better developed testicles of some cases. Bornstein (1967) described two equine intersexes both with female karyotype and the same morphology as the 17 cases above. He found that the phallus on erection discharged a slimy, watery fluid from which spermatozoa were absent. Howell (1938) also mentioned a 4 year old hermaphrodite horse in which the enlarged clitoris could be erected. Kodagali (1969), in a recent case report, described an intersex horse ("Nord Svensk" breed) morphologically similar to the case described below and to some of those described by earlier workers; it exhibited intense libido and emitted an azoospermic discharge in drops during sexual excitement. White & Farebrother (1969), in a similar case, found evidence of a bulbourethral gland but no prostate or seminal vesicles. Two subcutaneous testicles were present in the midline with small and underdeveloped seminiferous tubules, between which Leydig

cells were numerous. The genetic sex was not determined.

By means of nuclear sexing Franz & Widmaier (1960) were able to identify their gonadal male intersex as a genetic female. In an attempt to explain the phenomenon, they raised the possibility of a Klinefelter type syndrome but lacked definite chromosomal proof, as chromosome spreads were not made. Morphologically and histologically it resembled the above 17 cases but in addition a small prostate and bulbourethral gland were present. Freudenberg (1960) described a female-like intersexual foal (freemartin) born as a twin to a male. Allantochorionic anastomoses between twin horse fetuses were mentioned by Keller (1934).

In view of the rarity of the intersex state in horses and the limited number of sex determinations done, it was considered worthwhile to describe a case which we have encountered and to compare its features with those of previous cases in order to assess similarities in morphology of the sexual tract of the equine intersex and its relation to genetic sex. This condition is always associated with cryptorchidism and should not be confused with uncomplicated cryptorchidism frequently encountered in the horse.

DESCRIPTION OF CASE AND METHOD OF EXAMINATION

An Arabian filly, about 1 year old, with an enlarged penis-like clitoris was referred to the Genesiology Department of this Faculty for clitorectomy for aesthetic reasons. For her age the filly was poorly grown out. Clinical examination of the external genitalia [Plate 2 (9)] revealed a rudimentary vulva with an enlarged penis-like clitoris within the labia. The clitoris measured about 4 cm in length and was 3 cm in diameter at the glans. The dorsal commissure of the vulva was connected to the ventral part of the anus by a firm funicular raphé, measuring about 12 cm in length. Smegma was found within the fossa clitoridis. The urethral opening was situated slightly towards the dorsal half of the caudal surface of the glans [arrow, Plate 2 (9)]. Two small, hard structures, apparently testes, were palpated at the opening of the external inguinal canal. Two well developed teats were present and rudimentary mammary tissue could be palpated [Plate 2 (8)].

For ethical reasons clitorectomy was not performed but bilateral castration was carried out instead. Both testes moved freely within the inguinal canal; a very strong retractile cremaster response did not facilitate surgery.

During her stay at the clinic the filly showed no distinctive behavioural pattern.

Chromosome spreads were prepared according to a previously described method (Gerneke, 1967) in which

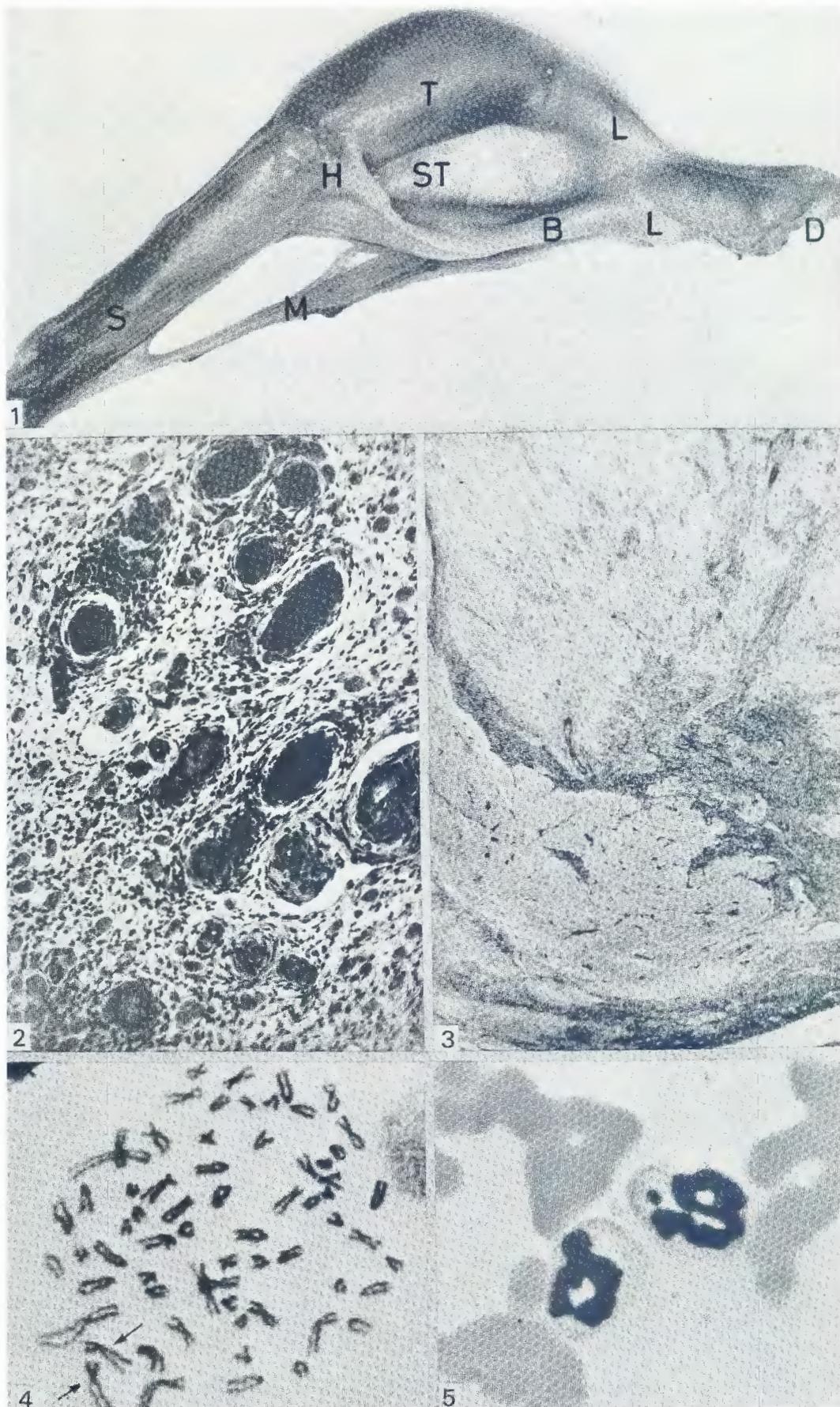


FIG. 1 The testis of the Arabian filly immediately after its removal. T, testis; H, head of epididymis; B, body of epididymis; L, tail of epididymis; D, ductus deferens cut; M, torn mesorchium; S, spermatic cord; ST, sinus testicularis

FIG. 2 The histological appearance of the testis: Numerous atrophic seminiferous tubules arranged in fibrous tissue in which pigment-rich Leydig cells are numerous



FIG. 3 The neurofibroma in which fibrous connective tissue and adipose tissue is intermingled with nerve cells and nerve fibres. A few mitotic figures revealing proliferation of nerve cells were identified

FIG. 4 A chromosome spread revealing the two x-chromosomes (at arrows) and a total of 64 chromosomes, the normal diploid number for the horse

FIG. 5 The neutrophil on the right shows a distinct "drumstick" attached to the nucleus whereas the red blood cells reveal "rouleaux" formation typical for the horse

FIG. 6 AND 7 Some nerve cells with sex-chromatin bodies of Barr distinctly seen lying against the nuclear membranes

FIG. 8 The inguinal region revealing an immature udder with two distinct teats and the penis-like clitoris in ventral view. The arrows indicate the subcutaneous position of the testes

FIG. 9 The photograph shows the penis-like clitoris in the vulva, the latter being joined to the anus by a median raphé. The arrow points to the urethral opening on the clitoris

bone marrow cells obtained by sternal puncture were the source of actively dividing cells.

Polymorph sexing was done on blood smears stained with Giemsa solution.

When examined about a year after castration, the animal had grown out well with a distinct feminine habitus. The labia of the vulva, although shorter in length than in a normal mare, were now more closely opposed, and slightly better defined than before. The penis-like structure had not changed appreciably in size. In relation to the normal growth of the body and surrounding structures, however, it had become smaller and lay concealed within the vestibulum. On urination a well defined stream instead of the normal female "gush" was still very distinctive. After urination the labia closed satisfactorily. Mammary and teat development was similar to that which would be expected in a 17 month old filly.

PATHOLOGICAL OBSERVATIONS

Both testes were very small (diameter 1.5 cm, length 2.5 cm) and compact [Plate 1 (1)], of a dark-brown colour and consisted mostly of immature and hypoplastic seminiferous tubules [Plate 1 (2)]. The latter were lined by Sertoli cells amongst which occasional degenerating cells could be identified as degenerating spermatogonia. No other indications of spermatogenesis were encountered. Around some tubules there was concentric fibrosis while in others the Sertoli cell epithelium was thrown into folds. The interstitial connective tissue was increased in amount and contained numerous large interstitial cells filled with a brown coarsely granular pigment assumed to be lipofuscin. They resembled Leydig cells in size and shape. A similar pigment was encountered in the lining cells of the numerous lymphatics present. An occasional artery was hyalinized.

The left testis contained a compact nodule about $\frac{1}{2}$ cm in diameter and of a white laminated appearance and hard consistency [Plate 1 (3)]. On histological examination it was diagnosed as an early neurofibroma. The numerous nerve cells present contained many Barr bodies, thus indicating the female genetic sex of the animal [Plate 2 (6 & 7)].

Immature and hypoplastic efferent and epididymal ducts were present, supported by a relatively increased amount of fibrous tissue. No sign of any ovarian tissue was encountered. The nature of the internal sexual organs was not determined.

In the 850 neutrophils counted, 11 drumsticks [Plate 1 (5)] and 11 sessile nodules were found. Only female sex chromosomes [Plate 1 (4) arrows] were encountered in the spreads prepared from the bone marrow biopsies. All spreads encountered contained 64 chromosomes, the normal diploid number for the horse (Hsu & Benirschke, 1967).

DISCUSSION

The clinical appearance of this Arabian filly generally concurs with that of some of the previously described cases.

The chromosome counts and nuclear and polymorph sexing have quite convincingly revealed the genetic sex of this animal to be female notwithstanding the presence of male gonads. In these respects there is, as far as can be deduced from the literature, a great similarity amongst intersex states in the horse. All apparently are genetic females. Previous reports (Krediet, 1939; Levens, 1911) have thus erroneously

listed them as males. They have hypoplastic male gonads or even an ovotestis (Krediet, 1939), a penis-like clitoris and male libido (Bornstein, 1967; Frans & Widmaier, 1960). It is thus a condition which very much resembles that occurring in pigs (Gerneke, 1967): intersex pigs are genetic females, usually with male gonads, although an occasional ovotestis can be found in some. They often reveal male libido. As the condition in pigs has been shown to be governed by a recessive gene (Johnston, Zeller & Cantwell, 1958) it may be presumed also to be the case in the horse. A recessive gene as the primary aetiological factor in the horse is suggested by the fact that inbreeding increases the incidence of intersexes in horses.

Because of the genetic sex and position of the testes it may be expected that typical cases will always be sterile. As in the case of the intersex pig, complete spermatogenesis cannot normally take place in an animal with a femal genetic constitution; regression of spermatogonia must occur. Levens (1911), however, found spermatozoa in the better developed testicles of some cases but he did not mention the degree of their development. As no spermatozoa have so far been seen in the droplet discharge, it must be assumed that they eventually undergo regression, being unable to reach maturity in a female genetic environment. Aberrant morphology and higher abdominal temperatures are contributory factors.

The presence of the neurofibroma is an interesting feature as it represents continued growth of nervous tissue in the absence of tissue to be innervated—giving thereby origin to the concentric arrangement. Krediet (cited by Bornstein, 1967) has also reported finding nodules in the testis of a hermaphrodite horse, but he did not present the histological picture. The effect of age on these nodules has not been studied.

With suppressed gonadal development, interstitial cells, normally present in the foetus in great numbers, are also suppressed, giving rise to the large, pigment-containing cells with pycnotic nuclei. The presence of such large, lipofuscin-filled cells are suggestive of foetal testicular degeneration. It is doubtful whether the interstitial cells in our case could ever have reached the massive degree of development that is normally present in the foetus—regressive changes could be expected to be preponderant.

The beneficial effect of castration on the subsequent feminine development may have been due to the absence of the suppressing influence that any male hormone produced by the testes would have had. The earlier the castration, therefore, the greater the expected aesthetic effect thereof.

SUMMARY

A brief review of the literature on intersexuality in the horse is given with a description of an intersex encountered in the Arabian breed. All intersexes of which the genotype has so far been described, have been found to be genetic females, notwithstanding the presence of male gonads. Polymorph sexing, in which drumsticks are found in polymorphs of the female, is an ideal and rapid method for determining the genetic sex in doubtful cases. Castration of the intersex resulted in an improved feminine appearance.

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