H-Y antigen in a fertile XY female horse*

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Summary. The presence of significantly reduced levels of H-Y antigen in the blood of an XY mare is consistent with the view that H-Y genes comprise a system of testis determinants. Loss or suppression of a critical portion of H-Y genes and sub-threshold expression of H-Y antigen could account for a failure of testicular differentiation, thereby allowing a measure of ovarian development in an XY embryo.

Introduction

In the horse (Equus caballus), as in man, the Y-chromosome determines maleness, presumably by causing the undifferentiated embryonic gonad to become a testis rather than an ovary. Thus embryos with two X chromosomes become females, and embryos with an X and a Y become males. Female horses with an XY karyotype have been reported, but these were infertile and in general resembled females with the XO karyotype (bearing small gonads and small uteri) (Chandley et al., 1975).

There is now a body of evidence indicating that it is not the Y chromosome per se that induces the primordial mammalian gonad to differentiate as a testis, but H-Y antigen, a serologically detectable molecule (or group of molecules) whose presence on the cell surface is determined by genes situated in the pericentric region of the Y chromosome (Wachtel & Ohno, 1979). Female development in the XY embryo could therefore represent deletion or inactivation of these genes, and indeed fertile XY females of the wood lemming (Myopus schisticolor) have been shown to be uniformly H-Y− (H-Y antigen negative) in serological tests (Wachtel et al., 1976). Here we describe a case of XY sex reversal in a fertile XY female horse that has retained an apparent, albeit reduced, H-Y+ phenotype.

Materials and Methods

The 5-year-old mare was referred to the University of Missouri Equine Center in March 1977 after a history of infertility during the previous (1976) breeding season. Rectal examination revealed small left and right ovaries (both approximately 2 × 2 cm). On palpation the uterus appeared underdeveloped; a uterine biopsy was unremarkable. Despite the small ovaries and underdeveloped uterus, the mare delivered a normal XX filly 1 year later. Whole blood was obtained from the mare before and after the birth of the filly. Cytogenetic studies revealed a male karyotype (64,XY). One hundred (100) metaphase spreads were examined. All were unequivocally XY, and in all cases the Y-chromosome appeared to be intact. Cells cultured from a skin biopsy were also 64,XY.

Tests for H-Y antigen were performed according to the procedures described by Goldberg, Boyse, Bennett, Scheid & Carswell (1971). Mouse H-Y antisera were selected, pooled and divided into four parts. One part was untreated and the other parts were absorbed with 20 × 10⁶

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white blood cells from normal stallions, normal mares and the XY mare. Each part was then incubated with mouse spermatozoa and rabbit serum (complement source). Spermatozoa reacting with the H-Y antibodies were killed. Dead spermatozoa were identified with trypan blue dye. Positive absorption of H-Y antibodies (indicating presence of H-Y antigen on absorbing white blood cells) was manifested as a fall in sperm cytotoxic titre.

Results

H-Y antigen was present in normal stallions but not in normal mares (Text-fig. 1). The cells of the XY female absorbed significantly more H-Y antibody than a corresponding number of cells from normal XX females ($P < 0.02$) but significantly less H-Y antibody than a corresponding number of cells from normal XY males ($P < 0.05$, two tails, as determined by the Mann–Whitney U-test). The results indicate that H-Y antigen is present in the cells of the XY mare, but not to the same extent as in cells of the normal stallion.

![Text-fig. 1. Cytotoxicity test on mouse spermatozoa with mouse H-Y antiserum absorbed with blood leucocytes from normal stallions and mares and from the XY mare. Unabsorbed denotes unabsorbed H-Y antiserum; Abs denotes absorption with cells of the indicated sex; C denotes control (antiserum omitted, complement included). The two values for C represent maximum and minimum readings above which sperm cell death is attributable to the cytotoxic action of H-Y antibody and complement. Each point is an average of values from 6 separate tests. Suspensions were read as coded samples.]

Discussion

The development of functional ovaries in the presence of ‘testis-determining’ H-Y antigen could be representative of: (1) hermaphroditism, (2) receptor defect, (3) cryptic mosaicism or (4) loss of a quorum of H-Y genes.

(1) Among a family of cocker spaniels, Selden, Wachtel, Koo, Haskins & Patterson (1978) found H-Y antigen in a fertile ‘female’. The bitch was actually a true hermaphrodite with bilateral ovotestes, but so far there is no direct evidence of testicular differentiation in the XY mare.

(2) Evidently testicular differentiation depends on the interaction of disseminated H-Y
antigen with its gonad-specific receptor (Muller, Aschmoneit, Zenzes & Wolf, 1978; Nagai, Ciccarese & Ohno, 1979). Failure of H-Y antigen to engage its receptor could thus account for failure of testicular differentiation in any XY mammal with the H-Y+ blood phenotype; subsequent development would depend, in part, on the ability of the gonadal primordium to organize an ovary in the absence of a second X-chromosome.

In addition to its gonad-specific receptor, H-Y antigen may utilize a ubiquitous non-specific 'receptor' consisting of β2-microglobulin in association with antigens of the major histo-compatibility complex (β2m-MHC), hence its expression in all tissues (Ohno, 1979). To the extent that this stable membrane anchorage site contributes to organogenesis in general, differentiation could be impeded by abnormal binding affinities of an inducer for the products of a particular MHC haplotype. It is open to question whether abnormal display of H-Y antigen in somatic tissues might reflect such a condition.

(3) Cytological investigation of an XY woman with H-Y+ blood cells revealed an XO cell line in fibroblasts cultured from one of the dysgenetic gonads (Wachtel, 1977). Accordingly, the reduced amount of H-Y antigen in the XY mare could reflect loss of H-Y genes from some of her cells. From this standpoint the gonads of the mare could have developed from a primordium containing XX or XO cells, but it is not clear how this would be related to the intermediate expression of H-Y in blood, because no XX or XO cells were detected in that tissue.

(4) In the apparent absence of the Y chromosome, XX males of mouse and man have been shown to express less H-Y antigen than is expressed by normal XY males (Wachtel & Ohno, 1979). This indicates that certain subnormal levels of H-Y antigen expression are compatible with successful organogenesis of the testis. On the other hand, low levels of H-Y antigen expression need not interfere with successful organogenesis of the ovary. Since H-Y+ phenotype and testes are inherited as an autosomal recessive trait by P/P ('poll') XX male goats, mothers of the XX males are obligate H-Y+ heterozygotes (Wachtel, Basur & Koo, 1978). Thus there must exist a threshold level of H-Y antigen above which testicular development is induced, and below which it is not.

It has been proposed that Y-situated testis determinants comprise a family of H-Y genes which may code for a family of H-Y molecules (possibly with related but discrete specificities) (Wachtel & Ohno, 1979). According to that scheme, mutational suppression or loss of a critical moiety from the Y could account for a failure of testicular differentiation; under conditions favouring feminization of the primordial gonad, ovarian differentiation would prevail. Reduced expression of H-Y antigen in the blood of a fertile mare is consistent with this scheme as the particular specificities represented, although insignificant functionally (being the products of a subcritical moiety of H-Y genes), might nevertheless be detected serologically. A reciprocal condition, inheritance of H-Y genes as part of a translocation, could account for sex reversal in XX phenotypic males and XX true hermaphrodites. And, depending on the particular portion of H-Y genes transferred, inheritance of a translocated chromosome could generate a recessive mode of male inheritance in which one might expect H-Y antigens in the occasional XX mother of an XX male (de la Chapelle, Koo & Wachtel, 1978).

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References


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