Fetal Ovotestes in XX $\rightleftharpoons$ XY Chimeric Mice Develop into Testes in Adults

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Abstract. It has been accepted that the majority of XX $\rightleftharpoons$ XY chimeric mice develop into adult males. The present study was designed to examine the gonads of XX $\rightleftharpoons$ XY chimeric mice to determine whether the co-existence of chromosomally female (XX) and male (XY) cells affects sex differentiation. Southern blot analysis using a Y chromosome specific DNA fragment as a probe revealed the sex chromosomal constitution of C57BL/6N $\rightleftharpoons$ DDD/1 chimeric mice. The histological features of the gonads in XX $\rightleftharpoons$ XY chimeric mice were observed at both fetal and adult stages. The gonads in XX $\rightleftharpoons$ XY chimeras were ovotestes at the fetal stage on 15 days *post coitum* (d.p.c.), had predominantly testicular components after 16 d.p.c., and were testes at adult stage. This observation indicates that the fetal ovotestes in XX $\rightleftharpoons$ XY chimeras develop into testes in adults. In addition, testes of some adult chimeras which were not dominantly composed of XY cells showed co-existence of gametic and agametic seminiferous tubules.

Key words: Sex differentiation, Y chromosome, Testis, Ovotestis, Chimeric mouse.

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In mammals, differentiation of the genital ridge into a testis or an ovary is regulated by the presence or absence of testis-determining gene(s) on the Y chromosome (*Tdy* in mice). Once a gonad is directed to develop into a male pathway, it autonomously develops into a testis [1–3]. In XX $\rightleftharpoons$ XY chimeric mice, chromosomally female (XX) and male (XY) cells may co-exist from early cleavage stages onward and may be closely intermingled in every organ of the body. This provides a particu-

larly favorable opportunity to study mammalian sex differentiation [4].

It is well known that most adult XX $\rightleftharpoons$ XY chimeric mice are males [5–11]. At the fetal stage, on the contrary, ovotestes were found in half the chimeras, although their sex chromosomal constitution was not analyzed [12]. Therefore, it is essential to examine the presence of the gonadal development in fetal XX $\rightleftharpoons$ XY chimeric mice.

In this study, we produced chimeric mice between C57BL/6N and DDD/1 which showed different patterns of Restriction Fragment Length Polymorphisms (RFLPs) detected by Y chromo-

some specific DNA fragment pY353/B [13] (hereafter Y-probe). Then, we analyzed the sex chromosomal constitution of the chimeras by Southern blot analysis using Y-probe. To determine whether the co-existence of chromosomally female (XX) and male (XY) cells has some effect on sex differentiation, we examined the gonads of XX $\leftrightarrow$ XY chimeric mice at both fetal and adult stages.

Materials and Methods

Mice

C57BL/6N (hereafter B6) and ICR mice were purchased from CLEA Japan, Inc. (Tokyo, Japan). DDD/1 (hereafter DDD) mice were purchased from the Laboratory of Animal Research Center, Institute of Medical Science, The University of Tokyo.

Preparation of 8- to 16-cell embryos

To produce balanced chimeras, the developmental stage of B6 embryos was adjusted with techniques of *in vitro* fertilization. Female B6 mice were superovulated by intraperitoneally (i.p.) injection of 5 I.U. pregnant mare serum gonadotropin (PMSG) at 1630 h followed by i.p. injection of 5 I.U. human chorionic gonadotropin (hCG) 48 h later. Unfertilized, cumulus-intact eggs were recovered from oviducts at 15–16 h after the hCG injection and immersed in TYH medium [14] under mineral oil (Squibb, Princeton, N.J., U.S.A.). Spermatozoa of B6 mice were recovered from *cauda epididymis* at 14 h after the hCG injection (i.e. at 0630 h), suspended in TYH medium, and incubated for 2 h at 37°C under 5% CO₂ in air. A spermatozoa suspension was added to the medium containing egg clot (finally to 100 sperm cells/ μ l) at 16 h after the hCG injection (i.e. at 0830 h) and incubated for 6–8 h. Fertilized eggs were transferred to Whitten's medium [15] containing 100 μ M EDTA [16] and developed into 8- to 16-cell embryos at 72 h after the hCG injection.

Female DDD mice were superovulated by i.p. injection of 5 I.U. PMSG at 1830 h followed by i.p. injection of 5 I.U. hCG 48 h later. These females were mated with DDD males. Eight- to 16-cell DDD embryos were collected from the oviducts and uteri at 68–70 h after hCG injection.

Production of B6 $\leftrightarrow$ DDD chimeras

Chimeras were produced by aggregation of 8- to 16-cell embryos according to the method previously described by Azuma *et al.* [17]. The 8- to 16-cell embryos were aggregated with 50 μ g/ml of phytohemagglutinin (PHA-P, Difco, Detroit, MI, U.S.A.) in Whitten's medium after removal of *zona pellucida* with 0.5% pronase E (Kaken Chem., Tokyo, Japan) in bovine serum albumin (BSA)-free Whitten's medium. The aggregated embryos were incubated overnight and then transferred into the uterus of 3 day *post coitum* (d.p.c.) pseudopregnant ICR female mice. The day when a vaginal plug was found in the ICR recipients was regarded as 1 d.p.c.

At the stages of sex differentiation, on 14, 15, and 16 d.p.c., the recipient mothers were sacrificed by cervical dislocation, and fetal chimeras were surgically excised. Fetal mice were judged as chimeric by eye pigmentation. The cranial part of the fetus was used for DNA preparation. The caudal part including a gonad was used for light microscopic observation.

Adult chimeras, identified by coat color, were sacrificed at the age of 6 weeks or 6 months by cervical dislocation. Skin samples were used for DNA preparation, and the gonads were used for light microscopy.

Southern blot analysis for sex chromosomal constitution

Fetal and adult DNA samples were prepared according to the method described by Hogan *et al.* [18]. Each sample of DNA (5 μ g) was digested with *Hind*III (Takara, Kyoto, Japan), separated in 0.7% agarose gel (Bethesda Research Laboratories, Gaithersburg, MD, U.S.A.), and transferred onto a nylon membrane (Hybond-N, Amersham, Buckinghamshire, U.K.). Membranes were fixed under U.V. irradiation and prehybridized in 0.5% sodium dodecyl sulfate (SDS), 10% dextran sulfate, 0.01 M wEDTA, 6XSSC (0.9 M NaCl and 90 mM sodium citrate), 0.1% sodium pyrophosphate, 100 μ g/ml of salmon sperm DNA, and 5X Denhardt's solution (0.1% Ficoll, 0.1% polyvinylpyrrolidone, 0.1% BSA) for 2 h at 68°C. Probe labeling with α -³²PdCTP (NEG-013H, NEN Research Products, Wilmington, DE, U.S.A.) was made by random priming labeling kit (Boehringer Mannheim, Mannheim, Germany). The membranes were hy-

bridized overnight at 68C, washed in 0.1X SSC-0.25% SDS-0.1% sodium pyrophosphate at 68C, and exposed to X-ray film

The sex chromosomal constitution of B6 $\leftrightarrow$ DDD chimeric mice was judged by the presence or absence of the B6 Y chromosome specific 8.6 kb fragment and the DDD Y chromosome specific 10.5 kb fragment. Chimeras with both fragments were regarded as B6(XY) $\leftrightarrow$ DDD(XY). The chimeras with B6 Y chromosome specific 8.6 kb fragment but without 10.5 kb were considered as B6(XY) $\leftrightarrow$ DDD(XX). Those with the DDD Y chromosome specific 10.5 kb fragment but without 8.6 kb were B6(XX) $\leftrightarrow$ DDD(XY), and the chimeras without any hybridization signals were regarded as B6(XX) $\leftrightarrow$ DDD(XX).

Estimation of the ratio of XX to XY of the chimeras

To clarify the chimerism, the relative intensity of the hybridization signal by Southern blot analysis was judged by naked eye according to the following codes:

XX>XY More than 70% of the sample was XX

XX=XY 30–70% of the sample was XX

XX<XY Less than 30% of the sample was XX

Light microscopy

The caudal part of fetuses and the gonads of adults were fixed in Bouin's solution overnight, dehydrated in graded ethanol, and embedded in paraffin using routine procedures. They were sectioned at 5 μ m. Fetal sections were stained with hematoxylin and eosin (H-E). Adult sections were stained with periodic acid Schiff (PAS) and hematoxylin to identify spermatids.

To distinguish spermatogenic cells from somatic testicular cells, some adult sections were stained with the lectin; *Griffonia simplicifolia* agglutinin-II (GS-II; specificity for N-acetyl-D-glucosamine). GS-II is useful as a specific marker for spermatocytes, spermatids, and spermatozoa in mouse testis (unpublished data). Deparaffinized sections were rehydrated, first treated with 1% BSA in 10 mM phosphate-buffered saline (PBS) pH 7.2 and then incubated with 25 μ g/ml of biotinyl GS-II (Vector, Burlingame, CA, U.S.A.) in 0.1% BSA-PBS for 30 min. After a rinse with PBS, the sections were incubated with avidin-biotin complex (ABC) conjugated with peroxidase (Vector) for 30 min. After washing with PBS, they were immersed in 3,3'-diaminobenzidine (DAB)-H₂O₂ (0.005%) for 10 min, rinsed with distilled water, and counterstained with hematoxylin. They were then mounted and examined under a microscope.

Results

Sex chromosomal analysis in B6 $\leftrightarrow$ DDD chimeric mice

Twenty-five fetal and 19 adult chimeric mice were observed. All 4 combinations of sex chromosomal constitution, B6(XY) $\leftrightarrow$ DDD(XY), B6(XX) $\leftrightarrow$ DDD(XY), B6(XY) $\leftrightarrow$ DDD(XX), and B6(XX) $\leftrightarrow$ DDD(XX), were identified by Southern blot analysis using the pY353/B DNA fragment as a probe (Table 1).

Figure 1 shows the RFLPs patterns in chimeric mice. In the first lane from the left, representing a B6 male (XY) as a control, the 8.6 kb and 9.4 kb fragments were recognized. In the second lane, rep-

Table 1. Generation of B6 $\leftrightarrow$ DDD chimeras

	Fetuses	Offsprings	
		6 weeks	6 months
Total number recovered	44	13	23*
Number of chimeras according to genotypes	25	9	10
B6(XX) $\leftrightarrow$ DDD(XX)	6	0	2
B6(XX) $\leftrightarrow$ DDD(XY)	5	2	1
B6(XY) $\leftrightarrow$ DDD(XX)	7	1	4
B6(XY) $\leftrightarrow$ DDD(XY)	7	6	3

* Although three out of 23 mice recovered were chimeric on the basis of coat color, their genotypes could not be determined by Southern blot analysis.

Fig. 1. An autoradiogram of DNA samples from B6 $\leftrightarrow$ DDD chimeras after digestion with *Hind*III and hybridization with Y-probe. In the first lane from left, representing B6 male (XY) as a control, the 8.6 kb and 9.4 kb fragments are recognized. In the second lane, representing DDD male (XY) as a control, the 10.5 kb and 9.4 kb fragments are recognized. In lane 3, the B6 Y chromosome specific 8.6 kb fragment is detected, while the DDD Y chromosome specific 10.5 kb fragment is not detected. The constitution of sex chromosome in this case is determined as B6(XY) $\leftrightarrow$ DDD(XX). Lane 4 showed both the 8.6 kb and 10.5 kb fragments which indicates that the constitution of sex chromosome is B6(XY) $\leftrightarrow$ DDD(XY). In lane 5, hybridization signals are not detected and the constitution of sex chromosome was determined as B6(XX) $\leftrightarrow$ DDD(XX).

representing a DDD male (XY) as a control, the 10.5 kb and 9.4 kb fragments were recognized. In lane 3, the B6 Y chromosome specific 8.6 kb fragment showed roughly half as intense a signal as the B6 XY control, while the DDD Y chromosome specific 10.5 kb fragment was not detected. The sex chromosome constitution in this case was B6(XY) $\leftrightarrow$ DDD(XX) with an equal number of XX and XY cells. Lane 4 showed both the 8.6 kb and 10.5 kb fragments, which indicates that the constitution of sex chromosome is B6(XY) $\leftrightarrow$ DDD(XY). In lane 5, no hybridization signals were detected, and the constitution of sex chromosome was classified as B6(XX) $\leftrightarrow$ DDD(XX).

Light microscopic observation of gonads in fetal B6 $\leftrightarrow$ DDD chimeric mice

Gonadal differentiation was evaluated by the existence of testicular cords. Testicular components were defined as the cord-structure composed of supporting (Sertoli) cells and germ cells; ovarian components were defined as the lack of testicular cord-structures. The characteristics of fetal gonads are summarized in Table 2.

In the gonads of XY $\leftrightarrow$ XY chimeric mice on 14 d.p.c., some distinct testicular cords were observed. As with normal testicular development, the formation of testicular cords was not complete at this stage. In two XX $\leftrightarrow$ XY chimeras from the same litter, no distinct cords were observed. Thus, the

Table 2. Characteristics of gonads of fetal XX $\leftrightarrow$ XY chimeras

Chimera No.	Stage (d.p.c.)	XX/XY ratio ^{a)}	Strain of XY embryo	Morphology of gonads	Meiosis
4	14	XX=XY	B6	undifferentiated	—
7	14	XX=XY	B6	undifferentiated	—
13	15	XX<XY	B6	ovo<tes ^{b)}	—
25	15	XX<XY	B6	ovo<tes	—
22	15	XX<XY	DDD	ovo<tes	—
16	15	XX<XY	B6	ovo=tes	—
11	15	XX>XY	DDD	ovo>tes	—
24	15	XX>XY	DDD	ovo>tes	—
31	16	XX<XY	DDD	testis	—
27	16	XX=XY	B6	testis	+
33	16	XX<XY	B6	ovo<tes	+
30	16	XX>XY	DDD	ovo=tes	+

^{a)} The sample of DNA was prepared from the cranial part of a body. The relative intensity of hybridization signal was judged with the naked eye according to the code described in Materials and Methods. ^{b)} Ovotestes were classified into three types according to the following code: ovo>tes, More than 70% of a gonad was composed of ovarian components; ovo=tes, 30–70% of a gonad was composed of ovarian components; ovo<tes, Less than 30% of a gonad was composed of ovarian components.

Fig. 2. Sagittal section of a testis in $XY \rightleftharpoons XY$ chimera No. 20 on 15 d.p.c. The testis forms distinct testicular cords (C) and contains a well-developed interstitial region (I). $\times 120$.

Fig. 3. Sagittal section of an ovotestis in $XX \rightleftharpoons XY$ chimera No. 16, which is predominantly composed of XY cells ($XX < XY$), on 15 d.p.c. Ovarian components (O) are present adjacent to the mesonephros (M), while testicular components (T) are along the tunica albuginea. $\times 120$.

gonadal differentiation of $XX \rightleftharpoons XY$ chimeric mice was difficult to determine, and gonads of $XX \rightleftharpoons XY$ chimeras on 14 d.p.c. were judged as an undifferentiated state.

On 15 d.p.c., the gonads of $XY \rightleftharpoons XY$ chimeras were oval shaped with complete formation of testicular cords that were surrounded with a well-developed interstitial region. They were judged as testes (Fig. 2). On the other hand, the gonads of $XX \rightleftharpoons XX$ chimeras at the same stage were flattened in shape and lacked the cord structure. Therefore, they were judged as ovaries. In the gonads of $XY \rightleftharpoons XY$ and $XX \rightleftharpoons XX$ chimeras, no difference was observed compared with the normal development of gonads. The gonads of $XX \rightleftharpoons XY$ chimeras were, however, different from normal ones. The gonads of $XX \rightleftharpoons XY$ chimeric mice No. 13, 22, 25, which were predominantly composed of XY cells ($XX < XY$), were mainly composed of round testicular cords and interstitial cells in the sagittal section

but were partly composed of an irregularly shaped mass of germ cells and somatic cells in an ovarian fashion. They were judged as ovotestes with a dominant testicular region. The gonad of $XX \rightleftharpoons XY$ chimera No. 16, which was predominantly composed of XY cells, had an ovotestis with ovarian components adjacent to the mesonephros and testicular components along the tunica albuginea (Fig. 3). In this case, the gonads were judged as an ovotestis equally occupied with testicular and ovarian regions. The gonads of $XX \rightleftharpoons XY$ chimeras No. 11 and No. 24, which were predominantly XX, were flattened in shape and mainly occupied with an ovarian cell mass, whereas some testicular cords were observed. They were judged as ovotestes with a dominant ovarian region.

On 16 d.p.c., most of the germ cells in the ovary of an $XX \rightleftharpoons XX$ chimera entered meiosis (Fig. 4). Strikingly abnormal features were observed in the

Fig. 4. Sagittal section of an ovary in XX $\leftrightarrow$ XX chimera No. 29 on 16 d.p.c. No clearly defined testicular cord is present. Most germ cells are in meiotic prophase. $\times 120$.

Fig. 5. Sagittal section of an abnormal testis in XX $\leftrightarrow$ XY chimera No. 27 on 16 d.p.c. Some germ cells within the testicular cords are in meiotic prophase in a female fashion (arrow). $\times 120$.

gonads of XX $\leftrightarrow$ XY chimeras at this stage. The gonad of XX $\leftrightarrow$ XY chimera No. 27 had an abnormal testis. Some of the germ cells within the testicular cords entered meiosis in a female-like fashion (Fig. 5). The gonads of XX $\leftrightarrow$ XY chimeras No. 30 and 33 were ovotestes with both testicular and meiotic ovarian regions (Fig. 6). However, the gonad of XX $\leftrightarrow$ XY chimera No. 31, which was composed mainly of XY cells by Southern blot analysis, was a normal testis without any ovarian components.

Light microscopic observation of gonads in adult B6 $\leftrightarrow$ DDD chimeric mice

All eight XX $\leftrightarrow$ XY adult chimeras had testes instead of ovotestes. Testes of five XX $\leftrightarrow$ XY chimeras showed a spermatogenic abnormality. The characteristics of adult gonads in XX $\leftrightarrow$ XY chimeras were summarized in Table 3.

At 6 weeks, all XY $\leftrightarrow$ XY chimeric mice and No. 114 XX $\leftrightarrow$ XY had normal testes. In the testes of No.

115 and 120 XX $\leftrightarrow$ XY chimeric mice, however, a spermatogenic failure was observed. Some cords were completely devoid of germ cells and were small in diameter. In some seminiferous tubules, clusters of Sertoli cells were sloughed off into the lumen. In some tubules, round spermatids were sloughed off.

At 6 months, the testes of XX $\leftrightarrow$ XY chimeras of No. 110 and 112, which were mainly composed of XX cells, suffered a severe atrophic change. A large part of the testes was occupied with agametic seminiferous tubules, and the interstitial region was hyperplastic throughout the testes (Fig. 7). Although some germ cells still remained to be degenerated in parts of agametic seminiferous tubules, most of agametic ones were small in diameter due to the lack of germ cells. Moreover, most of agametic tubules contained whorls of Sertoli cells and showed vacuolation in varying degrees (Fig. 8).

Discussion

Sex chromosomal analysis is the first step in the study of sex differentiation of chimeric mice. The sex chromosomal constitution of chimeric mice has been analyzed using various methods: cytogenetic techniques combined with a translocation marker chromosome [19], electrophoretic polymorphisms of X chromosome linked isozyme [10], and *in situ* DNA-DNA hybridization with a Y chromosome specific DNA fragment [20]. In the present study, Southern blot analysis with Y-probe could determine all four patterns of sex chromosomal constitution {B6(XY) $\leftrightarrow$ DDD(XY), B6(XY) $\leftrightarrow$ DDD(XX), B6(XX) $\leftrightarrow$ DDD(XY) and B6(XX) $\leftrightarrow$ DDD(XX)} in B6 $\leftrightarrow$ DDD chimeric mice.

In this study, XX $\leftrightarrow$ XY chimeras had ovotestes at fetal stage 15 d.p.c., whereas XX $\leftrightarrow$ XY chimeras had testes at adult stage. This result indicates that the testes in adult XX $\leftrightarrow$ XY chimeric mice develop from fetal ovotestes.

In XX $\leftrightarrow$ XY chimeras on 15 d.p.c., the ratio of ovarian to testicular components in gonads was consistent with the ratio of XX to XY cells, except for No. 16 (Table 2). Provided that the ratio of XX to XY cells in the cranial part of the body reflects the ratio in the gonads themselves, this results may suggest that ovarian components are mainly composed of XX cells while testicular ones are composed mainly of XY cells. Detailed investigation is required to define the relationship between gonad morphology and the ratio of XX to XY cells in the gonads of XX $\leftrightarrow$ XY chimeric mice.

Fig. 6. Sagittal section of an ovotestis in XX $\leftrightarrow$ XY chimera No. 30 on 16 d.p.c., showing both testicular region (T) and ovarian region (O). The majority of germ cells in the ovarian region is in meiotic prophase (arrow). $\times 120$.

Table 3. Characteristics of gonads of adult XX $\leftrightarrow$ XY chimeras

Chimera No.	Age at autopsy	XX/XY ratio ^{a)}	Strain of XY	Testicular weight ^{b)} (mg)	Morphology ^{d)}
114	6 weeks	XX=XY	DDD	98	testis
120	6 weeks	XX=XY	B6	66	agametic<gametic
115	6 weeks	XX=XY	DDD	90	agametic<gametic
106	6 months	XX<XY	DDD	NT ^{c)}	testis
103	6 months	XX=XY	B6	NT	testis
105	6 months	XX>XY	B6	NT	agametic<gametic
112	6 months	XX>XY	B6	38	agametic=gametic
110	6 months	XX>XY	B6	24	agametic=gametic

^{a)} The sample of DNA was prepared from skin. ^{b)} Mean weight of XY $\leftrightarrow$ XY chimera testes on 6 weeks was 95 mg.

^{c)} Not tested. ^{d)} Adult XX $\leftrightarrow$ XY chimeras had testes. Testes were classified into three categories according to the following code: testis, Normal; agametic<gametic, Less than 30% of a testis was composed of agametic region; agametic=gametic, 30–70% of a testis was composed of agametic region.

Fig. 7. Section of a testis in adult $XX \rightleftharpoons XY$ chimera No. 110, which is mainly composed of XX cells ($XX > XY$), at six months. PAS staining, a specific marker for the acrosome over the spermatid head, shows co-existence of gametic (G) and agametic (A) seminiferous tubules. Most of the agametic cords are completely devoid of germ cells, are small in diameter. The interstitial region is hyperplastic throughout the testis. $\times 40$.

Fig. 8. Higher magnification of the testis in the adult $XX \rightleftharpoons XY$ chimera No. 110, shown in Fig. 7. The agametic cords contain whorls of Sertoli cells and show vacuolation at various degrees. In some agametic cords, Sertoli cell clusters are sloughed off into the lumen (S). In some, germ cells still remain to be degenerated (D). $\times 190$.

In $XX \rightleftharpoons XY$ chimeras on 16 d.p.c., the ratio of ovarian to testicular components was lower than XX to XY cells (Table 2). In gonads of adult $XX \rightleftharpoons XY$ chimeras, moreover, there were no ovarian components. This indicates that from 16 d.p.c. onward testicular components gradually predominated the gonads, and fetal ovotestes in $XX \rightleftharpoons XY$ chimeras develop into testes in adults.

The co-existence of gametic and agametic seminiferous tubules was previously reported in F1 ($C57BL \times BALB/c$) $\rightleftharpoons$ Swiss white adult chimeric mice (this work lacked sex chromosomal analysis) [21] and in 129/Ola (XY) $\rightleftharpoons$ F2 ($C57BL/6J$ Lac $\times$ CBA/Ca Lac) (XX) adult chimeric mice [20]. This change was also observed in the $XX \rightleftharpoons XY$ chimeras between B6 and DDD (Table 3). The agametic

region was restricted to a small part of the testes in 6-week-old $XX \rightleftharpoons XY$ chimeric mice No. 115 and 120, which had well-balanced chimerism. A large part of the testes was occupied with agametic tubules in 6-month-old $XX \rightleftharpoons XY$ chimeras No. 110 and 112, which were mainly composed of XX . This severer atrophic change at 6 months may be partly caused by more dominant participation of XX cells in the gonads of No. 110 and 112 compared to No. 115 and 120. The existence of degenerating germ and Sertoli cells, however, indicates the expansion of the atrophic area in adults.

The formation of seminiferous tubules devoid of germ cells in adult $XX \rightleftharpoons XY$ chimeras may be partly involved with meiotic germ cells in (ovo)testes at the fetal stage on 16 d.p.c. (Figs. 4, 5). The meiosis

in fetal XX $\leftrightarrow$ XY chimeric mice was previously reported, and the meiotic germ cells in fetal XX $\leftrightarrow$ XY chimeras have been postulated not to participate in adult testes but to regress [22, 23, 26]. The tubules without germ cells in adult XX $\leftrightarrow$ XY chimeras may be the remnant of testicular cords with fetal meiotic germ cells.

Transformation of the ovary into a testicular cord-like structure devoid of germ cells was previously observed in fetal rat ovaries cultured in the presence of Anti-Müllerian hormone (AMH or Müllerian inhibiting substance, MIS) [24] and in transgenic XX mice expressing AMH [25]. Therefore, in XX $\leftrightarrow$ XY chimeric mice, AMH may trans-

form ovarian components in fetal ovotestes into the agametic cords in adult testes.

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