Development 107, 373-380 (1989)
Printed in Great Britain © The Company of Biologists Limited 1989

Analysis of the testes of H-Y negative XOSxr^b mice suggests that the spermatogenesis gene (*Spy*) acts during the differentiation of the A spermatogonia

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Summary

H-Y antigen negative XOSxr^b mice, like their H-Y positive XOSxr^a counterparts, have testes; but, in contrast to XOSxr^a males, XOSxr^b testes almost totally lack meiotic and postmeiotic stages of spermatogenesis. The quantitative analysis of the testes of XOSxr^b males and their XY±Sxr^b sibs, described in the present study, identified two distinct steps in this spermatogenic failure. First, there was a reduction in mitotic activity among T_1 prospermatogonia, so that approximately half the normal number of T_2 prospermatogonia were produced. Second, there was a dramatic decrease in the number of A_3 and A_4 spermatogonia and no Intermediate or B spermatogonia. These reductions were also largely due to decreased mitotic activity, there being a shortage of A_1 and A_2 spermatogonial divisions and no divisions among A_3 or A_4 spermatogonia. Mitotic activity among the T_2 prospermatogonia and the undifferentiated A spermatogonia was normal. This means that the spermatogonial stem cells, which are a subset of the undifferentiated A spermatogonia, are unaffected in XOSxr^b mice. Sxr^b is now known to have arisen by deletion of DNA from Sxr^a. It is clear from the present findings that a gene (or genes) present in the deleted DNA plays a major role in the survival and proliferation of the differentiating A spermatogonia.

Key words: mice, spermatogenesis, sex reversal, H-Y antigen negative, *Spy*.

Introduction

Sex-reversed (Sxr) is a factor that causes an inherited form of sex-reversal, such that XX and XO mice carrying Sxr develop as phenotypic males (Cattanach *et al.* 1971). In 1982 evidence was obtained that Sxr was in fact an extra copy of the testis-determining region of the mouse Y chromosome which had become located distal to the pairing and exchange region of the Y, so that it regularly crossed over onto the X chromosome during male meiosis (Singh and Jones, 1982; Evans *et al.* 1982; Burgoyne, 1982; Eicher, 1982; Hansmann, 1982).

In addition to testis-determining information, the original Sxr (now termed Sxr^a – McLaren *et al.* 1988) included information required for H-Y antigen expression (Bennett *et al.* 1977). In 1984 McLaren *et al.* discovered a variant of Sxr^a (originally designated Sxr', but now Sxr^b) that retained the testis-determining information, but which had lost the Y-chromosomal gene required for transplantation H-Y antigen expression (Simpson *et al.* 1981, 1986). This finding, recently confirmed by the separation of TDF from H-Y loci in humans (Simpson *et al.* 1987), negated the hypothesis of Wachtel *et al.* (1975) that H-Y antigen was the primary testis determinant (at least in so far as the transplantation H-Y antigen is concerned).

XXSxr males differ genetically from normal males not only in that they lack most of the Y chromosome, but also in having two X chromosomes. The presence of two X chromosomes is incompatible with male germ cell survival beyond the perinatal period (reviewed by McLaren, 1983) so that in order to investigate the effects of the Y-chromosomal deficiencies associated with Sxr^a and Sxr^b, it is necessary to produce Sxr males with single X chromosomes.

XOSxr^a mice were first described by Cattanach *et al.* (1971) and although all stages of spermatogenesis are represented in their testes, the later stages are severely depleted so that the testes are small and the mice are sterile. The majority of the spermatids are in fact diploid and the few sperm produced, whether haploid or diploid, are abnormal (Levy and Burgoyne, 1986*a*).

XOSxr^b mice have a more severe spermatogenic impairment with only a few germ cells reaching early meiotic prophase (Burgoyne *et al.* 1986). The XO germ cells in an XO/XY/XYY mosaic male described by Levy and Burgoyne (1986*b*) suffered a similar fate despite a normal XY Sertoli cell environment. These

findings led Burgoyne *et al.* (1986) to suggest that Sxr^a carries a spermatogenesis gene (*Spy*) that is lacking in Sxr^b, and that *Spy* is expressed cell-autonomously in the germ line. Recent studies have shown that the Sxr^b variant arose by deletion of DNA from Sxr^a (Bishop *et al.* 1988; Mardon *et al.* 1989).

The purpose of the present study was to define the spermatogenic block in $XOSxr^b$ mice by a quantitative analysis of germ cells in the two weeks following birth (when the block first becomes apparent) and from this deduce the function of *Spy*. During the course of the experiment, the finding of a significant body weight difference between $XOSxr^b$ and $XOSxr^a$ mice supported a hypothesis, under separate study, that a growth and development gene (dubbed *Gdy*) may also be deleted.

Materials and methods

Mice

$XYSxr^b$ males were mated with females heterozygous for the inversion In(X)1H. In(X)/X females produce some nullo-X eggs following crossing-over within the inversion (Evans and Phillips, 1975), and approximately 1 in 19 of the progeny from this cross have the $XOSxr^b$ genotype. The In(X)/X females were checked for vaginal plugs each morning, and coitus was presumed to have taken place at the midpoint of the previous dark cycle. Ages were calculated from conception, rather than birth, because it is known that the duration of pregnancy is affected by litter size. The majority of litters were born about 19½ days *post coitum* (dpc), so in what follows this is equated with the day of birth. 157 litters were bred of which 59 included $XOSxr^b$ males. Litters were processed from 19½ dpc (day of birth) through 30½ dpc (11 days *post partum*), 32½ dpc (13dpp) and 59½ dpc (40dpp).

A similar breeding cross was set up to produce $XOSxr^a$ males as controls for a possible XO effect. Data from 35 litters are included in this study. The litters were processed at 19½, 21½ through 24½, 27½, 29½, 31½ and 33½ dpc.

Body weights were recorded at autopsy. Following exclusion of 'runts' (Burgoyne *et al.* 1983*b*), litters were evaluated provided at least one XOSxr and one XY±Sxr male was present. Since a qualitative analysis suggests that XY and XYSxr testes are not significantly different during the premeiotic stages (results not shown) XY and XYSxr males were not separately identified. 52 Sxr^b and 35 Sxr^a litters finally provided data.

Karyotyping

Mitotic spreads were prepared either by dissociating liver fragments (19½ and 20½ dpc) or by flushing out bone marrow cells (21½ dpc onwards) in 0.04% colcemid in Hepes-buffered Eagle's minimal essential medium, and incubating at 32°C for 60 min (liver) or 15 min (bone marrow). Cells were then treated with 0.56% KCl for 20 min followed by five changes of 3:1 methanol:glacial acetic acid. The cells were then air-dried on slides and stained for 15 min in 2% Giemsa in pH 6.8 buffer. XOSxr males were identified by scoring at least 5 consecutive spreads with 39 chromosomes and no evidence of a Y chromosome. XY±Sxr males were identified by 40 chromosomes, with a Y recognised by size and the presence of splayed short arms (Ford, 1966).

Histology

Both testes from each male were weighed using a Cahn electrobalance, and were then retained in Bouin's fixative awaiting the results of karyotyping. Testes from XOSxr and XY±Sxr littermates were dehydrated and cleared according to standard procedures, embedded in paraffin wax, serially sectioned at 3 μm and stained with haematoxylin and eosin.

Quantitative analysis

This analysis was carried out 'blind' with respect to genotype of the mice from which the sections were taken. The sampling was one tubule cross-section from every 20th section, or every 10th section for smaller testes, such that between 25 and 35 tubule cross-sections were analysed per testis. The procedure for selecting tubules for analysis was as follows: (1) A 0.25 mm square grid (R-4 grid, Graticules Ltd, Tonbridge, Kent) was 'stuck' to the bottom of the microscope slide with a film of water and a Chalkley grid (G52, Graticules Ltd) was inserted in the eyepiece. (2) When a section was selected, the square grid was focused under low power with a ×10 objective and a square chosen at random. The central cross of the Chalkley grid was centered over the square and the section was brought back into focus. (3) The tubule cross-section adjacent to the central cross was analysed under oil immersion, provided it could be encompassed within the field of view.

This selection procedure ensures that all regions of the gonad have an equal chance of being sampled. Once a tubule was selected, all cells within the tubule cross-section were classified as to cell type except dead or dying cells which could not be classified. Sertoli cells were scored as being in interphase or division. Gonia were scored as being in interphase or division, and were also classified as to stage (i.e. T_1 prospermatogonia, T_2 prospermatogonia, undifferentiated A spermatogonia, differentiating A_1 or A_2 spermatogonia, A_3 or A_4 spermatogonia, Intermediate or B spermatogonia) using the criteria described by Clermont and Perey (1957), Oakberg (1971), Hilscher *et al.* (1974), Hilscher and Hilscher (1976), Bellve *et al.* (1977), Huckins and Oakberg (1978) and Kluin and de Rooij (1981). It was often difficult to assign divisions to specific spermatogonial stages and in these cases they were classified according to the adjacent interphase stages in the same tubule. A category existed for cells that could not be classified. This group formed less than 0.5% of germ cells scored and have been omitted from the analysis. It should be pointed out that these cell counts are crude counts, uncorrected for cell sizes and thickness of the sections.

Results

The body weight data for the Sxr^a and Sxr^b litters are given in Table 1. The best estimates for the body weights of the four genotypes ($XOSxr^a$, $XY±Sxr^a$, $XOSxr^b$, $XY±Sxr^b$) at the various ages studied are provided by the means of litter means. In order to compare the two genotypes in each cross, mean weighted differences between these genotypes and the significance of these differences have been calculated from 'within litters' as described by Burgoyne *et al.* (1983*b*). From these mean weighted differences it is clear that $XOSxr^b$ mice are underweight when compared with $XY±Sxr^b$ mice. Despite the limited number of mice at each age, the difference is significant for 5/13 age groups, and pooling across age groups (the mean weighted differences are similar throughout the age

Table 1. *Mean body weights for (A) XOSxrb and XY±Sxrb, and (B) XOSxra±Sxra mice and the estimated difference between them for the period 19½–33½ dpc*

(A)

Days *post coitum*	No. of Mice		Mean±S.E.M. body weights (g)*		Mean±S.E.M. weighted XOSxrb−XY±Sxrb difference (g)	Significance of XOSxrb−XY±Sxrb difference (*P*)
	XOSxrb	XY±Sxrb	XOSxrb	XY±Sxrb		
19½	3	5	1.570±0.03	1.693±0.08	−0.120±0.046	NS
20½	3	9	1.917±0.10	2.200±0.10	−0.284±0.070	<0.005
21½	4	12	2.065±0.15	2.175±0.10	−0.120±0.180	NS
22½	7	16	2.436±0.12	2.862±0.11	−0.496±0.160	<0.005
23½	11	14	3.297±0.24	3.645±0.30	−0.237±0.135	NS
24½	6	19	3.876±0.16	4.240±0.13	−0.347±0.197	0.05–0.025
25½	5	19	4.094±0.19	4.932±0.26	−0.842±0.108	<0.005
26½	3	8	5.130±0.66	5.500±0.79	−0.330±0.266	NS
27½	4	8	5.290±0.25	5.525±0.16	−0.324±0.144	<0.005
28½	3	7	6.385±0.11	6.500±0.10	−0.115±0.194	NS
29½	6	12	6.618±0.66	6.675±0.47	−0.219±0.284	NS
30½	4	6	7.505±0.71	8.025±0.67	−0.498±0.332	NS
32½	4	6	8.060±0.50	8.563±0.53	−0.483±0.407	NS
			Pooled mean weighted difference		−0.359±0.059	<0.005

(B)

Days *post coitum*	No. of Mice		Mean±S.E.M. body weights (g)*		Mean±S.E.M. weighted XOSxra−XY±Sxra difference (g)	Significance of XOSxra−XY±Sxra difference (*P*)
	XOSxra	XY±Sxra	XOSxra	XY±Sxra		
19½	3	6	1.467±0.04	1.450±0.01	+0.010±0.050	NS
21½	4	6	2.727±0.27	2.927±0.01	−0.241±0.103	0.05–0.025
22½	5	14	3.142±0.43	3.148±0.45	−0.003±0.102	NS
23½	4	12	3.448±0.14	3.465±0.25	+0.004±0.082	NS
24½	4	12	4.123±0.31	4.213±0.17	−0.130±0.219	NS
27½	4	10	5.868±0.40	6.273±0.54	−0.404±0.162	0.025–0.010
29½	6	17	6.658±0.45	6.305±0.32	+0.529±0.230	0.025–0.010
31½	4	15	7.918±0.38	8.285±0.47	−0.367±0.275	NS
33½	4	12	9.145±0.62	9.295±0.33	−0.092±0.157	NS
			Pooled mean weighted difference		−0.044±0.069	NS

*Mean of litter means.

range studied) gives an overall estimated weight deficit of −0.359±0.059 g ($P<0.005$). XOSxr^a mice are not significantly underweight when compared with XY±Sxr^a mice (pooled mean weighted difference= −0.044±0.069 g).

The testis weight data for the Sxr^a and Sxr^b litters are given in Table 2. XOSxr^a testes (Table 2B) are not underweight when compared with XY±Sxr^a litter mates, but XOSxr^b testes (Table 2A) are significantly underweight for 9/13 of the ages studied. Since XOSxr^b mice are underweight, this testis weight deficit could simply be a reflection of the overall reduction in body weight. The XOSxr^b testis weights were therefore corrected by dividing by individual body weight and multiplying by the mean XY±Sxr^b body weight for the relevant litters. The mean weighted XOSxr^b−XY±Sxr^b differences for these corrected testis weights are plotted in Fig. 1. XOSxr^b testes are significantly underweight by 23½ dpc and the weight deficit rapidly increases thereafter.

The reason for the reduced testis weight in XOSxr^b mice is apparent in Fig. 2, which gives the mean number of germ cells and Sertoli cells per tubule cross-section in XOSxr^b and XY±Sxr^b mice, throughout the period

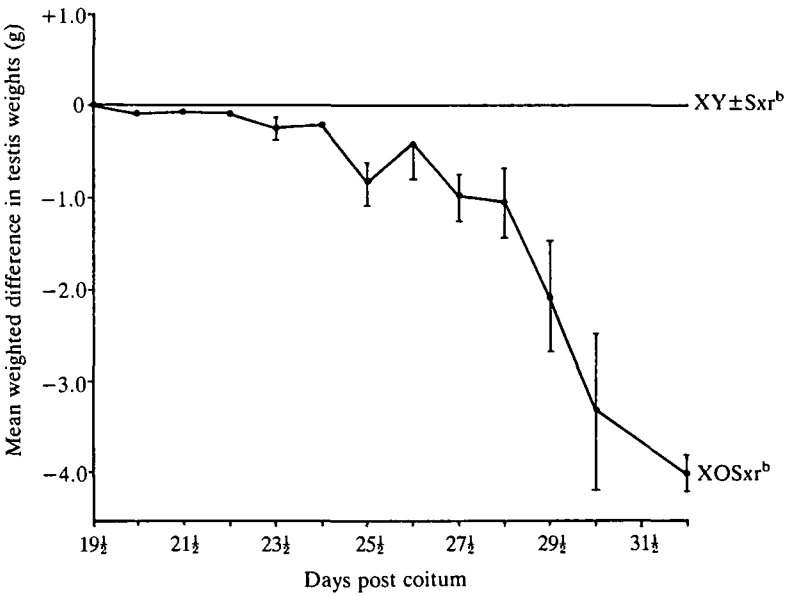

Fig. 1. Mean weighted differences in testis weights (corrected for body weights) for XOSxr^b and XY±Sxr^b mice for the period 19½–32½ dpc. Where error bars are shown the differences are significant (*t*-test, 1-tailed).

Table 2. *Mean testis weights for (A) XOSxrb and XY±Sxrb, and (B) XOSxra±Sxra mice and the estimated difference between them for the period 19½–33½ dpc*

(A)

Days *post coitum*	No. of mice		Mean±S.E.M. testis weights (mg)*		Mean±S.E.M. weighted XOSxrb−XY±Sxrb difference (mg)	Significance of XOSxrb−XY±Sxrb difference (*P*)
	XOSxrb	XY±Sxrb	XOSxrb	XY±Sxrb		
19½	3	5	0.883±0.01	0.960±0.26	−0.063±0.019	0.05–0.025
20½	3	9	0.980±0.17	1.167±0.18	−0.185±0.167	NS
21½	4	12	1.183±0.10	1.260±0.02	−0.091±0.163	NS
22½	7	16	1.440±0.10	1.782±0.17	−0.380±0.167	0.025–0.010
23½	11	14	2.190±0.14	2.622±0.20	−0.373±0.117	<0.005
24½	6	19	2.528±0.13	2.942±0.05	−0.402±0.268	NS
25½	5	19	2.734±0.18	4.148±0.22	−1.396±0.239	<0.005
26½	3	8	3.580±0.18	4.207±0.25	−0.606±0.405	NS
27½	4	8	3.708±0.27	5.003±0.23	−1.232±0.255	<0.005
28½	3	7	3.385±0.68	4.555±0.48	−1.114±0.383	0.025–0.010
29½	6	12	4.555±0.40	6.248±0.41	−1.974±0.613	<0.005
30½	4	6	4.615±0.16	7.685±1.80	−3.617±0.896	<0.005
32½	4	6	6.027±0.19	10.347±0.64	−4.356±0.293	<0.005

(B)

Days *post coitum*	No. of Mice		Mean±S.E.M. testis weights (mg)*		Mean±S.E.M. weighted XOSxra−XY±Sxra difference (mg)	Significance of XOSxra−XY±Sxra difference (*P*)
	XOSxra	XY±Sxra	XOSxra	XY±Sxra		
19½	3	6	0.837±0.02	0.757±0.08	+0.068±0.106	NS
21½	4	6	1.863±0.18	1.940±0.10	−0.097±0.091	NS
22½	5	14	2.108±0.33	2.104±0.30	−0.036±0.105	NS
23½	4	12	2.608±0.24	2.563±0.26	+0.078±0.188	NS
24½	4	12	2.818±0.22	3.030±0.27	−0.226±0.228	NS
27½	4	10	4.938±0.56	4.898±0.40	+0.059±0.156	NS
29½	6	17	6.358±0.69	5.655±0.38	+0.907±0.192	<0.005
31½	4	15	8.898±1.20	9.323±1.15	−0.435±0.620	NS
33½	4	12	12.688±2.87	12.343±1.05	+0.691±0.944	NS

* Mean of litter means.

studied. As expected, there is a marked increase in the number of germ cells per tubule cross-section in XY±Sxr^b mice, but by contrast there is no increase in XOSxr^b mice. There is no deficiency of Sertoli cells in XOSxr^b mice. Indeed the mitotic index for Sertoli cells during the period 19½–23½ days was found to be very similar in XOSxr^b mice (0.85%) and XY±Sxr^b mice (0.90%). The mitotic index for Sertoli cells drops to less than 0.3% after 24½ dpc in both genotypes. Clearly, the testis weight deficiency in XOSxr^b mice is due to germinal failure.

In view of the normal numbers of Sertoli cells in XOSxr^b mice, in the more detailed analysis of the germ cell deficiency that follows, germ cell numbers are expressed per 100 Sertoli cells, rather than per tubule cross-section.

In Fig. 3, germ cell numbers are plotted against age for the various classes of germ cells identified in the scoring procedure. The numbers of T_1 prospermatogonia are indistinguishable in XOSxr^b and XY±Sxr^b mice. However, XOSxr^b mice clearly have fewer T_2 prospermatogonia than the controls and pooling over the period 20½–24½ dpc reveals that XOSxr^b have only 39% of the control value. By contrast, XOSxr^a mice have 91% of the control value. Since T_2 prospermato-

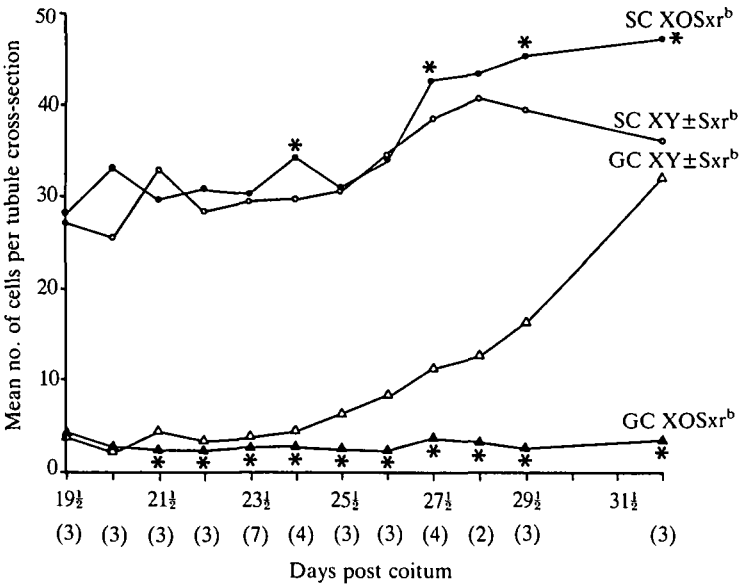

Fig. 2. Mean number of Sertoli cells (SC) and germ cells (GC) per tubule cross-section in XOSxr^b and XY±Sxr^b mice for the period 19½–32½ dpc. The numbers in parentheses are the numbers of litters scored at each age. Asterisks indicate XOSxr^b points which are significantly different from controls (*t*-test, 2-tailed). The significantly *higher* numbers of Sertoli cells in XOSxr^b tubules at 29½ and 32½ dpc is a scoring artifact: at these ages some large tubule cross-sections from the controls had to be excluded because they would not fit in the field of view, resulting in an underestimate of the numbers of Sertoli cells and germ cells for controls at these ages.

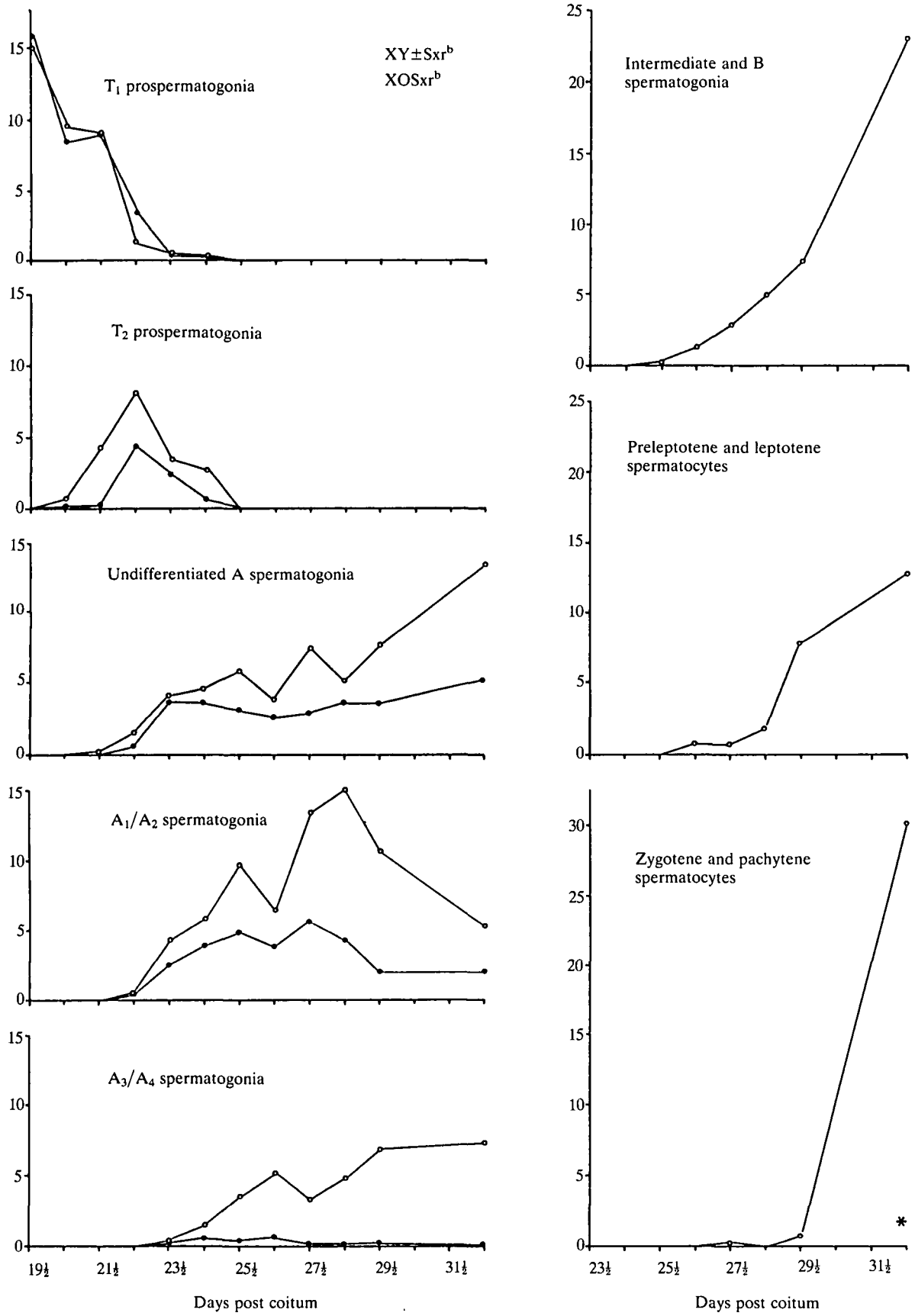

Fig. 3. Number of germ cells per 100 Sertoli cells for each germ cell stage during the period 19½–32½ dpc. The asterisk denotes occasional XOSxr^b zygotene or pachytene cells.

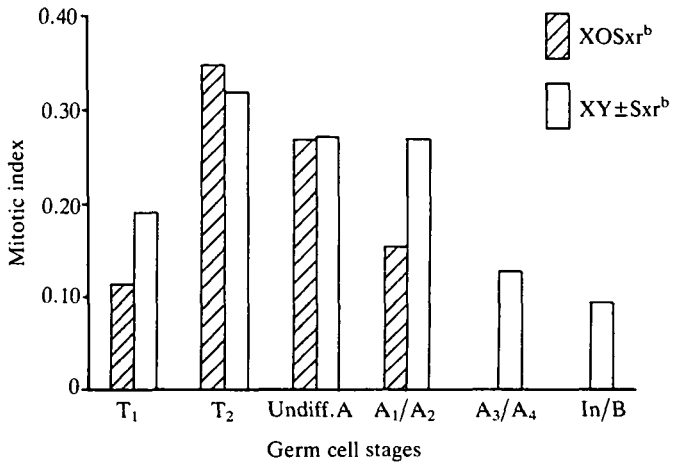

Fig. 4. Histogram showing the mitotic index according to germ cell stage of XOSxr^b and XY±Sxr^b mice.

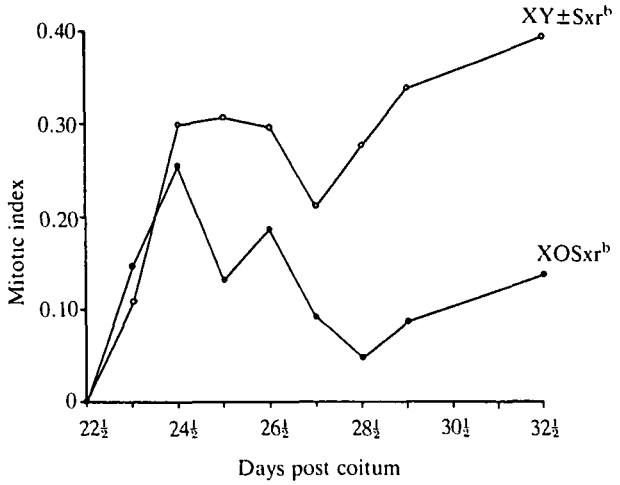

Fig. 5. Mitotic index of A$_1$/A$_2$ spermatogonia in XOSxr^b and XY±Sxr^b mice during the period 19½–32½ dpc.

gonia are assumed to be the progenitors of the undifferentiated A spermatogonia, a deficit of undifferentiated A spermatogonia is expected in XOSxr^b mice, and is indeed observed (XOSxr^b is 54% of XY±Sxr^b). Similarly, there is the expected deficit of differentiating A$_1$/A$_2$ spermatogonia (XOSxr^b is 42% of XY±Sxr^b). The number of A$_3$/A$_4$ spermatogonia, however, is reduced much more than expected (XOSxr^b is 7% of XY±Sxr^b) and there are no Intermediate or B spermatogonia.

This pattern of germ cell deficiency in XOSxr^b mice is largely accounted for by observations on mitotic index (Fig. 4). That is to say, there is a shortage of dividing T$_1$ prospermatogonia, accounting for the drop in the number of T$_2$ prospermatogonia; a reduced frequency of divisions among A$_1$/A$_2$ spermatogonia accounting for the much more severe shortage of A$_3$/A$_4$ spermatogonia; and no dividing A$_3$/A$_4$ spermatogonia accounting for the absence of In/B spermatogonia.

During the scoring procedure the gonia with the morphological characteristics of A$_1$ and A$_2$ spermatogonia were pooled, although it is assumed that they are distinct generations of spermatogonia, as in the adult. When the mitotic index of the A$_1$/A$_2$ spermatogonia is plotted against age (Fig. 5), there is no marked shortage of divisions in XOSxr^b mice until 25½ dpc, raising the possibility that it is the A$_2$ rather than the A$_1$ spermatogonia that are affected.

If A$_1$/A$_2$ spermatogonia rarely divide to give A$_3$ or A$_4$, but the undifferentiated A spermatogonia continue to divide, one might expect a 'piling up' of A$_1$/A$_2$ stages. This is not observed, implying that the cells that fail to divide are degenerating. This is supported by observations on the germ cell degeneration index (Fig. 6), which has been calculated on the assumption that all the dying cells observed were germ cells. The degeneration index is very low in XOSxr^b and XY±Sxr^b mice. Nevertheless, from 26½ days onwards XOSxr^b mice clearly have more degenerating cells than controls, which is consistent with the increased degeneration of A$_1$/A$_2$ spermatogonia. It is tempting to suggest that the increased degeneration index in

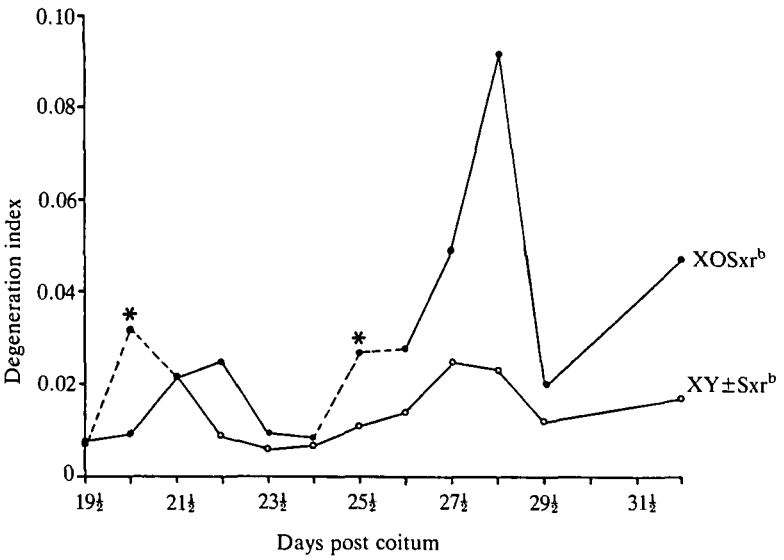

Fig. 6. The germ cell degeneration index for the period 19½–32½ dpc was calculated on the assumption that all dying cells were germ cells. The two points marked with an asterisk are artifically high, in that only one of the males at each of these points showed an elevated degeneration index.

XOSxr^b mice at 22½ days is similarly due to the death of T$_1$ prospermatogonia that failed to divide.

Although no Intermediate or B spermatogonia were scored during the quantification, very rare patches of these spermatogonia, and also of early meiotic stages, can be found in 32½ dpc and adult (59½ dpc) XOSxr^b testes. They occur without the normal hierarchy of stages, and in small patches, as if an occasional A$_3$/A$_4$ spermatogonium divides and the products proceed *via* the usual stages up to early pachytene.

Discussion

The present results show that XOSxr^b testes have normal numbers of germ cells at birth, but become

severely deficient in germ cells in the ensuing two weeks. During the same period the numbers of Sertoli cells remain normal. These findings are consistent with the view of Burgoyne *et al.* (1986) and Levy and Burgoyne (1986*b*) that the spermatogenic failure in XOSxr^b mice is due to the loss of a gene (*Spy*) that acts cell autonomously in the germ line.

The quantitative analysis of the germ cell deficiency in XOSxr^b mice revealed a reduction in mitotic activity among T_1 prospermatogonia, which resulted in a shortage of T_2 prospermatogonia, and consequently a reduced pool of undifferentiated A spermatogonia. However, mitotic activity among the undifferentiated A spermatogonia, which include the spermatogonial stem cells, was found to be normal. It was during the early differentiating spermatogonial stages that the spermatogenic block occurred, with mitotic failure leading to an almost complete absence of Intermediate and B spermatogonia and subsequent meiotic stages.

XO female mice are developmentally retarded in early pregnancy (Burgoyne *et al.* 1983*b*) and are significantly underweight postnatally (Burgoyne *et al.* 1983*a*). It was anticipated that XOSxr^b mice would also be underweight from birth, and this proved to be the case. Unexpectedly, however, the XOSxr^a mice originally included as controls for this 'XO effect' showed little, if any, postnatal weight deficit. Coincidentally, the genetic basis for the early developmental advantage of XY over XX embryos (Tsunoda *et al.* 1985; Seller and Perkins-Cole, 1987) was being investigated in this laboratory, concurrently with the present study of XOSxr^b mice, and the findings may provide an explanation for this difference in postnatal weight between XOSxr^b and XOSxr^a mice. Briefly, it was shown that the Y chromosome carries a factor that accelerates the early growth and development of XY embryos, and it appears that this factor (*Gdy*) may be present in Sxr^a (P. S. Burgoyne, S. Kalmus, E. P. Evans, K. Holland and M. J. Sutcliffe, unpublished) but deleted from Sxr^b (P. S. Burgoyne and C. E. Bishop, unpublished). Thus it may be that the 'XO effect' is ameliorated by the presence of Gdy in XOSxr^a but not XOSxr^b mice.

The deletion of Y-chromosomal material involved in the generation of Sxr^b has thus removed genetic information required for H-Y antigen expression (McLaren *et al.* 1984), for spermatogenesis (Burgoyne *et al.* 1986) and for an early acceleration of growth and development (P. S. Burgoyne *et al.* unpublished). Burgoyne *et al.* (1986) pointed out that the spermatogenesis gene (Spy) and the gene controlling H-Y expression (*Hya*) might be one and the same, and this possibility still holds. Similarly, *Gdy* may not be a separate gene from *Hya* and/or *Spy*. At the molecular level, it has been shown that *Zfy-2*, one of the Y-chromosomal copies of a gene encoding a zinc finger protein, present along with *Zfy-1* in Sxr^a, has been deleted from Sxr^b (Roberts *et al.* 1988; Mardon *et al.* 1989; Nagamine *et al.* 1989*a*). Because it is strongly expressed in testes, probably in germ cells (Mardon and Page, 1989; Nagamine *et al.* 1989*b*), it is an obvious candidate for *Spy*.

As to the function of the 'spermatogenesis gene' *Spy*, we have clearly shown that the spermatogenic failure seen in XOSxr^b mice is due to a failure of proliferation during the differentiating A spermatogonial stages, and so by definition *Spy* is important for the survival/proliferation of these spermatogonial stages. Whether the deficiency of T_1 or prospermatogonial divisions in XOSxr^b mice is also a consequence of the deletion of *Spy*, or whether it is due to the deletion of a gene separate from *Spy*, remains to be determined.

M.J.S. is very grateful to Drs W. and B. Hilscher for training in the recognition of the germ cell stages. M.J.S. is a recipient of an MRC Studentship.

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(*Accepted 19 June 1989*)