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# Rat spermatogenesis in mouse testis

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## Abstract

Recently, transplantation of mouse donor spermatogonial stem cells from a fertile testis to an infertile recipient mouse testis was described[1,2]. The donor cells established spermatogenesis in the seminiferous tubules of the host, and normal spermatozoa were produced. In the most successful transplants, the recipient mice were fertile and sired up to 80 per cent of progeny from donor cells[2]. Here we examine the feasibility of transplanting spermatogonial stem cells from other species to the mouse seminiferous tubule to generate spermatogenesis. Marked testis cells from transgenic rats were transplanted to the testes of immunodeficient mice, and in all of 10 recipient mice (in 19 of 20 testes), rat spermatogenesis occurred. Epididymides of eight mice were examined, and the three from mice with the longest transplants (≥110 days) contained rat spermatozoa with normal morphology. The generation of rat spermatogenesis in mouse testes suggests that spermatogonial stem cells of many species could be transplanted, and opens the possibility of xenogeneic spermatogenesis for other species.

Spermatogenesis is a complex process in which spermatogonial stem cells adjacent to the seminiferous tubule basal membrane of the testis undergo division, to renew the population of stem cells and to generate progeny cells that develop into spermatozoa simultaneously[3–6]. The process is thought to be regulated by the somatic Sertoli cells that line the seminiferous tubules and that support and nurture germ cells during spermatogenesis[7,8]. Unlike mammalian female germ cells, which cease dividing before birth, male spermatogonial stem cells continue to divide throughout adult life[6,9].

To extend the spermatogonial transplantation technique to other species and assess the feasibility of using the mouse as a repository of xenogeneic spermatogenesis, we transferred donor cells from the testes of fertile transgenic Sprague-Dawley rats into the testes of immunodeficient mice sterilized by busulphan treatment. We used both nude and severe

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*Note added in proof:* 'Intraluminal spermatogenesis' after transfer of male germ cells from Sprague–Dawley rats to the seminiferous tubules of busulphan-treated Long–Evans rats has recently been described[21]. Spermatogonial stem cells can now be cryopreserved, making the germ line of individual males immortal[22].

combined immunodeficient (SCID) mice as recipients, as transplantation of donor cells into immunocompetent mice (C57BL/6 × $SJLF_1$) did not result in rat spermatogenesis. Donor rats carried the *MT-lacZ* transgene[10], which was expressed in Sertoli cells and germ cells allowing them to be identified by their blue staining after incubation with X-gal (Fig. 1*a*). Recipient mice were killed between 42 and 127 days after cell transfer, and their testes examined for evidence of rat spermatogenesis. In rat, the length of time from initiation of stem cell division to formation of mature spermatozoa is 52 days, compared with 35 days in the mouse)[3,6]. Thus, in these experiments, the time available for xenogeneic sperm development was 80 to 240 per cent of the time necessary for rat spermatogenesis. To identify spermatogenesis arising from rat stem cells, recipient testes were incubated with X-gal, which results in blue staining of transgenic rat but not control rat or recipient mouse germ cells (Fig. 1*a, b*). The seminiferous tubules of busulphan-treated recipient mice do not generally contain male germ cells (Fig. 1*c*). Furthermore, any endogenous mouse germ cells would not stain blue, because they do not carry the transgene[1,2]. Mouse seminiferous tubules in which rat spermatogenesis occurred were stained blue from the earliest time point examined, indicating that rat testes cells survived isolation and transplantation and could subsequently populate mouse seminiferous tubules (Fig. 1*d*, Table 1).

To determine the efficiency of colonization of mouse seminiferous tubules by rat testis cells, microscopic sections of recipient testes were examined. In transgenic rat donor testes, Sertoli cells and germ cells expressed *β*-galactosidase and stained blue (Figs 1*a*, 2*a*). There was no staining in non-transgenic rat testes (Figs 1*b*, 2*b*) or unrepopulated immunodeficient busulphan-treated mouse testes (Figs 1*c*, 2*c*). However, areas in recipient testes colonized by transgenic donor testis cells were stained blue. As in donor rats, germ cell stages were stained (Fig. 2*d*, *e* and *f*). Mature forms of rat spermatozoa could also be identified, displaying the characterisitic long, thin rat spermatozoa head (compare Fig. 2*d*, *e* and *f* with Fig. 2*a* and *b*). Although the donor cell population contained both Sertoli cells and germ cells, blue-stained rat Sertoli cells were not seen in the recipient tubules (Fig. 2*d*, *f*), suggesting that they did not colonize the recipient tubule and that rat spermatogenesis was being supported by mouse Sertoli cells.

Spermatozoa were recovered from the epididymides of eight of the ten recipient mice and examined using phase contrast and fluorescence microscopy (Fig. 3*a–f*). In the best recipient mouse (767, Table 1), roughly one in 39 epididymal spermatozoa arose from rat donor cells, and in the right epididymis from recipient 775, one in 53 spermatozoa were from rat. The three recipients killed at the longest time post-transplantation (≥ 110 days) contained normal rat spermatozoa in the epididymides (Table 1). In a fourth animal killed after 104 days, a rat spermatozoon with a normal tail and an abnormal head was found. Thus, appearance of rat spermatozoa in the epididymides is favoured by a long period of colonization (≥ 104 days), extensive colonization of recipient seminiferous tubules (≥ 12 tubules), and use of nude rather than SCID recipients.

Although the general process of spermatogenesis is highly conserved, many characteristics, including timing of the differentiation process and spermatozoa morphology, are unique and species-specific[3–6]. Thus, the capacity of the mouse seminiferous tubule to support production of spermatozoa that normally require 50 per cent longer to develop and have a

different morphology is remarkable. Whereas Sertoli cells were present with transplanted donor germ cells and may be necessary to support rat spermatogenesis, the absence of blue-staining rat Sertoli cells in association with rat spermatogenesis in recipient mice suggests considerable flexibility in the supporting role of the Sertoli cell and in its interaction with the differentiating stages of male germ cells. As the mouse and rat are believed to have diverged 11 million years ago[11], this reflects a striking conservation of a very specific and highly organized process.

A practical application of our results may be to establish the mouse as an *in vivo* host for spermatogenesis of other species. Immunodeficient mice could prove useful for developing spermatozoa from subfertile or valuable males, in species where immunologically tolerant recipients are not available. It may not be essential to produce many spermatozoa from the transplanted donor cells or for the final stages of maturation to be completed, as it has been demonstrated that round spermatids as well as mature spermatozoa can fertilize eggs after intracytoplasmic injection in mice and humans[12–14]. Finally, although culture systems are not currently available for spermatogonial stem cells, if this were achieved, modification of the genes in these cells should be feasible. In combination with xenogeneic spermatogonial transplantation, this would facilitate the extension of the full range of transgenic technology[15] to many, if not all, mammalian species. Because of its ability to replicate throughout life, the male germ line stem cell may prove useful in biology, medicine and agriculture.

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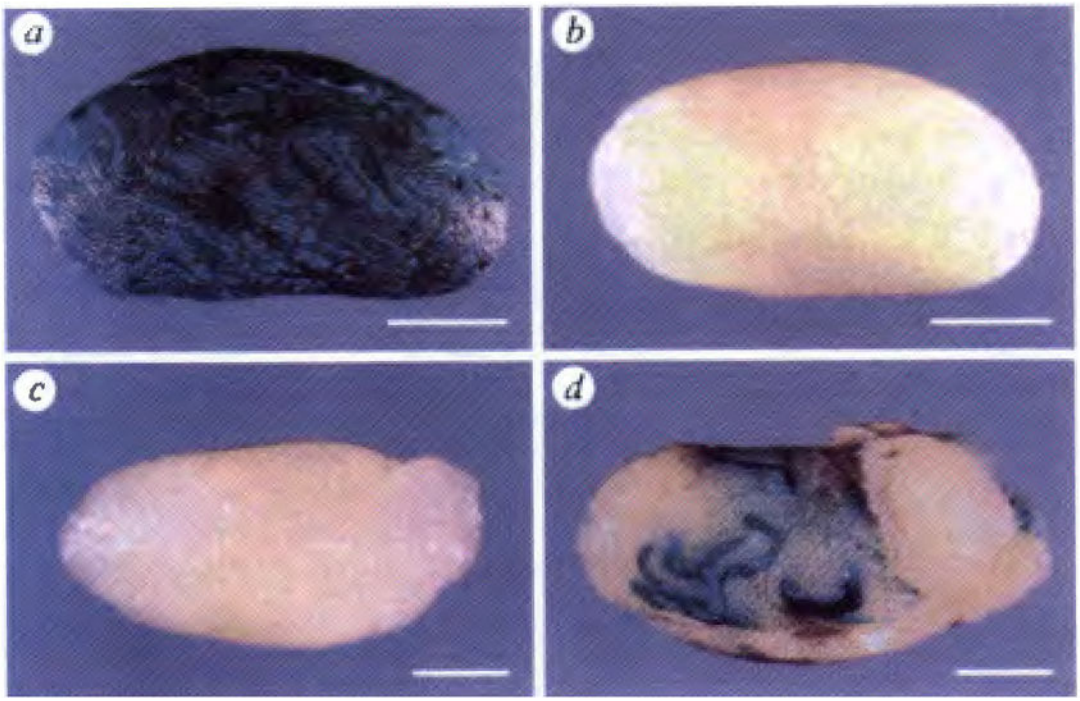


**FIG. 1.**

Generation of rat spermatogenesis in mouse testes. a, Testis from 8-week-old transgenic rat (line 14-2) incubated with 5-bromo-4-chloro-3-indole-*β*-D-galactosidase (X-gal). The transgene was composed of the mouse metallothionein-1 promoter and flanking regions (MT) fused to the *Escherichia coli lacZ* (*lacZ*) structural gene, which results in expression of *β*-galactosidase (*β*-gal) in the liver and testes of rats, causing blue staining of these tissues after incubation with X-gal (refs [1,2,10] and unpublished results). In rats, Sertoli cells are uniformly stained (light areas) and all germ cells will stain blue depending on substrate penetration (dark areas). *b,* Testis from 8-week-old non-transgenic rat which does not stain blue after incubation with X-gal. *c*, Testis from 16-week-old immunodeficient nude (nu/nu) mouse treated with busulphan (32 mg per kg) to destroy endogenous mouse spermatogenesis[1,2,16]. *d*, Testis from busulphan-treated nude mouse that was injected with transgenic rat testis cells (Left testis of 777, Table 1). Enlarged area is site of injection from which some tubules were extruded. Blue tubules represent areas of rat spermatogenesis in the mouse testis. Scale bars: *a*, *b,* 5 mm; *c*, *d*, 1 mm.

METHODS. Transgenic rats were generated as previously described[17]. Technique for isolating testis cells[18,19], microinjection into recipient mouse seminiferous tubules and analysis of testes have also been described[1,2]. Roughly 0.5 ml of cell suspension was used to inject the testes of each recipient. Recipient Swiss nude mice were injected with bone marrow cells (~ $3 \times 10^6$ per mouse) into the tail vein, 3 days after busulphan treatment, to reduce mortality.

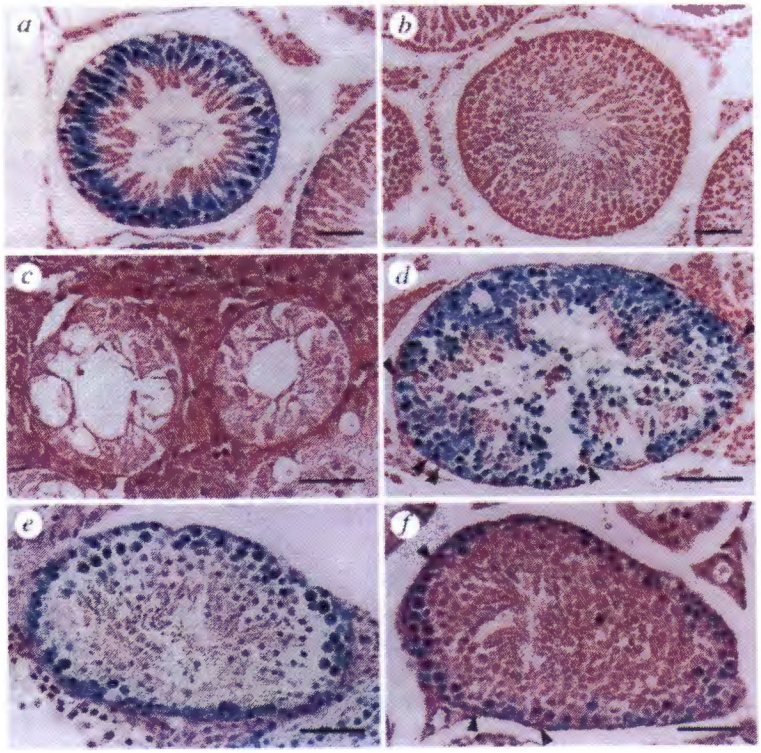


**FIG. 2.**

Microscopic appearance of rat spermatogenesis in mouse seminiferous tubules. a, Seminiferous tubule of transgenic (*MT-lacZ*) rat. Sertoli cells along basement membrane are uniformly stained. All germ cell stages stain depending on substrate penetration. Centre tubule shows staining of pachytene spermatocytes. Stain, X-gal and neutral fast red (NFR). *b*, Seminiferous tubule of control rat. No cells are stained blue by X-gal. The long, thin heads of mature rat spermatozoa are evident. Stain, X-gal and NFR. *c*, Seminiferous tubule of nude mouse treated with busulphan. No germ-cell stages are present; only Sertoli cells can be seen on the basement membrane of the tubule. Stain, NFR. *d*, Seminiferous tubule of busulphan-treated recipient nude mouse showing rat spermatogenesis from transplanted cells (left testis from mouse 777). Rat pachytene spermatocytes and round spermatids are heavily stained blue. Sertoli cells (arrowheads) do not stain blue. Stain, X-gal and NFR. *e*, Seminiferous tubule from left testis of mouse 777. Rat preleptotene and pachytene spermatocytes are stained blue. Stain, X-gal plus periodic acid Schiff and haematoxylin, which emphasizes acrosome and head shape of rat spermatozoa. *f*, Seminiferous tubule from left testis of mouse 775. Rat preleptotene and pachytene spermatocytes are stained blue. Sertoli cells (arrowheads) do not stain blue. Stain, X-gal plus haematoxylin and eosin to emphasize normal morphology of spermatogenesis arising from transplanted rat stem cells. Scale bar, 50 μm.

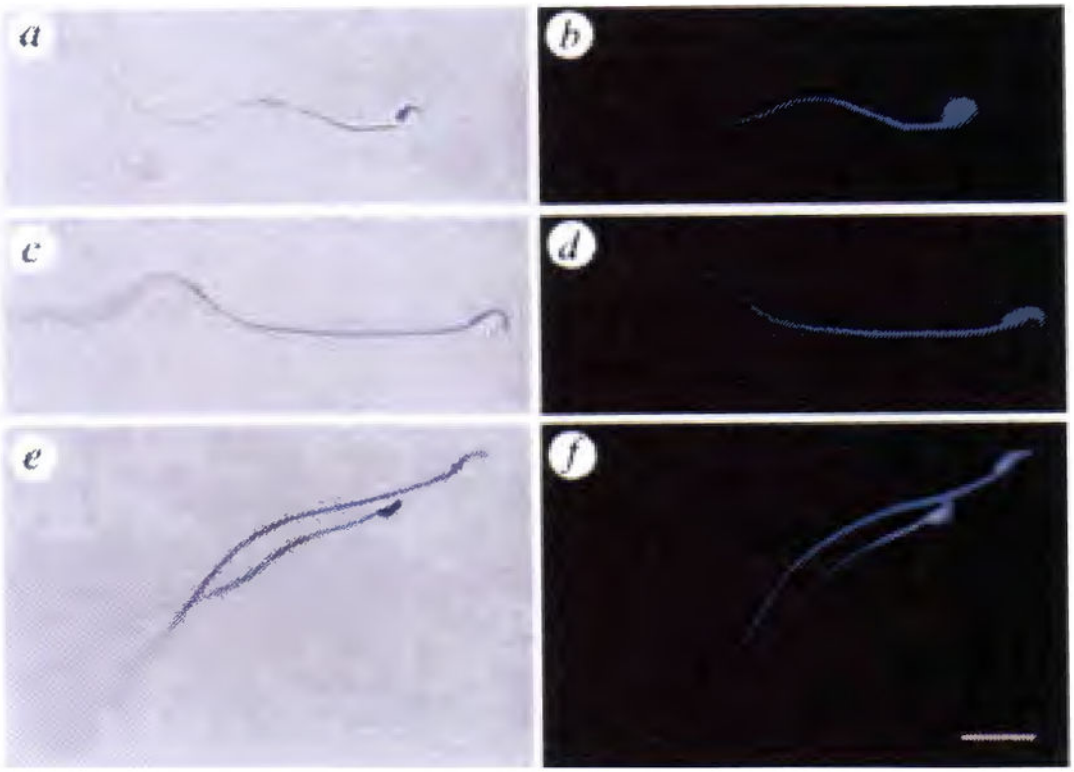


**FIG. 3.**

Epididymal spermatozoa. *a*, *c* and *e* are phase contrast and *b, d* and *f* are fluorescence photomicrographs. *a, b* Normal mouse spermatozoa, with short, thick, sickle-shaped head. *c*, *d* Normal rat spermatozoa, with long, thin head, and tail that is longer and thicker than mouse. *e*, *f* Spermatozoa from epididymides of recipient mouse 767, with distinct rat and mouse morphologies. Ratio was 1 rat to 39 mouse spermatozoa in this animal.

METHODS. To examine and count spermatozoa, epididymides were placed in phosphate-buffered saline and cut in several places[20]. Mouse and rat spermatozoa were distinguished by their characteristic head morphology and tail size. For fluorescence photography, the spermatozoa solution was treated with 20 μg per ml Hoecsht 33258 dye[20]. Scale bar, 25μm.

**TABLE 1**

Rat spermatogonial stem cell transfer into mouse recipient testes

| Experiment | Recipient mouse | Genotype | Per cent testis surface area covered* right, left | Time to analysis, days† | Number of testis tubules with donor cells‡ right, left | Rat sperm in epididymides§ |
|---|---|---|---|---|---|---|
| 1 | 761 | SCID | 50, 25 | 46 | 10, 10 | ND |
| | 763 | | 50, 50 | 97 | 5, 4 | 0/852 |
| 2 | 766 | Nude | 80, 80 | 91 | 8, 3 | 0/399 |
| | 767 | | 80, 90 | 110 | ≥12, ≥12 | 7/270 |
| 3 | 769 | SCID | 60, 25 | 42 | 4, 0 | ND |
| | 770 | | 50, 30 | 104 | ≥12, ≥12 | 1/1832// |
| 4 | 775 | Nude | 90, 50 | 127 | ≥12, ≥12 | 24/1273, 4/2041 |
| | 776 | | 80, 50 | 77 | 3, 4 | 0/1284 |
| | 777 | | 90, 90 | 121 | 8, ≥12 | 3/2370 |
| | 778 | | 50, 50 | 96 | 8, 6 | 0/189 |

SCID mice, which lack B-cells and T-cells. Nude, Swiss nude mice, which lack T-cells. Recipient mice received 32 mg per kg busulphan, which destroys endogenous spermatogenesis but allows partial regeneration with time. This system provides carrier mouse spermatozoa and results in a mixed population of spermatozoa in the epididymis[2]. Transgenic rat donor testis cells were collected and microinjected into the seminiferous tubules of recipient mice as previously described[2]: Experiment 1, $40 \times 10^6$ cells per ml from 6 hemizygous transgenic 17-day-old rat testes; Experiment 2, $79 \times 10^6$ cells per ml from 6 hemizygous transgenic and 6 control 12-day-old testes; Experiment 3, $65 \times 10^6$ cells per ml from 4 hemizygous transgenic and 8 control 12-day-old testes; Experiment 4, $59 \times 10^6$ cells per ml from 4 hemizygous transgenic and 4 control 13-day-old testes. Roughly 0.5 ml of cell suspension was used to inject each mouse.

*Percentage of the surface seminiferous tubules in recipient testes filled by the injected rat cell suspension.

†Number of days from injection of donor cells to analysis of the recipient testis for presence of spermatogenesis; the time available for spermatogenesis.

‡Number of mouse seminiferous tubules with evidence of rat spermatogenesis as indicated by blue staining (Fig. 1). Tubules in testes with more than 12 stained could not be counted accurately because of convolution of the tubules[2].

§ND, not determined. Numerator, number of rat spermatozoa; denominator, number of mouse spermatozoa. In all cases except 775, the spermatozoa from both epididymides were pooled. In 775 the first number is for right, the second for left. Spermatozoa were identified by characteristic morphology (see Fig. 3).

//Rat spermatozoa had normal tail and small round head. Epididymides were fixed in neutral buffered formalin before dissection, which makes release of spermatozoa more difficult.