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Editorial: What Can Spermatogonial Transplants Teach Us about Male Reproductive Biology?

In this issue of *Endocrinology*, there is a rapid communication by Mahato *et al.* (1) that addresses the issue of estrogen action directly on germinal cells in the testis. The authors transplanted germ cells from the testis of the estrogen receptor α (ERα) knockout mice into the seminiferous tubules of germ cell-depleted wild-type mice. The germ cells carrying the knockout mutation underwent qualitatively normal spermatogenesis, and the recipients were fertile. Offspring derived from some of the recipient mice were shown by coat color and PCR to be derived from sperm carrying the disrupted gene for the ERα.

The transplantation of testicular germ cells from a donor to recipient of the same or a closely related species was pioneered in the laboratory of Dr. Ralph Brinster at the University of Pennsylvania (2–4). The initial reports on the successful transplants from donor mice and rats into recipient mice generated a great deal of interest and excitement from both basic and clinical scientists. In the technique developed in Brinster's laboratory, mixed germ cells, including an unknown number of spermatogonial stem cells, are introduced into the lumen of the seminiferous tubules. To initiate spermatogenesis, some of the stem cells introduced into the tubular lumen had to negotiate through Sertoli-Sertoli junctions to the basal lamina. This was a novel concept to most reproductive scientists because there was no reason to predict that this type of relocalization of germ cells could occur. Brinster and Avarbock (1994) also showed in mating experiments that the bacterial marker LacZ gene in transplanted cells that developed into sperm was passed to successive generations (2). This report also described how transplantation was successful when the endogenous germ cells in the recipient animals were reduced or eliminated using the chemotherapeutic agent, busulfan. At the levels used, busulfan does not kill all endogenous spermatogonia, but some stem cells remain and will reinitiate spermatogenesis in the recipient. Because a busulfan-treated recipient testis will simultaneously develop spermatogenesis via the transplanted spermatogonia and via endogenous stem cells, a genetic or morphological marker is required to identify the sperm arising from the transplanted stem cells.

The prevailing theory is that spermatogenesis in the transplants begins from a single seeded stem cell. The stem cell in the testis is generally thought to be the A_s ($A_{isolated}$) spermatogonium. The division of A_s cells yields more A_s cells and spermatogonia committed to the spermatogenic process. By transplanting germ cells from mice carrying the bacterial Lac Z marker, Nagano *et al.* (5) showed that transplanted cells, presumably stem cells, reach the basement membrane within a few days after transplantation and form chains of cells within the first month. These chains grow in length along the basal aspect of the tubule and then develop toward the lumen where spermatocytes are found within 1 month after transplantation. Within 2 months, sperm are produced, and after 3 months an average about 1/3 of the testis contained donor-derived spermatogenesis. The Brinster laboratory has extended these initial reports and has developed methods to improve transplantation efficiency and application. They have developed methods for enriching the stem cell population, for preserving donor cells by freezing, and for increasing the transplantation efficiency by treatment with an GnRH agonist, leuprolide (6–9). Additionally, xenogeneic spermatogonial transplantation (rat, hamster, rabbit, and dog into mouse, mouse into rat) has given insight into the cross-species limitations of the technology and the similarities and differences in the testicular environment between species (4, 10, 11).

A potential specific clinical use of this technique is the replacement of the germ line in patients whose endogenous stem cells had been eliminated as a result of chemotherapy. Other potential clinical applications all involve the replacement of a defective germ line or the surrogate production of spermatozoa in the case of a somatic cell defect. Animal scientists envisioned applying this technique to the preservation of the germ line of valuable animals. The clinical and practical applications of the transplantation technology will be realized in time, whereas most of the advances since 1994 involve improvements and descriptions of the transplantation technology.

Some studies, again originating in the Brinster laboratory, provided insights into basic questions about male reproductive biology. Xenogeneic spermatogonial transplantation (rat germ cells into mouse recipients) was used to examine a very basic question about the timing of germ cell development (12). The time required for spermatogonia to develop into sperm is different for different species. In the mouse, about 35 days are necessary, whereas it takes 52–53 days in the rat. Franca *et al.*, 1998, showed that rat germ cells developing in the mouse testis took about 52–53 days; thus, the rate of development was inherent in the germ cells, and the somatic cells had no influence over this rate.

It is relatively common that gene knockout experiments lead to male infertility. Stem cells may not be present, germ cell development may be blocked at any of several stages, or functional sperm may not be produced. In most cases, the identity of the testicular cell type(s) where the disrupted gene is phenotypically important is not readily apparent. The inability to determine whether somatic or germ cells are responsible for a particular organ or tissue phenotype is a common problem in any multicellular tissue, but it is espe-

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cially acute in the testis and epididymis where germ cell development into functional sperm is directly dependent on Sertoli cells and epididymal epithelial cells. Transplantation of germ cells carrying the disrupted gene into wild-type recipients and transplantation of wild-type germ cells into recipients carrying the knockout can provide this information. Ogawa *et al.* (13) applied this type of approach by transplanting germ cells from infertile mice carrying the Steel (Sl) mutation to infertile white spotting (W/Wv or Wv/W54) mutant male mice and the recipient mice were shown to be fertile. Thus, transplantation of spermatogonial stem cells from an infertile donor to an infertile recipient that had a permissive testicular somatic cell environment restored fertility.

The application of testicular transplantation described by Mahato *et al.* (1) is one of the first to correct male infertility (13) and is the first reported use of the transplantation technique to demonstrate that a gene knockout that disrupts spermatogenesis has no direct action on germ cells. In addition, the authors used coat color differences in offspring to prove that not only was spermatogenesis qualitatively normal but functional fertile sperm were produced that carried the ERα knockout gene. Both of the known estrogen receptors, ERα and ERβ, have been reported in multiple cell types in the testis and epididymis including germ cells (14, 15). Separate knockouts of both the ERα and ERβ genes confirmed a functional role for ERα in spermatogenesis (16, 17). However, with all of this information in hand, it was not clear whether estrogen was acting on the germ cells or the somatic cell components of the testis and epididymis. This experiment provides biological proof that the direct action of ERα on the cells of the germ line is not required for either complete spermatogenesis or sperm maturation. It also reinforces the effectiveness of the technique of spermatogonial transplantation to answer important questions in male reproductive biology.

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