Making male gametes in culture

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Making sperm in a culture dish has been a long-term goal of reproductive biologists. In 2010, a group of investigators from Yokohama, Japan, reinvestigated an organ culture system that was originally used for testicular tissue in the 1960s (1–3). By using better molecular markers, they were able to demonstrate that some cells in this culture system underwent complete meiosis to form round spermatids (4). This work was followed by a study from the same research group that showed that the efficiency of the completion of meiosis and even the formation of flagellated cells was increased by the addition of knockout serum replacement to the organ culture medium (4). The investigators also showed that cryopreserved testicular tissue could be used in these cultures, and healthy, reproductively competent murine offspring were obtained after microinsemination using the haploid cells produced in culture. This technique was further extended when it was demonstrated that spermatogonia grown in culture in the absence of somatic cells could be transplanted into recipient testes and proceed through meiosis to form reproductively functional haploid gametes (5). Sato et al. now expand their observations again by showing that their system can be used to correct spermatogenic defects in vitro (6). The kit ligand (KITL), a cytokine type of growth factor, is produced by Sertoli cells in the testis and must interact with the kit receptor found on differentiating spermatoagonia and spermatocytes for spermatogenesis to be completed (7, 8). These authors report that the addition of recombinant KITL and colony stimulating factor-1 (CSF-1) to the medium of organ cultures of testis tissue derived from mice deficient in the production of KITL induced spermatogenesis to proceed through meiosis (6). The efficiency of male gamete production in these organ cultures, and in all the earlier studies described here, is very low. Only a few tubules in the cultured testes contain spermatogenic cells, and the cells completing meiosis and becoming 1N are rare. However, haploid cells recovered from the cultures of normal testes, testes that received transplanted cultured spermatogonia, and testes from mutant mice treated with KITL and CSF-1 were then used to produce healthy offspring by microinsemination, thereby establishing proof of principle.

Spermatogenesis in mammals takes place in the seminiferous tubules of the testis and is under strict endocrine and exocrine control (9). The seminiferous tubules contain somatic cells (Sertoli cells and peritubular myoid cells) in addition to the germ cell component (Fig. 1). Spermatogonial stem cells can be differentiated and differentiating spermatogonia, spermatocytes undergoing meiosis, spermatids undergoing morphological remodeling, and, finally, spermatozoa released into the tubule lumen.

Successful spermatogenesis requires the orchestrated expression of hundreds of unique testicular gene products. The function of some of these gene products is known but many remain on array spreadsheets as “unknown” or “unannotated.” Hidden among these genes are likely required products for male fertility or potential targets for contraception. At some point in the future, it is likely that the intricacies and mysteries for efficiently

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making functional male gametes from human or animal stem cells in a culture dish will all be revealed. Ideally, the efficient production of spermatozoa in culture from spermatogenic stem cells—or, more generally, from pluripotent stem cells—has important applications. Human spermatogenic stem cells in culture could be a source of gametes from individuals with a number of infertility issues. As described by Sato et al. (6), defects in endocrine or paracrine factors could be corrected, and the potential exists for transgenesis to correct any known genetic defect. Spermatogenic stem cell cultures could be used to replace stem cells destroyed by toxic agents or anticancer drugs. This may be a technique useful in the expansion and the maintenance of germplasm from endangered species. The ability to maintain cultures of spermatogenic stem cells from a variety of animal species that can be induced to efficiently undergo meiosis and can be genetically modified by transgenesis opens a world of possibilities to many groups, including research scientists, clinicians, and livestock producers.