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RESEARCH HIGHLIGHT

In vitro production of functional sperm in mice

Kathleen Hwang[1] and Dolores J Lamb[2,3]

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Spermatogenesis refers to the process by which the production and development of the spermatozoa (the mature male gamete of most sexually reproducing species) occurs. It is a complex, stepwise process of sperm development, taking more than a month depending upon the species involving three specific functional phases: spermatogonial proliferation, meiosis and metamorphosis.[1] Investigators have been successful in recreating this process in some species of fish; however, this whole process has never been reproduced *in vitro* in mammals.[2,3]

In a recent issue of *Nature*, Sato and colleagues[4] attempted to produce functional sperm *in vitro* with cultured neonatal mouse tissue. Using two different transgenic mouse lines that incorporated a green fluorescent protein (GFP) marker, the researchers could follow the relative expression of GFP to define the presence of meiosis and haploid cells. Organ culture methods made spectacular progress in the 1960s, particularly in testicular culture; however, it was not possible to promote spermatogenesis beyond the pachytene stage.[5] Over the next few decades, researchers then focused on cell culture methods to achieve spermatogenesis *in vitro*. Despite such endeavors, progress was limited and Sato and colleagues re-evaluated the feasibility of organ culture methods to produce mature sperm *in vitro*.

Utilizing the organ culture method, elongated spermatids and flagellated sperm were produced using 1–3 mm tissue fragments from 0.5 to 2.5 days postpartum pup mouse testes. Sato *et al.* cultured testicular fragments to maintain the proper microenvironment for cell differentiation. Surprisingly, the authors discovered that the use of knockout serum replacement, used widely for the serum-free culture of embryonic stem cells, was more efficient and demonstrated more success in inducing and extending the duration of GFP expression in comparison to fetal bovine serum.

Confirmation of genuine meiosis was accomplished with examination of the expression of meiotic marker proteins (SYCP1 and SYCP3). These experiments of extended culture resulted in induction and maintenance of GFP expression for a period of over 2 months. Histological examinations revealed the presence of flagellated sperm and elongated spermatids, which was further supported by flow cytometric analysis of dissociated cells from cultured tissues identifying cells with 1N ploidy as a marker for the spermatid cell population. The subsequent fertility potential of these sperm-like cells was tested *via* intracytoplasmic sperm injection with the flagellated sperm and the round spermatid injection technique was used with the elongated spermatids. Using 23 and 35 oocytes for round spermatid injection technique and intracytoplasmic sperm injection, respectively, seven and five live offsprings were delivered and weaned at 3 weeks. They further demonstrated that cryopreservation of the neonatal testis tissue resulted in full spermatogenesis later *in vitro*. The authors also concluded that the most critical component of knockout serum replacement media was indeed that of lipid-rich bovine serum albumin (AlbuMAX), by which they were able to demonstrate its ability to successfully induce spermatogenesis when adding AlbuMAX to fetal bovine serum. Overall, Sato and colleagues showed that organ culture conditions, without a circulatory system as *in vivo*, can support the complete spermatogenesis of mice.

Despite many previous imaginative and creative attempts, there has been rare success in producing fully differentiated haploid spermatids by culture.[6] Promising results with rat germ cells in culture were previously reported, but those methods have not been generally applied.[7] Previous efforts to cultivate sperm *in vitro* were unsuccessful and unable to support the entire complex process of spermatogenesis. This achievement has eluded reproductive biologists for years, until now.

Preservation of fertility is a major concern for patients requiring treatment, such as radiation and chemotherapy that can inadvertently destroy germ cells. In adults, this obstacle is addressed with cryopreservation of sperm prior to treatment. The fertility status of childhood cancer survivors is now the focus of attention as a result of the overwhelming improvements in cancer treatment. Nevertheless, the solution to secondary infertility following cancer treatment is less obvious and direct in prepubescent boys. The authors demonstrated the ability to produce functional sperm in a test tube. The potential of this astounding accomplishment is quite exciting in regard to the preservation of future fertility in cancer patients. Sato and colleagues defined not only the first necessary step in achieving this goal, but also a viable alternative to earlier studies that promised the hope of utilizing spermatogonial stem cell transplantation in treating pre-pubescent boys, work pioneered by Brinster and Steinberger[8] in 1994.

Of note, this study also highlights the need for further investigations into the consequences of *in vitro* spermatogenesis not only at the molecular and cellular levels, but also to evaluate the future health and function of the progeny. Earlier data have suggested that there are potential adverse epigenetic effects that occur when cells, such as gametes, are maintained in culture.[9] With future refinements and customization of culture conditions, the ability to translate this critical success to

[1]Scott Department of Urology, Baylor College of Medicine, Houston, TX 77030, USA; [2]Scott Department of Urology and Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX 77030, USA and [3]Laboratory for Male Reproductive Research and Testing, Baylor College of Medicine, Houston, TX 77030, USA
Correspondence: Professor DJ Lamb (dlamb@bmc.tmc.edu)

human/adolescent testicular samples prior to gonadotoxic therapy would revolutionize our ability to preserve fertility in this patient population.

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