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RESEARCH HIGHLIGHT

Generation of fertile sperm in a culture dish: clinical implications

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Spermatogenesis is a tightly regulated process of development for the generation of mature spermatozoa. Mammalian spermatogenesis occurs in the convoluted seminiferous tubules of the testes, where spermatogenic stem cells (SSCs) reside on sustentacular Sertoli cells of somatic origin, forming a germinal epithelial structure. Spermato genesis is accomplished in a complicated, timely controlled differentiation of SSCs into premeiotic primary spermatocytes, followed by formation of postmeiotic round spermatids and their subsequent maturation into spermatozoa.

Spermatogenesis *in vitro* has long been attempted, but it remains a challenge due to the limitation of culture systems. *In vitro* differentiation of SSCs is technically difficult because development of gametes requires a unique, microenvironmental niche, at which Sertoli cells play important roles in structural support, secretion of factors for maintenance and control of spermatogenesis, clearance of apoptotic germ cells by phagocytosis, as well as acting as a blood–testis barrier. Despite the technical challenge, differentiation of isolated SSCs was achieved by coculture with Sertoli cells.[1,2] Alternatively, tissue culture of testicular biopsy specimens provides an *in vitro* environment which maintains the niche and structural integrity similar to *in vivo* spermatogenesis.[3] It has also been reported that immortalized spermatogonial cell lines could differentiate to haploid spermatids in the absence of supportive cells.[4] The success of *in vitro* differentiation of germ cells is encouraging; however, the current obstacle to define a culture system which can reproducibly generate fertile, matured spermatozoa for *in vitro* fertilization remains.

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The recent report by Sato *et al.*[5] of successful development of a culture system for *in vitro* production of functional sperm from neonatal mouse testes is a unique achievement. Unlike conventional tissue culture media which usually contain fetal bovine serum, Sato *et al.*[5] tested the feasibility of knockout serum replacement (KSR) for culturing testicular tissue. To their surprise, KSR can efficiently increase spermatogenesis in culture dish. Importantly, their culture system enabled production of mature haploid spermatids which could be used to fertilize eggs and produce live offsprings.

Several points highlighted here distinguish their work. First, the use of KSR for tissue culture of testis is unexpected. KSR is usually used for culturing embryonic stem cells to maintain the undifferentiated state. Withdrawal of KSR and replacement with fetal bovine serum results in spontaneous differentiation of embryonic stem cells. Thus the use of KSR for differentiation of SSCs, though unanticipated, is interesting. With KSR, neonatal testicular tissues lacking postmeiotic germ cells differentiated to the stage of meiosis-committed spermatocytes and finally haploid spermatids. Histological analysis revealed complete spermatogenesis upon extended culture to 38 days, and flagellated cells were observed. Further examination of the active components in KSR identified AlbuMAX, a lipid-rich bovine serum albumin, as a critical factor for inducing spermatogenesis. It should be remarked that in addition to the differentiation of germ cells, Sertoli and peritubular myeloid cells of somatic origin also underwent developmental changes, as demonstrated by the expression of androgen receptor, a nuclear receptor protein important for mediating the effect of testosterone. The support of *in vitro* spermatogenesis is more likely to provide the testicular tissues with a culture niche for the spontaneous progression of spermatogenesis, not merely providing growth factors for the differentiation of spermatogonia. Nevertheless, the current culture system requires the integrity of testicular tissues, and spermatogenesis of isolated SSCs alone has not been made possible. Second, the *in vitro* differentiated spermatids and sperms are fertile, as shown by the production of live offspring. These animals were fertile, since interbreeding of F1 gave rise to normal pups. Their result is exciting and opens opportunities for its application to treat male infertility in the human; however, rigorous assessment must be applied prior to its application in clinical practice. Although promising, it is not clear whether *in vitro* differentiated spermatid and sperm will display the same genetic profile as *in vivo* germ cells. Genome-wide transcriptome analysis is necessary to ensure genetic fidelity. Furthermore, since spermatogenesis involves a sequential DNA metabolism, it will be important to perform cytogenetic analysis to ensure the absence of aneuploidy in haploid germ cells. In the late stage of spermatogenesis (spermiogenesis), male germ cells undergo rapid chromatin remodeling, during which histones are replaced with transition proteins and eventually protamines.[6] These molecular events are highly orchestrated to guarantee proper organization of the compacted nuclei in elongating and condensing spermatids, allowing efficient packaging and increased stability of the paternal genome. It is therefore critical to review any genetic,epigenetic or genomic mistakes occurring in the *in vitro* system to establish a clinically safe protocol for humans. Third, cryopreserved testicular tissues were able to resume spermatogenesis after thawing, suggesting that cryopreservation has little effect on cell viability. It is a common practice to cryopreserve human sperm or

testicular biopsies for future clinical use. In laboratory embryos, organs, tissues or cells are frequently cryopreserved for banking of animal lines. Successful recovery of differentiation-prone organs/tissues in this report has clinical implications as its preservation (human testicular tissue) would allow management of clinical specimens to derive mature male germ cells available for assisted reproductive techniques.

In summary, Sato *et al.*[5] demonstrated an improved organ culture system for derivation of mature spermatid and sperm *in vitro*. This may facilitate future studies to elucidate the molecular mechanisms of spermatogenesis and the development of new therapeutic strategies.

Future studies should focus on human spermatogenesis. The direct impact of human spermatogenesis *in vitro* allows manipulation of matured sperm in the culture dish. One clinical application for this technique is to generate fertile sperm from testicular biopsies taken from infertile men. Together with the well-developed *in vitro* fertilization technique, *in vitro* spermatogenesis is hopefully the promise for treating male infertility.

1 Steinberger A, Steinberger E. Tissue culture of male mammalian gonads. *In Vitro* 1970; **5**: 17–27.
2 Stukenborg JB, Wistuba J, Luetjens CM, Elhija MA, Huleihel M *et al*. Coculture of spermatogonia with somatic cells in a novel three-dimensional soft-agar-culture-system. *J Androl* 2008; **29**: 312–29.
3 Tesarik J, Mendoza C, Anniballo R, Greco E. *In-vitro* differentiation of germ cells from frozen testicular biopsy specimens. *Hum Reprod* 2000; **15**: 1713–6.
4 Feng LX, Chen Y, Dettin L, Pera RA, Herr JC *et al*. Generation and *in vitro* differentiation of a spermatogonial cell line. *Science* 2002; **297**: 392–5.
5 Sato T, Katagiri K, Gohbara A, Inoue K, Ogonuki N *et al*. *In vitro* production of functional sperm in cultured neonatal mouse testes. *Nature* 2011; **471**: 504–7.
6 Govin J, Caron C, Lestrat C, Rousseaux S, Khochbin S. The role of histones in chromatin remodelling during mammalian spermiogenesis. *Eur J Biochem* 2004; **271**: 3459–69.