Cell Reports Medicine

CellPress
OPEN ACCESS

Preview

# Gene therapy for female infertility: A farfetched dream or reality?

Subhajit Pathak,[1,2] Pratiksha Sarangi,[1,2] and Giridhara R. Jayandharan[1,*]
[1]Department of Biological Sciences and Bioengineering, Indian Institute of Technology Kanpur, Kanpur, Uttar Pradesh, India
[2]These authors contributed equally
*Correspondence: jayrao@iitk.ac.in
https://doi.org/10.1016/j.xcrm.2022.100641

**A major cause of infertility in women is impaired ovulation or oogenesis. In this issue of *Cell Reports Medicine*, Kanatsu-Shinohara et al.[1] demonstrate the potential of gene delivery with adeno-associated virus that can cross the blood-follicle barrier and restore oogenesis in congenitally infertile mice.**

Infertility is a primary concern for more than 48 million couples and 186 million individuals worldwide.[2] The most common treatment option is assisted reproduction technology. However, the complexity involved in embryo implantation procedures typically lead to a low rate of success (~25% live births). There have been attempts to find a long-lasting therapy to restore spermatogenesis by utilizing different viral vectors to deliver the functional copy of the mutated gene responsible for infertility in mice models. The expression of c-kit ligand via a lentiviral vector in Sertoli cells of $Sl/Sl^{d}$ mice restored spermatogenesis and generated offspring after intracytoplasmic sperm injection into the oocytes.[3] Another study from Takehashi et al.[4] showed that adenovirus could infect spermatogonial stem cells (SSCs) and further rescue spermatogenesis after transplantation into seminiferous tubules of infertile mice. While the use of these viral vectors has shown promise, certain limitations exist in translating these to clinical applications. Insertional mutagenesis, cell-specific targeting, and pronounced inflammation are some of the major concerns. To overcome these barriers, adeno-associated-virus (AAV)-based gene delivery is a promising option with its unique features like low immunogenicity, broader tissue tropism, and long-term targeted gene expression.[5] For example, AAV2 and AAV9 were shown to infect intertubular testosterone-producing Leydig cells among the five different serotypes (AAV2, -5, -8, -9, and rh10) assessed. Interestingly, a phosphomutant vector of AAV2 was able to cross the myoid cell barrier and infect Sertoli cells.[6] In another study, AAV1 and AAV9 were shown to cross the blood-testes barrier and transduce the Sertoli cells, SSCs, Leydig cells, and peritubular cells. The recombinant AAV vector was able to restore spermatogenesis in congenitally infertile $Kitl^{Sl}/Kitl^{Sl\text{-}d}$ mice and produce offspring.[7]

Only a few studies have attempted to restore fertility in females using a gene therapy approach.[8] In this issue of *Cell Reports Medicine*, Kanatsu-Shinohara et al.[1] have demonstrated an elegant approach to augment expression of Kit ligand (*Kitl*) in granulosa cells, which led to a successful ovulation in congenitally infertile female mice ($Kitl^{Sl\text{-}t}/Kitl^{Sl\text{-}t}$). KITL-KIT interaction between granulosa cells and oocytes is important during several stages of oocyte development.[9] Because of AAV's unique ability to undergo transcytosis (transcellular transport), the authors investigated the ability of an AAV-based vector to cross the blood-follicle barrier (BFB) and express Kitl in granulosa cells. Several AAV serotypes (AAV1, AAV6, AAV6.2, AAV7M8, AAV9, AAVDJ, and AAVDJ8) containing a reporter gene (*mCherry*) were assessed by microinjection into the ovaries of normal mice (Figure 1A). Of these, the AAV9 serotype showed the highest transduction efficiency in granulosa cells, and this was further enhanced in the presence of a neuraminidase enzyme.

Significantly, although the authors report here the first evidence of breakthrough of BFB and the infection of granulosa cells by the AAV9 vector, these vectors did not infect the oocytes. This is important, as it dramatically reduces the risk of vertical gene transfer. By utilizing this unique ability of the AAV9 vector, the authors subsequently modulated the oocyte microenvironment by expressing *Kitl* in infertile mice ($Kitl^{Sl\text{-}t}/Kitl^{Sl\text{-}t}$) and further assessed its impact on oogenesis and fertility. *Kitl* overexpression did not lead to oocyte apoptosis despite a significant increase in theca cells. Furthermore, there was no evidence of $CD4^{+}$ or $CD8^{+}$ cell infiltration. In the control female mice ($Kitl^{Sl\text{-}t}/Kitl^{Sl\text{-}t}$) that did not receive any gene therapy, histological staining showed that the ovaries were relatively small and that the follicles were halted at an early stage with a single layer of granulosa cells. However, upon administration of AAV9-*Kitl*, the ovaries of $Kitl^{Sl\text{-}t}/Kitl^{Sl\text{-}t}$ mice had growing follicles, signifying *Kitl* expression and its role in oogenesis. Peripheral blood samples from infertile ($Kitl^{Sl\text{-}t}/Kitl^{Sl\text{-}t}$) mice had lower estrogen levels than wild-type mice but significantly greater follicle-stimulating hormone (FSH) and luteinizing hormone (LH). This phenomenon mirrors the early ovarian insufficiency in humans. After administration of AAV9-*Kitl*, FSH levels in the infertile mice dropped dramatically within the first 10 days, whereas LH was unaltered until 70 days. The levels of estrogen did not vary significantly even after gene therapy. A total of eight out of the 19 females (42.1%) injected with the AAV-*Kitl* vector were pregnant (Figure 1B). The first litter was delivered at an average of 65.6 days after vector microinjection; however, after 126 days, no offspring was delivered. All the offspring born were morphologically normal at birth, and upon sexual maturation, the females

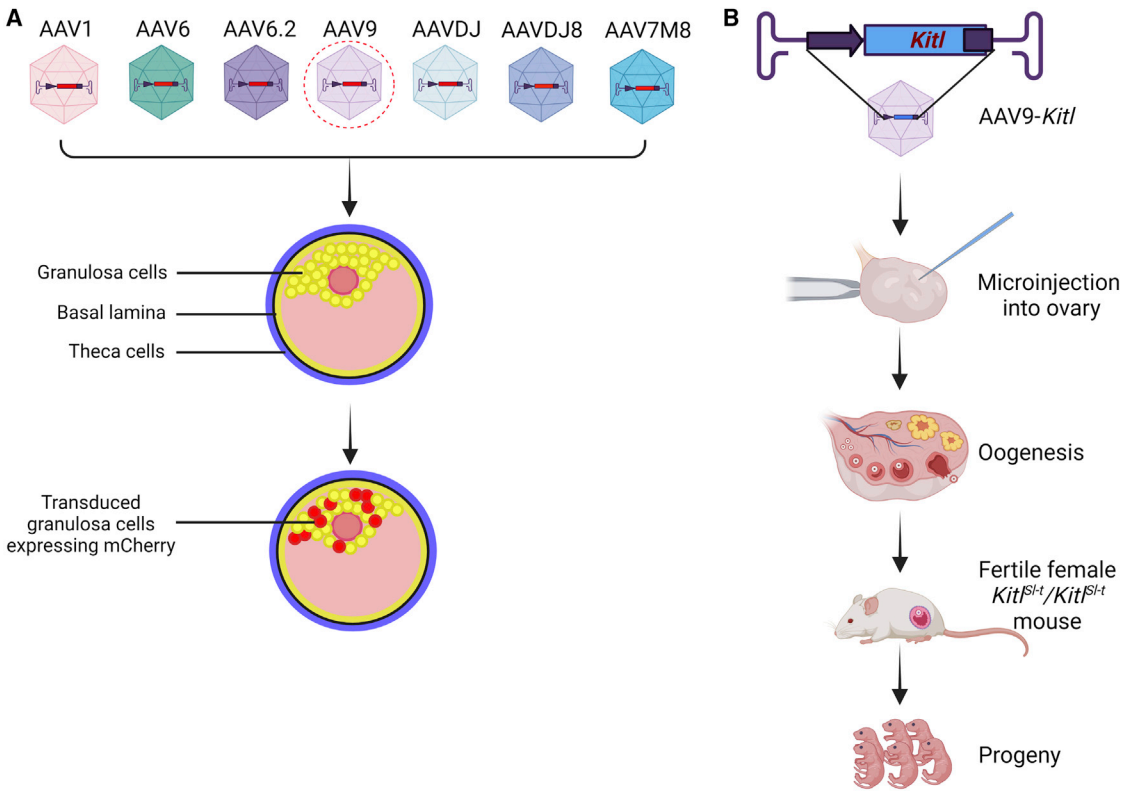


**Figure 1. AAV-mediated gene delivery into ovaries for restoration of oogenesis in congenitally infertile *Kitl*$^{Sl\text{-}t}$/*Kitl*$^{Sl\text{-}t}$ mice**

(A) Screening of different AAV serotypes containing *mCherry* in ovaries.

(B) AAV9-*Kitl* restores oogenesis by crossing the BFB and transducing granulosa cells in ovaries of infertile *Kitl*$^{Sl\text{-}t}$/*Kitl*$^{Sl\text{-}t\ l}$ mice.

Image created with BioRender.com.

were infertile because of *Kit*$^{Sl\text{-}t}$ mutations, whereas males were fertile. Genetic analysis of the offspring DNA by polymerase chain reaction revealed no integration event in the germ cells. The possibility of genome imprinting in the offspring DNA was ruled out further by a combined bisulfite restriction analysis and DNA sequencing. The specificity of AAV9 serotype to restore fertility was confirmed by a series of control experiments utilizing a lentivirus and a different AAV7 serotype vector expressing *Kitl*. However, all 12 female mice administered with lentivirus-*Kitl* and all eight females receiving AAV7M8-*Kitl* remained infertile. This highlights the fact that it is very critical to select the appropriate AAV serotype to cross the BFB and rescue fertility.

While these data are promising and a new experimental approach is proposed here, further studies are warranted prior to clinical application, as highlighted by the authors. The possibility of inadvertent oocyte transduction with long-term AAV exposure needs to be investigated with sensitive detection techniques. A high-throughput DNA-sequencing analysis of both the parental oocyte-granulosa complex and the progeny will be crucial to document the viral integration events into the host genome. The use of additional AAV serotypes and well-characterized capsids[10] that are dose optimized for infectivity of granulosa cells, but not theca cells, may also be beneficial. Another important parameter is to establish the molecular mechanisms of transcytosis and the long-term fertility in the recipient mice.

In summary, this study[1] has demonstrated that AAV9 can be used in the presence of neuraminidase to specifically infect granulosa cells *in vivo,* and this elegant strategy can be used for genetic manipulation to promote oogenesis and restore fertility in female mice. Because no vertical transmission of vector was discovered in the progeny, these findings attest that AAV-based gene therapy is relatively safe and has a great potential to treat female infertility.

## ACKNOWLEDGMENTS

G.R.J. is supported by the DBT Wellcome Trust India Alliance Senior fellowship (IA/S/16/1/502355) and ASPIRE research award from Pfizer, Inc. USA (#5359919). S.P. and P.S. were supported by a PhD fellowship (MHRD grant to IIT-Kanpur).

## DECLARATION OF INTERESTS

The authors (G.R.J.) have filed for patent applications for improved AAV vectors for gene therapy through IIT Kanpur. None of them are related to this present article.

## REFERENCES

1. Kanatsu-Shinohara, M., Lee, J., Miyazaki, T., Morimoto, H., and Shinohara, T. (2022). Adeno-associated-virus-mediated gene delivery to ovaries restores fertility in congenital infertile mice. Cell Rep. Med. *3*, 100606-1–100606-10.
2. World Health Organization. (2021). WHO fact sheet on infertility. Global Reproductive

Health *6*, e52. https://doi.org/10.1097/grh.0000000000000052.

3. Ikawa, M., Tergaonkar, V., Ogura, A., Ogonuki, N., Inoue, K., and Verma, I.M. (2002). Restoration of spermatogenesis by lentiviral gene transfer: Offspring from infertile mice. Proc. Natl. Acad. Sci. U S A *99*, 7524–7529. https://doi.org/10.1073/pnas.072207299.

4. Takehashi, M., Kanatsu-Shinohara, M., Inoue, K., Ogonuki, N., Miki, H., Toyokuni, S., Ogura, A., and Shinohara, T. (2007). Adenovirus-mediated gene delivery into mouse spermatogonial stem cells. Proc. Natl. Acad. Sci. U S A *104*, 2596–2601. https://doi.org/10.1073/pnas.0609282104.

5. Watanabe, S., Kanatsu-Shinohara, M., Ogonuki, N., Matoba, S., Ogura, A., and Shinohara, T. (2017). Adeno-associated virus-mediated delivery of genes to mouse spermatogonial stem cells. Biol. Reprod. *96*, 221–231. https://doi.org/10.1095/biolreprod.116.143495.

6. Rajasekaran, S., Thatte, J., Periasamy, J., Javali, A., Jayaram, M., Sen, D., Krishnagopal, A., Jayandharan, G.R., and Sambasivan, R. (2018). Infectivity of adeno-associated virus serotypes in mouse testis. BMC Biotechnol. *18*, 70. https://doi.org/10.1186/s12896-018-0479-1.

7. Watanabe, S., Kanatsu-Shinohara, M., Ogonuki, N., Matoba, S., Ogura, A., and Shinohara, T. (2018). In vivo genetic manipulation of spermatogonial stem cells and their microenvironment by adeno-associated viruses. Stem Cell Rep. *10*, 1551–1564. https://doi.org/10.1016/j.stemcr.2018.03.005.

8. Ghadami, M., El-Demerdash, E., Salama, S., Binhazim, A., Archibong, A., Chen, X., Ballard, B., Sairam, M., and Al-Hendy, A. (2010). Toward gene therapy of premature ovarian failure: intraovarian injection of adenovirus expressing human FSH receptor restores folliculogenesis in FSHR (−/−) FORKO mice. Mol. Hum. Reprod. *16*, 241–250. https://doi.org/10.1093/molehr/gaq003.

9. Yoshida, H., Takakura, N., Kataoka, H., Kunisada, T., Okamura, H., and Nishikawa, S.I. (1997). Stepwise requirement of c-kit tyrosine kinase in mouse ovarian follicle development. Developmental Biol. *184*, 122–137. https://doi.org/10.1006/dbio.1997.8503.

10. Mary, B., Maurya, S., Arumugam, S., Kumar, V., and Jayandharan, G.R. (2019). Post-translational modifications in capsid proteins of recombinant adeno-associated virus (AAV) 1-rh10 serotypes. FEBS J. *286*, 4964–4981. https://doi.org/10.1111/febs.15013.