Infertility is a primary concern for more than 48 million couples and 186 million individuals worldwide. 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/Sld mice restored spermatogenesis and generated offspring after intracytoplasmic sperm injection into the oocytes. Another study from Takehashi et al. 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 inflammations 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. 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. 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 KitlSl-t mouse and produce offspring. Only a few studies have attempted to restore fertility in females using a gene therapy approach. In this issue of Cell Reports Medicine, Kanatsu-Shinohara et al. demonstrated the potential of gene delivery with adeno-associated virus that can cross the blood-follicle barrier and restore oogenesis in congenitally infertile mice. 

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

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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.

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