Adenovirus-mediated gene delivery restores fertility in congenitally infertile female mice

Mito KANATSU-SHINOHARA1, 2), Jiyoung LEE3), Takehiro MIYAZAKI1), Hiroko MORIMOTO1) and Takashi SHINOHARA1)

1)Department of Molecular Genetics, Graduate School of Medicine, Kyoto University, Kyoto 606-8501, Japan
2)AMED-CREST, AMED 1-7-1 Otemachi, Chiyodaku, Tokyo 100-0004, Japan
3)Advanced Multidisciplinary Research Cluster, Institute of Research, Tokyo Medical and Dental University (TMDU), Tokyo 113-8510, Japan

Abstract. Oogenesis depends on close interactions between oocytes and granulosa cells. Abnormal signaling between these cell types can result in infertility. However, attempts to manipulate oocyte-granulosa cell interactions have had limited success, likely due to the blood-follicle barrier (BFB), which prevents the penetration of exogenous materials into ovarian follicles. Here, we used adenoviruses (AVs) to manipulate the oocyte-granulosa cell interactions. AVs penetrated the BFB and transduced granulosa cells through ovarian microinjection. Although AVs caused transient inflammation, they did not impair fertility in wild-type mice. Introduction of Kitl-expressing AVs into congenitally infertile KitlSl−t/KitlSl−t mutant mouse ovaries, which contained only primordial follicles because of a lack of Kit expression, restored fertility through natural mating. The offspring showed no evidence of AV integration and exhibited normal genomic imprinting patterns for imprinted genes. These results demonstrate the usefulness of AVs for manipulating oogenesis and suggest the possibility of gene therapies for human female infertility.

Key words: Adenovirus, Gene therapy, Kitl, Ovary

Oogenesis is a long and complex process that depends on both intra- and extra-ovarian factors [1, 2]. At the time of birth, all oocytes are arrested at prophase I of the first meiotic division as primary oocytes in primordial follicles. Oocytes are surrounded by a layer of somatic cells called granulosa cells (GCs). A small number of primordial follicles are continuously recruited for fertilization. Recruitment of primordial follicles depends on the secretion of follicle stimulating hormone (FSH). Increases in circulating FSH levels during each reproductive cycle induce development of a cohort of follicles. GCs surrounding the oocyte increase in size and number, and begin to secrete estrogen. While most oocytes degenerate during oogenesis, stimulation by luteinizing hormone (LH) initiates ovulation of mature oocytes for subsequent fertilization.

Among the molecules involved in oogenesis, the interaction between the KIT tyrosine kinase receptor on oocytes and its ligand KITL on GCs has been extensively studied [3]. Mice with mutations in either of these molecules often exhibit impaired fertility, as the KITL-KIT interaction promotes both oocyte growth and survival. KITL is expressed in GCs in soluble (KITL1) or membrane-bound (KITL2) forms. Growth and differentiation factor 9 (GDF9) expression in oocytes inhibits the expression of both Kitl and Kitl2. In contrast, bone morphogenetic protein 15 (BMP15), another molecule expressed in oocytes, promotes the expression of both Kitl1 and Kitl2 in monolayers of GCs [3]. Neutralizing antibody against KIT administered in vivo inhibited the onset of primordial follicle development, primary follicle growth, follicular fluid formation, and preovulatory follicle development [4]. These findings demonstrate the critical role of KITL-KIT signaling in oogenesis.

The development of a gene delivery method would be valuable for dissecting the complex molecular interactions between oocytes and GCs. However, the delivery of exogenous molecules into ovarian follicles is limited by the blood-follicle barrier (BFB), which consists of vascular endothelium, sub-endothelial basement membrane, thecal interstitium, follicular basement membrane, and membrane granulosa [5, 6]. BFB restricts access of molecules > 500 kDa into ovarian follicles [6]. Therefore, BFB likely prevents gene transduction into the oocyte-granulosa complex. However, we recently found that adeno-associated viruses (AAVs) can rescue the fertility of KitlSl−t/KitlSl−t mutant mice with defective Kitl expression [7]. The ovaries of KitlSl−t/KitlSl−t mice completely lack Kitl expression, and oogenesis is arrested at the primordial follicle stage [8]. Microinjection of AAVs expressing the Kitl gene into their ovaries initiated oogenesis and allowed the production of offspring through natural mating. DNA analysis of the offspring revealed no evidence of germline integration of the transgene.

Although these results provide evidence that AAVs can penetrate the BFB and deliver a transgene to GCs, there are severe critical drawbacks. AAVs have a very limited cargo capacity (4.7 kb) for transgenes [9]. Moreover, transgene expression occurs more slowly than with other viral vectors (3–21 days in vivo) [10]. Thus, although AAVs are considered the most promising gene therapy vectors, these drawbacks restrict their application. Among other gene therapy vectors, adenoviruses (AVs) appear to be good candidates for gene therapy in the oocytes, as they usually do not integrate into the host genome, which is the most serious side effect for germ cell lines. However, there are at least two problems associated with AV-mediated gene delivery. The first is inflammation, which often occurs in target tissue following AV-mediated gene delivery [11]. The death of one patient

Received: August 10, 2022
Accepted: September 21, 2022
Advanced Epub: October 13, 2022
©2022 by the Society for Reproduction and Development
Correspondence: T Shinohara (e-mail: tshinohara@virus.kyoto-u.ac.jp)
This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial No Derivatives (by-nc-nd) License. (CC-BY-NC-ND 4.0: https://creativecommons.org/licenses/by-nc-nd/4.0/)
Measurement of hormones

Virus production

Animals and microinjection procedure

DNA analysis

Materials and Methods

Animals and Methods

Viruses production

Measurement of hormones

Analysis of ovaries

Combined bisulfite restriction analysis (COBRA)

Bisulfite sequencing

Statistical analyses

Results
Therefore, in the present study we used R26R-Eyfp mice [15]. These mice contained a loxP-flanked STOP sequence followed by the Eyfp gene, which was inserted into the Gt(ROSA)26Sor locus (Fig. 1A). These mice allow for more sensitive detection of gene expression in a variety of tissues, including the testes [18]. Even if originally infected cells are lost, their progeny may contribute to enhanced yellow fluorescent protein (EYFP) expression. We produced Cre-expressing AV (AxCAN-Cre) and monitored the expression of EYFP after microinjection into the ovaries of R26R-Eyfp mice.

When the mice were sacrificed 1 week after microinjection, a strong EYFP signal was detected (Fig. 1B). Histological sections of the ovaries revealed EYFP signals in the cells within the ovarian follicles. Double immunostaining of R26R-Eyfp mouse ovaries with ovarian cell markers indicated that the EYFP signal was present in extrafollicular HSD3B+ theca cells and intrafollicular AMH+ GCs (Figs. 1C, D). However, no signal was observed in oocytes. These results suggest that AVs can penetrate the BFB and infect GCs without transduction of oocytes.

Comparison of AVs and AAVs for ovarian transduction

Because the results presented in the preceding section demonstrate successful infection of ovarian follicles by AVs, we compared the effects of AVs and AAVs. For this experiment, we used AVs that express Kitl (AxCAN-Kitl) [19]. In our previous study of this virus for male infertility treatment, we were able to restore spermatogenesis in congenitally infertile Kitl+/Kitl− mutant mice, which contained only undifferentiated spermatagonia due to a lack of membrane-bound Kitl [20]. Although this vector was useful for restoration of spermatogenesis, we observed infiltration of lymphocytes into the seminiferous tubules, and it was impossible to obtain offspring through natural mating. This result suggested that transgene expression may not be sustained long enough to generate a sufficient amount of sperm [19]. Nevertheless, we tested whether exogenous Kitl overexpression stimulates oogenesis.

To examine the differences between AVs and AAVs, we microinjected AVs and AAVs into the same mice (AVs into right ovaries and AAVs into left ovaries). Both viruses were used at the same concentrations. Ovaries recovered 7 and 14 days after microinjection and were examined for signs of inflammation. We found no differences in the macroscopic appearance between ovaries treated with AVs or AAVs at either time point. However, histological analysis revealed that AxCAN-Kitl caused inflammation. Although lymphocyte invasion was not evident 7 days after infection, many lymphocytes were found after 14 days (Figs. 2A–C). Immunostaining of the ovaries confirmed the presence of both CD4+ and CD8+ lymphocytes in the ovaries 2 weeks after infection (Figs. 2B, C). These lymphocytes were found mostly in the interstitial areas, and no invasion into the follicles was apparent. In contrast, no lymphocyte infiltration was observed in ovaries after AAV transduction. These results suggested that AV transduction induces inflammation, confirming our previous observations in the testes [19].

As these results suggest that AAVs are superior to AVs, we examined the number of apoptotic cells using terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) (Supplementary Fig. 1). Although the number of TUNEL+ cells was slightly higher for AAVs among both AMH+ GCs and HSD3B+ theca cells, the differences were not statistically significant. We found no apparent effect on oocytes despite inflammation, suggesting that ovarian inflammation does not cause oocyte depletion. These results suggest that AVs cause inflammation and apoptosis in ovarian somatic cells and have effects comparable to those of AAVs.

Impact of Kitl overexpression on reproductive performance

Since oocytes could survive after AxCAN-Kitl expression, it is possible that the animals would retain fertility despite the observed inflammatory reactions. Based on this possibility, we examined the impact of AxCAN-Kitl overexpression on reproductive performance in wild-type mice. In this study, we microinjected AxCAN-Kitl into the ovaries of mature female mice. AxCAN-LacZ at the same viral concentration was used as a control. These females were housed with wild-type males for at least 7 days after microinjection, and the number of offspring and timing of birth were recorded.

Overall, four of the five females microinjected with AxCAN-Kitl were fertile, as were all five females microinjected with AxCAN-LacZ (Table 1). In Kitl-overexpressing females, the first offspring were born as early as 22 days after mating, with an average timing of 26.5 ± 3.9 days. In contrast, the first offspring were born 28 days after mating in mice with AxCAN-LacZ transduction, with an average of 60.8 ± 11.7 days. The difference in the timing of offspring production was statistically significant. For litter size, an average of 5.1 ± 0.9 offspring were born after AxCAN-Kitl transduction. Although AxCAN-LacZ transduction led to a larger number of offspring (6.1 ± 0.8), there was no statistical difference between the two groups. AxCAN-Kitl transduction did not deplete the oocyte pool, as all four fertile females bore additional litter. Together, these results show that AxCAN-Kitl transduction does not impair fertility in wild-type animals.

Fig. 1. AV-mediated gene delivery into GCs. (A) Experimental diagram. R26R-Eyfp mice were microinjected with AxCAN-Cre. CRE-mediated recombination removed the neo cassette, and EYFP expression was initiated under control of the ROSA26 promoter. (B) Macroscopic appearance of R26R-Eyfp ovary 7 days after AxCAN-Cre injection. (C) Immunostaining of R26R-Eyfp ovary. (D) Quantification of cells expressing EYFP (n = 12 for AMH; n = 18 for HSD3B). Bar = 1 mm (B), 50 μm (C). Counterstain, Hoechst 33342 (C).
Histological analysis of ovaries after AxCAN-Kitl transduction

To evaluate the impact of AxCAN-Kitl transduction on oogenesis, we collected ovaries from both types of mice and performed histological analysis 3 months after microinjection. Because we observed lymphocyte infiltration 2 weeks after AV injection, we expected that AV transduction would induce fibrosis in the long term. However, no fibrosis or lymphocyte infiltration was observed at this stage. Instead, we observed large clusters of extrafollicular cells (Fig. 3A). Because these cell clusters were found in control ovaries that received AxCAN-LacZ, it is likely that they resulted from AV infection rather than Kitl overexpression. Although Kitl overexpression can cause tumors [21], no evidence of tumor formation was found in AxCAN-Kitl-transduced ovaries.

When we conducted immunostaining to confirm the cell type, the cell clusters were reactive with anti-HSD3B antibody (Fig. 3B), suggesting that they were derived from thecal cells. However, because these cells expressed low levels of MKI67, they did not actively proliferate at the time of analysis. In contrast, MKI67 signals...
were stronger in AMH+ GCs in both ovarian types. However, no significant difference was observed between the two vectors. Likewise, TUNEL staining showed that there was no significant enhancement of apoptosis due to AxCAN-Kitl transduction (Supplementary Fig. 2).

Despite the development of theca cell clusters, histological analysis showed normally growing oocytes in the control ovary that received the AxCAN-LacZ injection (Fig. 3A). Oogenesis also appeared normal in ovaries that received AxCAN-Kitl injections with many antral follicles. However, quantitative examination of oogenesis revealed that the number of secondary follicles was slightly, but significantly, reduced after AxCAN-Kitl transduction (Fig. 3D). This finding raised the possibility that Kitl overexpression might have influenced oocyte differentiation. To exclude the possibility of germline integration of the transgene, we examined the presence of adenovirus DNA by PCR. We collected tail DNA from all offspring and performed PCR using a virus-specific primer set. None of the offspring from AxCAN-Kitl- or AxCAN-LacZ-transduced ovaries showed evidence of germline integration (Fig. 3D), suggesting that the reduced number of secondary follicles might have been an indirect effect of Kitl expression in surrounding cells.

**Rescue of infertility in Kitl(−/−)/KitlSl-t mice**

Because experiments using wild-type ovaries showed that AxCAN-Kitl transduction does not impair fertility, we next tried to restore fertility in congenitally infertile Kitl(−/−)/KitlSl-t mice [8]. The size of the ovaries was significantly reduced in these mice (Fig. 4A). Histological analysis of the ovaries showed that they contained only primordial follicles (Fig. 4B). Owing to the lack of growing oocytes, none of the more than 100 female mutant mice housed with wild-type males produced offspring. Although the exact nature of this mutation remains to be determined, we recently found that KitlSl-t/KitlSl-t female mice completely lacked Kitl expression in their ovaries [7]. Therefore, the mutation responsible for the Kitl(−/−)/KitlSl-t phenotype likely disrupts the regulatory mechanism, thereby reducing the levels of Kitl mRNA in the ovary. In contrast, Kitl(−/−)/KitlSl-t male mice exhibit normal spermatogenesis and are fertile, despite two point mutations in the Kitl gene [7]. Analysis of the peripheral blood of these mice revealed elevated FSH and LH levels [7]. In contrast, the estrogen levels were significantly reduced. Therefore, these mice can serve as models for primary gonadal failure in humans.

We microinjected AxCAN-Kitl into the ovaries of Kitl(−/−)/KitlSl-t mice and examined the impact of Kitl overexpression. Histological analysis of the ovaries at 1 month after microinjection showed evidence of resumed oogenesis (Fig. 4B). Although only primordial follicles were found in the uninjected ovaries, a small number of oocytes grew in secondary follicles. No evidence of lymphocyte infiltration or tumor formation was observed. No abnormal theca cell proliferation was observed in the ovaries. As these results suggested that AxCAN-Kitl overexpression can stimulate oogenesis, we microinjected AxCAN-Kitl into the ovaries of five mature female mice (Table 1). One week after the microinjection, the mice were housed with KitlSl-t/KitlSl-t male mice to produce offspring. Although four mice remained infertile, one female bore two female offspring 63 days after AxCAN-Kitl microinjection, demonstrating restoration of normal fertility (Fig. 4C). Despite successful offspring production, analysis of the peripheral blood of AxCAN-Kitl-transduced mice showed no apparent changes in the levels of estrogen, FSH, or LH (Fig. 4D).

**DNA analysis of offspring**

To test whether AV transduction resulted in integration of the transgene, we collected DNA from the offspring produced by treated mice and examined their transgene integration patterns. Using an AV-specific probe, we first performed a southern blot analysis and found no evidence of transgene integration (Fig. 5A). We then performed more sensitive PCR analysis, which confirmed the lack of transgene integration (Fig. 5B).

Because genomic imprinting in the female germline is initiated after birth [22], it is possible that forced expression of Kitl causes abnormal imprinting. To test this possibility, COBRA was performed on the DNA of the mature offspring. Control DNA from germline stem (GS) cell cultures showed complete androgenetic DNA methylation patterns. Although the DMR of H19 was heavily methylated, no methylation was apparent in the DMR of Igf2r. In contrast, the offspring of Kitl(−/−)/KitlSl-t mice exhibited somatic cell methylation patterns, and both the H19 and Igf2r DMRs were partially digested with methylation-specific restriction enzymes, similar to those found
in offspring born after natural mating (Fig. 5C). Bisulfite sequencing of the H19 and Igf2r DMRs also confirmed the somatic cell type imprinting patterns (Fig. 5D). These results suggest that AV-mediated ovarian rescue does not induce abnormalities in offspring.

Discussion

Oogenesis involves numerous genes that require complex cellular and hormonal interactions [1, 2]. Therefore, it is not surprising that a large proportion of couples have impaired fertility. Currently, approximately 10% of women of reproductive age are unable to conceive or carry a pregnancy to term [23]. Owing to rapid advances in elucidating the mechanism of oogenesis in recent decades, the basic mechanism of oogenesis in mice is now well understood. However, few attempts have been made to restore fertility caused by defective oogenesis in vivo. The current study aimed to establish a strategy to rescue oogenesis in KitlSl-t/KitlSl-t mice, which only have primordial follicles. These mice resemble the primary gonadal failure in humans, which accounts for approximately 1% of human infertility cases [24]. This defect is based on the deregulation of Kitl expression in GCs, which is likely the most intensively studied signal in the crosstalk between oocytes and GCs. Therefore, the analysis of this mouse model will provide a useful platform for the development of gene therapies for ovarian defects.

Our results using wild-type mice suggest that KITL overexpression did not deplete the oocyte pool after transduction. This was one of the greatest concerns in the current study because we expected that excessive KITL expression might trigger the activation of numerous oocytes or interfere with ovulation. However, we did not find such abnormalities, but instead noticed that they sired their offspring in relatively shorter periods after AV transduction. Considering that the size of the litters was comparable between control and KITL-expressing animals, these results suggest that KITL is not directly involved in determining the number of activated oocytes. Rather, it promotes the development of oocytes that are committed to activation. Although the number of animals in the current study may be too small to conclude such an effect, further investigation of this point by experiments on a larger scale will clarify the role of the KITL-KIT interaction in oocyte development. As the aim of this experiment was to confirm the normal fertility of animals with AVs, we applied AVs to infertile KitlSl-t/KitlSl-t mice. Our successful fertility restoration in KitlSl-t/KitlSl-t mice suggests that AVs could be used to restore fertility in animals with ovarian defects. Because AVs cannot penetrate the blood-testis barrier [18], we initially thought that AVs are not useful for ovarian gene therapy. However, we found evidence of follicular infection with AVs in R26R-Eyfp mice. We do not currently know how AAVs (25 nm) or AVs (90 nm) can penetrate BFB, which serves as a molecular sieve.
to prevent the diffusion of proteins > 20 nm in size [25–27]. One possibility for this is transcytosis. Transcytosis pathways are utilized by blood components and by viruses, such as human immunodeficiency virus and poliovirus [28]. AAVs have been shown to penetrate the epithelial barrier through transcytosis [29]. However, transcytosis of AAVs across the blood–brain barrier appears to be inefficient or impossible [28]. Because the composition and nature of barrier function likely differ in the ovary, whether AAVs employ transcytosis to penetrate the BBB remains to be determined.

The birth of offspring after AV transduction confirms our previous observation that oogenesis can resume with appropriate stimulation even in mature infertile female mice [7]. The remarkable flexibility of the oocyte-GC interaction is reminiscent of our previous research on the testes. Adult Sertoli cells were found to support spermatogenesis in undifferentiated spermatogonia that had never been exposed to membrane-bound Kitl during postnatal testicular development. To date, three types of viruses—AAVs, AVs, and lentiviruses—have been used for fertility restoration in male animals [18, 19, 30]. However, the efficiency of this method in males may be insufficient because no offspring are produced through natural mating. All offspring were born by collecting sperm from testes for microinsemination. This low efficiency is likely due to the relatively small amount of sperm that could mature through simple Kitl overexpression. In this sense, the present results in female mice are striking, as they produce offspring through natural mating. This finding is even more surprising considering that KitlSl-t/KitlSl-t mice may have additional defects in the oviduct, as neural crest cells, which also depend on KIT, colonize the oviduct and serve as pacemaker cells [31].

Fertility restoration by AVs was unexpected because of the previous failure to restore fertility in Fshr knockout mice via AV transduction [32]. Because the types of mutations and vectors used in the study are different, a direct comparison between the two studies is not easy. However, the constitutive CMV5 promoter used in a previous study was speculated to have been downregulated to support the increased expression of Lhr in the later phase of oogenesis. Although the use of the authentic human Fshr promoter may prevent such problems, the reconstruction of the complex inverse regulatory loop between Fshr and Lhr in the growing follicle that orchestrates successful maturation and ovulation is a daunting task. Although we were able to rescue fertility in the present study by simply overexpressing Kitl, these results suggest that the development of vectors reflecting endogenous gene expression patterns may be a more appropriate method, depending on the source of the infertility. Therefore, AVs may be preferable to AAVs because of their larger cargo size, which would allow a larger promoter to be incorporated into the virus.

One important concern in AV-mediated gene therapy is inflammation, which is a major problem with this vector [11]. In our previous study on male mice [19], we used AVs to treat spermatogenic defects because the testis is an immune-priviledged part of the body [33]. Therefore, we hypothesized that the suppression of immunity may modulate inflammation caused by AVs. Although AV infection attracted lymphocytes after microinjection in the seminiferous tubules of the testis, spermatogenic recovery was not inhibited, and it was possible to obtain sperm for offspring production. Because the ovary is also considered an immune-privileged site [34], we expected that AV infection might also be attenuated in the ovary relative to other organs. As expected, infiltration of lymphocytes was observed but did not persist in the long term, and the ovaries were functionally normal in the production of offspring. Therefore, AVs did not compromise fertility despite the apparent induction of inflammation.

Given these results, the most important concern for human applica-

tion is germline integration of the transgene. Fortunately, offspring born after gene therapy did not carry the transgene, indicating no integration into the germline. As the life cycle of wild-type AV is extrachromosomal [11], AV vectors are considered non-integrating vectors. However, it was previously observed that injection of AVs can result in chromosomal integration in vivo [35]. In a previous study, 0.0001–1% of cells showed evidence of integration when the frequency was estimated in vitro [36]. In vivo integration has also been reported for AAVs [37]. Similar to AVs, most AAVs exist in an extrachromosomal state. AAV integration can occur at non-homologous locations that are sites of DNA damage, or at specific sites via homologous recombination [38]. Therefore, further studies are required to examine the safety of gene therapy in fertility treatment.

We observed impairment of the hormonal response despite successful offspring production. KitlSl-t/KitlSl-t mice have elevated levels of FSH and LH, resembling primary gonadal failure in humans [7]. Hormonal regulation is important not only for ovulation, but also for oviduct transport in embryos. Since pacemaker cells that drive such transport also depend on Kit signaling [31], it is possible that KitlSl-t/KitlSl-t mice have an abnormal oviduct. However, the birth of offspring suggests that an appropriate hormonal response and gamete/embryo transport occurred in KitlSl-t/KitlSl-t mice. Although we do not yet know the mechanisms underlying these observations, we speculate that AV transduction transiently restores the hormonal response and allows for normal ovulation and offspring production. Because oogenesis is accompanied by GC proliferation, and AVs cannot integrate into the host genome, it is likely that Kitl-expressing GCs cannot maintain transgene expression at a constant level throughout oogenesis. It is possible that AVs may gradually become depleted in infected cells after several rounds of cell division, resulting in an impaired hormonal response. However, further research is required to examine these mechanisms.

Our results on AV-mediated gene delivery raise the possibility that gene therapy may be applied to human female infertility in the future. Through extensive analysis of the genes involved in fertility, the number of candidate genes for female infertility is increasing rapidly [23]. Our strategy based on AV- or AAV-based gene delivery into ovaries may be applicable to some of these genes. Because AVs can be applied locally to the ovarian stroma, they are safer than systemic injections, which often cause severe side effects in other organs. Our successful rescue of infertility by AVs increases the range of target diseases because they have a large cargo size, which allows the incorporation of large promotors to achieve sufficient or physiological gene expression levels. The identification of appropriate target genes is necessary at this stage of research, as it investigates the risk of transgene integration through large-scale experiments with other animal models. Such endeavors may eventually overcome fertility problems that cannot be treated using conventional assisted reproduction technologies.

Conflicts of interests: The authors declare no conflicts of interest associated with this manuscript.

Acknowledgments

We thank J. Yang for the technical assistance. Financial support for this research was provided by AMED (1733225, JP21gm1100008) and MEXT (19K22512, 19H05750, 19H04906, 18H04882, 18H05281, and 18H02935).
References

1. Gooden RG. Oocyte development and loss. Semin Reprod Med 2013; 31: 393–398. [Medline] [CrossRef]
2. Matozak MM, Burns KH. Vulneros MM, Eppig JJ. Intercellular communication in the mammalian ovary: oocytes carry the conversation. Science 2002; 296: 2178–2180. [Medline] [CrossRef]
3. Kiddie GM, Naucke-Cohen BC. Bidirectional communication between oocytes and follicle cells: ensuring oocyte developmental competence. Can J Physiol Pharmacol 2010; 88: 399–413. [Medline] [CrossRef]
4. Yoshida H, Takakura N, Kataoka H, Kuniyoda T, Okamura H, Nishikawa S. Stepwise requirement of k-catenin kinase in mouse ovarian follicle development. Dev Biol 1997; 184: 122–137. [Medline] [CrossRef]
5. Zachariás F. Studies on the mechanism of ovulation: permeability of the blood-liquid barrier. Acta Endocrinol (Copenh) 1958; 27: 339–342. [Medline] [CrossRef]
6. Siu MKY, Cheng CY. The blood-follicle barrier (BBF) in disease and in ovarian function. Adv Exp Med Biol 2012; 763: 186–192. [Medline] [CrossRef]
7. Kanaori-Shinohara M, Lee J, Miyazaki T, Morimoto H, Shinohara T. Adeno-associated-virus-mediated gene delivery to ovaries restores fertility in congenitally infertile mice. Gene therapy treats congenital female infertility in mice. Cell Rep 2022; 3: 100606. [Medline] [CrossRef]
8. Kusohra T, Yokoyama M, Taguchi T, Kitamura Y, Tsuchikawa K. Effect of the Slt mutant allele on the production of tissue mast cells in mice. J Histochem 1985; 74: 175–376. [Medline] [CrossRef]
9. Wang D, Tai PWL, Gao G. Adeno-associated virus vector as a platform for gene therapy delivery. Nat Rev Drug Discov 2019; 18: 388–378. [Medline] [CrossRef]
10. Collada P, Ronzulli G, Minguasso F. Emerging issues in AAV-mediated in vivo gene therapy. Mol Ther Methods Clin Dev 2017; 5: 87–104. [Medline] [CrossRef]
11. Lee CS, Bishop, Zongh R, Xu Y, Farina EM, Yun S, Zhao C, Zheng Z, Shu X, Wu X, Lei J, Li Y, Zhang W, Yang C, Wu K, Wu Y, Ho S, Athirivatham A, Lee ML, Wol JMF, Reid RR, He TC. Adenovirus-mediated gene delivery: Potential applications for gene and cell-based therapies in the new era of personalized medicine. Genes Dis 2017; 4: 45–63. [Medline] [CrossRef]
12. DeMatteo RP, Chu G, Ahn M, Chang E, Barker CF, Markmann JF. Long-lasting adenovirus transgene expression in mice through neonatal intrathymic tolerance induction without the use of immunosuppression. J Virol 1997; 71: 3350–3353. [Medline] [CrossRef]
13. Bouard D, Alazard-Dany D, Cossel FL. Viral vectors: from virology to transgene expression. Br J Pharm 2009; 157: 153–165. [Medline] [CrossRef]
14. Pfeil N, Wudrich H. Imaging the adenovirus infection cycle. FEMS Lett 2019; 593: 3419–3448. [Medline] [CrossRef]
15. Serranas S, Watanabe T, Lin CS, William CM, Tanabe Y, Jessell TM, Costantini F. Cre reporter strains produced by targeted insertion of EVFP and ECFP into the ROSA26 locus. BMC Dev Biol 2001; 1: 1. [Medline] [CrossRef]
16. Takehashi M, Kanatsu-Shinohara M, Inoue K, Ogounoki N, Mikih H, Toyokuni S, Ogora A, Shinohara T. Adenovirus-mediated gene delivery into mouse spermatogonial stem cells. Proc Natl Acad Sci USA 2007; 104: 2596–2601. [Medline] [CrossRef]
17. Watanabe S, Kanatsu-Shinohara M, Ogounoki N, Matoba S, Ogura A, Shinohara T. Adeno-associated virus-mediated delivery of genes to mouse spermatogonial stem cells. Biol Reprod 2017; 96: 221–231. [Medline] [CrossRef]
18. Watanabe S, Kanatsu-Shinohara M, Ogounoki N, Matoba S, Ogura A, Shinohara T. In vivo genomic manipulation of spermatogonial stem cells and their microenvironment by adenovirus-associated viruses. Stem Cell Reports 2018; 10: 1551–1564. [Medline] [CrossRef]
19. Kanatsu-Shinohara M, Ogora A, Ikegawa M, Inoue K, Ogura N, Tashiro K, Toyokuni S, Honjo T, Shinohara T. Adenovirus-mediated gene delivery and in vivo microinjection produce offspring from infertile male mice. Proc Natl Acad Sci USA 2002; 99: 1383–1388. [Medline] [CrossRef]
20. Flanagan JG, Chan DC, Ledet P. Transmembrane form of the kit ligand growth factor is determined by alternative splicing and is missing in the Slt mutant. Cell 1991; 64: 1015–1035. [Medline] [CrossRef]
21. Rablin BP, Singer S, Taso C, Dusenian A, Lux ML, Ruiz R, Hibbard MK, Chen CJ, Xiao S, Tuveon DA, Demetri GD, Fletcher CD, Fletcher JAT. KIT activation is a ubiquitous feature of gastrointestinal stromal tumors. Cancer Res 2001; 61: 8118–8121. [Medline] [CrossRef]
22. Yang Y, Kono T. Maternal primary imprinting is established at a specific site for each gene throughout oocyte growth. J Biol Chem 2002; 277: 5285–5289. [Medline] [CrossRef]
23. Yatsenko SA, Rajkovic A. Genetics of human female infertility. Biol Reprod 2019; 101: 580–566. [Medline] [CrossRef]
24. Nelson LM. Clinical practice. Primary ovarian insufficiency. N Engl J Med 2009; 360: 506–614. [Medline] [CrossRef]
25. Gooden RG, Hunter RH, Telfer E, Torrance C, Brown N. Physiological factors underlying the formation of ovarian follicular fluid. J Reprod Fertil 1988; 82: 813–825. [Medline] [CrossRef]
26. Horwitz ED, Rahman KS, Bower BD, Dmasuke DF, Falvo MR, Griffith JD, Harvey SC, Asokan A. Biophysical and ultrastructural characterization of adeno-associated virus capsid uncoating and genome release. J Virol 2013; 87: 2994–3002. [Medline] [CrossRef]
27. Ahl YA, Mittal SK. Components of adenovirus genome packaging. Front Microbiol 2016; 7: 1503. [Medline] [CrossRef]
28. Tsang Y, Han T, Everts M, Zhu ZB, Gillespie CY, Curiel DT, Wu H. Directing adenovirus across the blood-brain barrier via melanotransferrin (P97) transcytosis pathway in an in vitro model. Gene Ther 2007; 14: 523–532. [Medline] [CrossRef]
29. Di Pasquale G, Chiorini JA. AAV transcytosis through barrier epithelia and endothelia. Mol Ther 2006; 13: 506–516. [Medline] [CrossRef]
30. Itaka M, Tergaonkar V, Ogura A, Ogounoki N, Inoue K, Verma IM. Restoration of spermatogenesis by lentiviral gene transfer: Offspring from infertile mice. Proc Natl Acad Sci USA 2002; 99: 7524–7529. [Medline] [CrossRef]
31. Dixon RE, Hwang SJ, Hennig GW, Ramsay KI, Schriepsema JH, Sanders KM, Ward SM. Chlamydia infection causes loss of pacemaker cells and inhibits oocyte transport in the mouse oviduct. Biolog Reprod 2009; 80: 665–673. [Medline] [CrossRef]
32. Ghadami M, Salama SA, Khatoum N, Chitvers R, Nagamani M, Cheedrese PJ, Alexander A. Toward gene therapy of primary ovarian failure: adenovirus expressing human FSH receptor corrects the Finnish C567T mutation. Mol Hum Reprod 2008; 14: 9–15. [Medline] [CrossRef]
33. Chen Q, Deng T, Han D. Testicular immunoregulation and spermatogenesis. Semin Cell Dev Biol 2016; 59: 157–165. [Medline] [CrossRef]
34. Newell MK, Villabosch-Meney E, Schweitzer SC, Harper ME, Camley RE. Cellular metabolism as a basis for immune privilege. J Immunol Based Ther Vaccines 2006; 4: 1. [Medline] [CrossRef]
35. Stephen SL, Montini E, Sivanandam VG, Al-Dhalmy M, Kestler HA, Finagold M, Groupe M, Kochanek S. Chromosomal integration of adenoviral vector DNA in vivo. J Virol 2010; 84: 9987–9994. [Medline] [CrossRef]
36. Mittani K, Kabo S. Adenovirus as an integrating vector. Curr Gene Ther 2002; 2: 135–144. [Medline] [CrossRef]
37. Li H, Malani N, Hamilton SR, Schlachterman A, Bussadori G, Edmonsson SF, Shah R, Arruda VR, Minguazzi F, Wright JF, Bushman FD, High KA. Assessing the potential for AAV-vector genotoxicity in a murine model. Blood 2011; 117: 3311–3319. [Medline] [CrossRef]
38. Doyle DR, Russell DW. Adeno-associated virus vector integration. Curr Opin Mol Ther 2009; 11: 442–447. [Medline]