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Would gene therapy for the treatment of male infertility be safe?

Dolores J Lamb

Lester and Sue Smith Chair in Urologic Research, Professor of Urology and Molecular and Cellular Biology, and Vice Chairman for Research at the Scott Department of Urology, Baylor College of Medicine, Houston, TX, USA

Gene therapy offers the potential to correct genetic defects, including reproductive defects in men with infertility. The safety of gene therapy for male infertility is a major issue that must be addressed, and the vector employed for gene therapy is of paramount importance. The ideal vector should be immunologically inert, target only cell types with the deficiency, be capable of delivering large pieces of genetic material, integrate into an exact site in a chromosome or work as an episome, have a mechanism to limit or even destroy the function if necessary, and infect both dividing and nondividing cells.¹ This article will discuss the possible gene therapy vectors for male infertility, and the safety issues involved.

A range of approaches are under development for gene therapy, including chemical methods (e.g. liposomes, microparticles, nanoparticles, microadhesive particles and gels and peptide ligands), mechanical approaches (e.g. gene gun, liquid jet injection, nebulizer and microenhance arrays), physical techniques (e.g. electroporation, sonoporation, magnetofection and laser irradiation) and biological processes (e.g. bacterial vector, bacterial ghost and viruses).² Not all approaches, however, would be useful for male infertility, given their mode of administration and pharmacokinetics.²

The most commonly used method for gene therapy employs viral vectors. Some retroviral vectors preferentially integrate reverse transcribed DNA into genes, others into 5' ends of transcription units, while others show no preference.³ Integration "hotspots", however, have been recognized, such as the region at 11q13. The site of integration may cause adverse and unexpected consequences, as evidenced in a study for children with X-linked severe combined immunodeficiency. When a murine-leukemia-virus-based vector was administered to correct this genetic syndrome, 10–35% of children developed leukemia. The vector integrated in a nonrandom manner to a "dangerous" region of the genome near the *LMO2* protooncogene.⁴ This process is well recognized, and is termed insertional mutagenesis.

Gene therapy with adeno-associated virus (AAV) has also resulted in unanticipated adverse events. A trial with AAV vectors for the treatment of hemophilia B resulted in rises in serum transaminidases, and contamination of semen with the vector.⁵ Other problems encountered after viral-based gene therapy have included toxicity and immune and inflammatory responses. The host immune system generates cytotoxic T cells and antibodies to viral proteins, and the transgene itself may be recognized as foreign, with induction of both the humoral and cellular immune response. Each type of viral vector varies in terms of immunogenicity. A notable failure during a phase I trial for gene therapy was the death of

Jesse Gelsinger, an 18-year-old volunteer with ornithine transcarbamylase deficiency.^{1,6} He died of a severe immune response to the vector, resulting in fulminate respiratory failure. Two other participants also experienced an adverse reaction,⁶ and two primates had previously died during preclinical testing. These cases highlight the risk of potential complications from unrecognized genetic variants.

Other concerns with viral vectors include the biodistribution of the vector, which varies by vector and mode of administration. Retroviral vectors are frequently observed in the gonads, and sometimes within the genital tract.⁷ Germ line transmission did not occur in the trials discussed above; however, inadvertent vector integration in the germ line could occur: endogenous retrotransposon insertional mutations in humans are estimated in about 12% of individuals.⁸ This frequency is higher than the upper limit of insertion events in sperm suggested by the FDA for gene therapy strategies, which is one per 6,000 sperm.⁸

Despite these concerns, agents and treatments that modify the germ line genome have been recognized for decades. Chemical and radiation mutagen exposures have resulted in male-mediated germ line modifications in humans,⁹ and naturally occurring retrotransposons can genetically alter the germ line⁸ in a manner analogous to retroviral vectors.¹⁰ Furthermore, human germ line genetic intervention has inadvertently occurred in studies of oocyte cytoplasmic transfer from ova of young women to ova of older women.^{11,12} Not only ooplasm proteins, but also mitochondria, which carry their own genome, were transferred. The discovery of this heteroplasmy led the FDA to restrict the use of this assisted reproductive procedure.

Nonviral vectors for gene therapy present different challenges from viral vectors. Nonviral vectors (including cationic polymers [nanoparticles] with cell-targeting functional groups, cationic lipids, gold, other nanoparticles and naked DNA) may provide a safer, more versatile approach than viral vectors.¹³ Nonviral vectors are not infectious, but deliver plasmids, oligonucleotides or small interfering RNAs to cells. Usually, they will block or silence expression of defective genes rather than replace them. However, like viruses, some nonviral vectors (e.g. lipoplexes, nanoparticles etc.) induce a rapid immune response, generating high levels of proinflammatory cytokines, which can detrimentally affect sperm function.¹⁴ The toxicity of nonviral vectors is not fully understood, particularly that of nanoparticles. Free radicals can be generated, inducing inflammatory reactions, with nanoparticles accumulating in the liver, spleen, lymph nodes and bone marrow.¹⁵ Free radicals also adversely affect sperm and can result in genotoxicity, inflammation, nuclear and DNA damage, mitochondrial disruption, protein denaturation and cell apoptosis. Other cytotoxic adverse effects vary according to the size and concentration of the nanoparticles, as well as their composition, solubility and geometry.¹⁵ Another issue with nonviral vectors is delivery: extracellular barriers can prevent delivery of nonviral vectors, or the delivery method could disrupt the blood–testis barrier. Furthermore, the efficiency of nonviral vectors is low compared with viral vectors, although nonviral vectors have the potential to correct disorders where low-level expression is sufficient to alleviate symptoms. A problem with all methods of gene therapy is that gene transfer efficiency may vary, resulting in a mosaic state, and in essence only partially correcting the defect. Preimplantation genetic diagnosis could conceivably be required to confirm the genetic status of the man's offspring at the embryo stage.

Other approaches to gene therapy, such as artificial chromosomes, xenotransplantation and cross-species transfer of viruses, have potential, but are far from clinical reality. Issues such as unrecognized viral pathogenicity raise concerns for xenotransplantation.¹⁶

The safety of gene therapy for male infertility should be considered in comparison with the safety of currently available treatments. For example, intracytoplasmic sperm injection (ICSI) is a treatment available to some infertile men, but children conceived by ICSI have an increased risk of chromosome abnormalities, birth defects, hormonal dysfunction and epigenetic risks. If a suitable vector could be developed, the genetic abnormalities associated with male infertility could be resolved, and gene therapy could be considered safer than ICSI. This article highlights, however, that a vector that meets all the requirements for safety and efficacy for gene therapy is lacking; until such a vector is available, gene therapy for the treatment of male infertility cannot be considered safe.

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