Biological therapy for non-obstructive azoospermia

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ABSTRACT

Introduction: Most male patients with non-obstructive azoospermia (NOA) have no therapeutic options outside of assisted reproductive techniques to conceive a biological child. If mature sperm cannot be obtained from the testes, these patients must rely on options of donor sperm or adoption. Several techniques are in the experimental stage to provide this patient population alternatives for conceiving. Areas covered: This review discusses three of the experimental techniques for restoring fertility in men with NOA: spermatogonial stem cell transplantation, the use of adult and embryonic stem cells to develop mature gametes and gene therapy. After this discussion, the authors give their expert opinion and provide the reader with their perspectives for the future. Expert opinion: Several limitations, both technical and ethical, exist for spermatogonial stem cell transplantation, the use of stem cells and gene therapy. Well-defined reproducible protocols are necessary. Furthermore, several technical barriers exist for all protocols. And while success has been achieved in animal models, future research is still required in human models.

1. Introduction

Infertility affects approximately 15% of couples of reproductive age. Male factor infertility plays a role in 50% of these cases. Among patients with male factor infertility, approximately 10–15% have azoospermia [1]. Azoospermia is defined as the absence of spermatozoa in the ejaculate. This condition is typically classified as obstructive azoospermia (OA) or non-obstructive azoospermia (NOA). After confirmation of NOA with a second semen analysis, the workup of azoospermia involves a detailed history and physical examination along with hormonal and genetic testing. Testicular biopsy allows for a histologic diagnosis although the underlying cause of the azoospermia may never be determined. OA is characterized by an obstructed flow of spermatozoa at any point along the male genital tract. Spermatogenesis in the testis is usually normal in these patients. The etiology of NOA is more varied. Normal spermatogenesis requires intact and functioning seminiferous tubules as well as functioning Leydig cells for hormonal support of spermatogenesis by producing testosterone. NOA can be broadly classified into primary testicular failure and secondary testicular failure. Primary testicular failure refers to pathology localized to the testis resulting in elevated Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH). Known genetic causes of infertility such as Klinefelter’s syndrome and Y chromosome microdeletion will cause primary testis failure. Local effects from chemotherapy or radiation can also cause primary testis failure and result in NOA. A significant proportion of primary testicular failure cases are diagnosed as idiopathic, which limits therapeutic options.

Secondary testis failure is caused by impaired pituitary secretion of gonadotropins, which leads to insufficient stimulation of the Sertoli and Leydig cells in the testis. Kallmann syndrome results from a lack of hypothalamic Gonadotropic Releasing Hormone (GnRH) secretion, which leads to insufficient production of LH and FSH. This subsequently leads to secondary testicular failure as the Sertoli cells are unable to support spermatogenesis and the Leydig cells are insufficiently stimulated to provide local testosterone secretion for spermatogenesis. Men who take supplemental testosterone will also develop secondary testicular failure because the supplemental circulating testosterone suppresses FSH and LH production.

Men with NOA have limited options for reproduction. Testicular sperm extraction with Intracytoplasmic Sperm Injection (ICSI) is possible in these patients, but it is expensive and associated with high morbidity in the female partner. If sperm cannot be retrieved by testicular sperm extraction, there are no current options to maintain the reproductive potential of these individuals. Therefore, there is significant clinical demand for alternate therapies for NOA that would allow the production of mature spermatozoa either using stem cells or by gene therapy for patients with idiopathic causes of infertility. Such a technique might allow for natural conception in a population that is otherwise relegated to assisted reproductive techniques. This review will cover three experimental approaches for restoring fertility in men with NOA: spermatogonial stem cell (SSC) transplantation, in vitro spermatogenesis using adult or embryonic pluripotent stem cells, and gene therapy.
2. SSC transplantation

SSCs are precursors to mature spermatids that reside in the basal compartment of the seminiferous tubules of the testis. SSCs have the capability to self-renew and enter into meiosis to become a spermatocyte [2]. The spermatocytes then differentiate into haploid spermatids, which are transformed into mature spermatozoa. The SSC population is therefore critical in the production of sperm.

Sertoli cells surround the SSCs and provide signaling molecules and factors to support both the self-renewal and differentiation process. This microenvironment is referred to as the stem cell niche [3]. Recreating the SSC niche in the laboratory setting is a critical step in the development of SSC transplantation. The concept of transplanting SSCs to enable normal spermatogenesis in an otherwise sterile individual has promising clinical applications in men with NOA.

Transplantation of SSCs was first described by Brinster and Zimmerman in 1994 when suspensions of testicular cells from fertile mice were transplanted into infertile mice resulting in fertility restoration and progeny [4]. Subsequently, it has been shown that cryopreservation of SSCs does not impair their ability to produce healthy spermatozoa capable of fertilization [5]. Currently, two strategies exist for transplantation of SSCs – testicular tissue harvest and grafting and SSC isolate injection.

Testicular tissue grafting retains the natural stem cell niche, which may be optimal. However, this approach has not yet been optimized for cancer patients due to concerns for cancer cell contamination. Cryopreservation protocols for testicular tissue are well established in most centers using a slow freezing protocol with dimethyl sulfoxide [6]. Keros et al. found that a slow freezing protocol with dimethyl sulfoxide led to improved tubule integrity, fewer damaged spermatogonia, and better maintenance of the lamina propria when compared to cryopreservation protocols with glycerol and propanediol [6]. Testosterone production was also better maintained in the Dimethyl sulfoxide (DMSO) protocol [6]. However, ideal concentrations of dimethyl sulfoxide remain to be established.

Xenograft models have been used to store and support SSCs and testicular tissue in several animal models [7]. Wyns et al. describe using a mouse xenograft model for cryopreserved immature testicular tissue harvested from prepubertal males [8]. This work still requires a major advancement to be clinically relevant: the transfer of the tissue back into the patient post-pubertally with successful regeneration of spermatogenesis.

Testicular tissue grafting can be performed in several locations. The ideal location for graft transplantation is the scrotum because its temperature is lower than that in other sites that have been applied in animal models such as the peritoneum or under the skin on the back [9]. Grafting in ectopic sites also requires that the patient use Assisted Reproductive Techniques (ART) for conception. Some authors recommend grafting to several sites to optimize yield [10]. Timing of grafting, prepubertal versus postpubertal, has also been debated. High FSH and LH concentrations are required for successful proliferation of the graft; therefore, the peri-pubertal period may be an ideal target time, but this has not been explored. Because prepubertal boys do not yet make enough FSH and LH to support spermatogenesis, the ideal timing for grafting may be after patients have reached sexual maturity [10].

Schlatt et al. performed a grafting procedure in neonatal mouse model after castration to maintain high levels of FSH and LH [11]. This method, of course, cannot be replicated in humans, but it provides evidence that high levels of gonadotropins are necessary for successful grafting.

SSC injection is the other method of transfer to the recipient. The rete testis, efferent ducts, or the seminiferous tubules have been identified as injection targets for SSCs in several species [12]. Upon injection, the SSCs migrate to the basement membrane of seminiferous tubules and subsequently self-renew and differentiate to establish spermatogenesis in the recipient. This process should theoretically allow for conception without the requirement of assisted reproductive techniques, which is a major advantage. Hermann et al. injected adult rhesus monkey SSCs into the rete testes of recipient prepubertal rhesus monkeys and showed that mature spermatozoa were present in the ejaculate when they reached maturity. These spermatozoa demonstrated fertilizing capabilities when used with ICSI [13].

At present, SSC transplantation is experimental and has not been done in human models, but research thus far is promising. Several challenges have plagued the transformation of this idea into a real clinical therapy for patients: identifying the SSC population in the testis, culturing the SSCs, storing of the SSCs, and reintroducing the cells safely into a recipient. Several groups have been able to isolate human SSCs and culture and store the cells. However, well-defined protocols that are easily replicated are needed. Additionally, confirming the successful introduction of the SSCs into a human recipient is difficult as one must distinguish between native SSCs and transplanted SSCs as well as native mature spermatozoa and transplanted spermatozoa [14]. Finally, after successful SSC transplantation, it is essential to prove the fertilization potential of the mature spermatozoa in the recipient. Because there is some evidence of SSC loss after transplantation, a sufficient population of SSCs must be harvested. However, the results have poor reproducibility and the process is inefficient as demonstrated by a study by Wyns et al. showing 14.5% of the spermatogonial population remained at three weeks after grafting in a mouse population [15].

SSC transplantation resulting in mature gametes with fertilization potential has not yet been replicated in the human
In vitro gene therapy could develop a potential benefit for men facing gonadotoxic treatment for malignancy who desire biological children in the future. Additionally, prepubertal boys with a cancer diagnosis might benefit from this option, particularly given the fact that obtaining mature spermatozoa prior to treatment is not an option. Similarly, adult azoospermic men with Sertoli cell-only syndrome could be treated with transplantation of SSCs to repopulate the germline epithelium. Patients with Klinefelter’s syndrome demonstrate progressive fibrosis of the seminiferous tubules over their lifetime and may also be candidates for SSC transplantation if harvested early in life. For patients with genetic defects causing NOA such as Y-microdeletions, genomic editing would be required to remove the mutation. If SSC transplantation combined with genetic editing was successful, this procedure could also be applied to fertile patients with genetic diseases at a single locus to prevent transmission to offspring [16].

3. In vitro spermatogenesis using pluripotent stem cells

An alternative method for developing mature gametes in men with NOA is the use of pluripotent stem cells. This technique could be applied to adult males with idiopathic NOA. In order to develop germ cells from pluripotent stem cells, several discrete steps are required, each with specific stimuli required for progression to the next stage of development. In addition, the differentiation process that occurs in vitro does not always emulate the process that occurs in vivo. Despite these challenges, pluripotent stem cell lines may enable development of disease models for infertility to allow for development of new therapeutics.

There are two stem cell sources (in addition to SSCs) that may be used for generating germ cells: human embryonic stem cells and adult pluripotent stem cells [17]. Human embryonic stem cell lines have been studied for several years. Toyooka et al. generated male germ cells from mouse embryonic stem cells. However, the fertilization potential of the mature sperm was not studied [18]. Subsequent studies have shown that haploid spermatids developed from mouse embryonic cells have fertilization potential as demonstrated by live births [19]. Studies with human embryonic stem cells have yet to yield functional haploid spermatozoa [17]. In addition, the use of embryonic stem cells is limited by the ethical concerns, further limiting the propagation of this technique toward clinical application.

Pluripotent adult stem cells are able to self-renew and differentiate into all three germ-layer cell types. These stem cells are developed from somatic cells, making them easily accessible. Zhu et al. demonstrated that adult mouse pluripotent stem cells can differentiate into SSCs and late-stage male germ cells with the combination of in vitro and in vivo harvest [20]. These authors could not demonstrate that these cells progressed to form mature spermatozoa; therefore, fertilization potential was not shown. Hayashi et al. successfully developed primordial germ cells from both embryonic stem cells and adult pluripotent stem cells in mice and were able to show fertilization potential with ICSI [21]. In order to prompt differentiation into germ cells from adult pluripotent stem cells, oncogenic factors must be used. Until another protocol is developed, adult pluripotent stem cells are not an option as a therapy for male infertility because of their tumorigenic risks [17].

4. Gene therapy

Gene therapy has already been applied in the research setting to many human disease processes. The technique involves adding a ‘normal’ gene to a patient’s genome, removing an ‘abnormal’ gene, or mutating an ‘abnormal’ gene to allow it to function appropriately. This requires a thorough understanding of the specific genetic defect underlying the pathologic process. Unfortunately, such knowledge is lacking in the majority of cases of NOA.

Germline genetic defects pose a unique challenge for gene therapy in that spermatogenesis originates from stem cells. Therefore, the SSCs would need to be genetically altered in order to pass the altered genetic material to all mature spermatozoa and progeny. SSC transplantation with genetic modification, as discussed previously, would be required.

Although gene therapy for male infertility has been successful in mouse models resulting in live progeny, it is not yet applicable for clinical practice in infertile patients with NOA for a number of reasons. As stated earlier, we currently do not have enough knowledge to fully understand the single gene defects that cause NOA. For example, altering germline genetics is illegal in humans at the present time in most countries, and insertion of genetic material into the germ cell line could lead to oncogenesis. Finally, it is evident that gene therapy – which is a very expensive therapeutic – would provide therapeutic benefit only for a small subset of patients with NOA. This includes some men with NOA caused by known genetic defects and those with primary ciliary dyskinesia, a genetic disorder that results in nonmotile spermatozoa. However, men with Klinefelter’s syndrome and Y-microdeletions would not be candidates for gene therapy due to the amount of DNA that would require addition or deletion. Without the knowledge of single gene defects that cause infertility in humans, gene therapy is not yet applicable for clinical practice. Substantial ethical and safety hurdles must be surpassed as well [22].

Promising research is being conducted to determine other genetic bases for NOA such as DNA methylation patterns and sperm mitochondrial genome deletions [23]. As further developments occur, more targets may develop for gene therapy. With time, the future may bring answers to these patients as to the cause of their fertility [24].

5. Conclusion

Existing therapies for patients with NOA who wish to conceive a genetically related child are limited and require harvesting mature spermatozoa from the male while the female endures the morbidity of assisted reproductive technology. The use of SSCs is promising, although their entrance into the clinical landscape awaits further optimization to ensure safety and reproducibility. The use of SSCs has the broadest applicability to subsets...
of the infertile male population and has been demonstrated successfully in animal models. The hurdles that remain include identifying ideal protocol for harvest, storage, and transfer in humans. Additionally, this option is not available to cancer patients, given the risk of oncologic recurrence. Further limiting the use of stem cell therapies for some patients with NOA is the potential requirement to manage the underlying genetic defect that lead to the disorder in addition to the stem cell transplantation. Stem cell therapy using embryonic stem cells or adult pluripotent stem cells is further from the clinical horizon as mature spermatozoa with capability to fertilize have not yet been developed, likely due to incomplete understanding of the stem cell niche required for spermatogenesis.

Gene therapy has been very promising to treat certain genetic diseases and does have some applicability to the male fertility stage for patients with single gene defects resulting in azoospermia. Until further single gene defects are identified and the ethical concerns over gene therapy for the germline are ameliorated, this option will not be widely applicable to the NOA population.

All novel therapeutics are expensive. Stem cell therapy at present is used off-label. Insurance does not usually provide any coverage, and the services can be cost prohibitive for many patients. If the biologic therapies discussed in this review reach the clinical setting, the procedures will undoubtedly be extremely costly, limiting the patients who can take advantage of these options. As supply increases and protocols are optimized, novel treatments tend to become more affordable. However, many existing therapeutics for both male and female fertility remain uncovered by insurance plans and require a significant payment from the patient. Not only will the treatments need to be more affordable, but the viewpoint that fertility treatments need not be covered by insurance in the United States will need to be modified. With regard to assisted reproductive techniques, data suggest that improved insurance coverage results in better outcomes in terms of fewer fresh embryos transferred and fewer multiple gestation pregnancies [25,26].

Despite the hurdles that remain, an impressive amount of progress has been made toward novel therapeutics for men with NOA who desire biological children.

6. Expert opinion

NOA is amenable to ICSI if mature spermatozoa can be obtained from the testicle. Unfortunately, not all men with NOA are candidates for ICSI, and the burden for the female is significant in terms of cost and morbidity. Men with maturation arrest and Sertoli cell-only pathology would benefit from the novel therapeutic options discussed here. The ability to repopulate the germinal epithelium of the seminiferous tubules with SSCs would drastically change the field of male infertility. Similarly, propagating adult or embryonic stem cells into mature spermatozoa would provide a therapeutic option for these patients. Gene therapy is applicable in a smaller population of patients but nonetheless would be a major advancement in the field. Despite the major advances made in the field of biologic therapy for male infertility, several obstacles exist to bring these to the clinical realm.

Ethical limitations create a significant barrier to performing the appropriate studies in humans. In the prepubertal patient population, storage of testicular tissue, SSCs, or stem cells is controversial, given the patient’s inability to provide his own consent or truly understand the implications of the procedure. Moreover, the use of embryonic stem cells is surrounded by controversy. Obtaining embryonic stem cells requires destruction of the embryo. The struggle to balance the need to cure disease with the moral obligation to protect future human life is inherent to the use of embryonic stem cells. Gene therapy for male infertility is similarly wrought with ethical concerns as manipulating the germline could have significant implications as a therapeutic option but also as a gateway to selecting characteristics to alter the genetics of populations.

Standardized protocols for tissue and cell culture and storage must be established prior to the use of these therapies in the clinical landscape. Although SSCs have been used with success in several animal models as well as across species, the results are not easily reproducible and the optimal site, method, and timing of injection or grafting are not yet well understood. The genetic stability of the cells after transplantation is critical as any acquired mutations will be passed to progeny. Pretransplantation culture and storage must be perfected to obtain genetic stability.

Future research is essential for further development of this promising field. Eliminating oncogenic risks must occur before studies in human subjects can take place. Of the existing novel therapeutics discussed, it is our opinion that SSC transplantation has the most promise and fewest limitations. It is reasonable to offer testicular tissue harvesting to prepubertal patients undergoing gonadotoxic treatments, ideally under a clinical research protocol, with the hope that these technologies will evolve to the clinical realm when these patients reach reproductive age.

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Declaration of interest

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References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (++) to readers.


• This paper is the first to describe spermatogonial stem cell transplantation in a mouse model.
- This is an important study demonstrating that healthy sperm can be derived from cryopreserved spermatogonial stem cells.


- An excellent summary of spermatogonial stem cell transplantation and the current challenges associated with this technique limiting use in humans.


- An important paper discussing the need for genomic editing in addition to spermatogonial stem cell autotransplantation in the treatment of NOA.


