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The Genetic and Phenotypic Basis of Infertility in Men with Pediatric Urologic Disorders

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Abstract

Of infertile couples, approximately one-third feature male factor issues. A subset of male factor infertility is attributable to congenital or acquired pediatric urologic conditions, and patients with these disorders are often challenging to treat. Prior to the development of in vitro fertilization (IVF) in the 1970s and intracytoplasmic sperm injection (ICSI) in 1992 [1], many forms of infertility due to pediatric urologic conditions were untreatable. These technologies have enabled many previously infertile men to father children, but the effectiveness of these techniques in men with pediatric urologic disorders has never been systematically examined. In this review we provide a contemporary survey of the fertility status of men with pediatric urologic disorders in light of advances in assisted reproductive technology (ART). We examine currently available treatments as well as developing technologies and discoveries that may apply to pediatric urologic disorders.

Keywords

Infertility; male; urologic diseases; child; azoospermia; birth defects; ICSI

The Society for Assisted Reproduction (SART) defines assisted reproductive techniques as any procedure requiring manipulation of gametes or embryos *in vitro*. By this definition, IVF is included but intrauterine insemination (IUI) is excluded.

IVF involves controlled ovarian stimulation followed by egg retrieval, *in vitro* fertilization and embryo transfer to the uterus. The goal of hormonal stimulation during IVF is to develop multiple mature follicles so that enough eggs are retrieved to counteract the inherent inefficiencies of fertilization, and the limitations of *in vitro* embryo culture. The process by which the menstrual cycle is regulated during ART procedures is controlled ovarian stimulation. Following ovarian stimulation and ovulation induction, egg retrieval is performed transvaginally by ultrasound-guided follicle aspiration. On the same day, a semen sample is

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obtained and washed, and sperm co-incubated with retrieved oocytes. After co-incubation, the eggs are observed for evidence of fertilization. Fertilized embryos are allowed to cleave and are transferred to the uterus from 1 to 5 days after fertilization. During embryo transfer the embryo is transferred to the uterine fundus. In general, more than one embryo is transferred because the implantation rate is not 100%.

ICSI, a revolutionary adjunct to IVF, was described in 1992. With this technique, the numerical sperm requirement for egg fertilization with IVF decreased from hundreds of thousands of viable sperm per egg to one viable sperm. This approach allows viable sperm with limited intrinsic fertilizing capacity to reliably fertilize eggs, including “immature” sperm obtained surgically from men with azoospermia. ICSI has become so popular that more than 56% of IVF cases performed in American clinics routinely use this technique to increase fertilization rates during IVF (<http://www.cdc.gov/reproductivehealth/art.htm>). In addition, the ability of ICSI to improve egg fertilization with reproductive tract sperm has allowed urologists to retrieve sperm from the vas deferens, epididymis and testicle for this technique.

These powerful ART techniques have proved invaluable to help men with pediatric urologic disorders father biological children. However, it is unclear what the long-term effects of ICSI, if any, have on the children who develop from manipulated gametes. Since the first child from IVF-ICSI was born, there is only 16 to 17 years of experience with the technique. Preliminary research suggests that children conceived by ICSI have increased incidence of congenital defects, multiple gestations, sex chromosome defects, and epigenetic syndromes[2]. Furthermore, ICSI by definition allows paternal genetic defects that would not otherwise have been transmitted to be passed to offspring. For example, microdeletions in the Y chromosome causing infertility are passed to boys conceived by ICSI [3]. The possibility of allowing otherwise sterile men to conceive children raises additional health questions.

Despite the questions about its ramifications, IVF-ICSI has been a breakthrough in ART. Many men with congenital malformations who were previously unable to conceive children are now successfully starting families. This breakthrough in ART forms the background of our discussion of congenital conditions that may result in infertility treatable through ART.

We examined pediatric urologic conditions that have associations with infertility in men. Disorders were organized by whether they induce infertility through pre-testis, testicular, or post-testis effects. A comprehensive MEDLINE database search (1950-present) was performed to assess the basis for infertility and the current and future prospects for infertility treatment in men with pediatric urologic diseases.

DISORDERS WITH INFERTILITY DUE TO PRE-TESTIS EFFECTS

Congenital Adrenal Hyperplasia

Hereditary defects in the adrenal enzymes regulating metabolism of steroids can result in syndromes collectively referred to as congenital adrenal hyperplasia (CAH). Five enzymes participate in the conversion of cholesterol to testosterone, and all can be altered by mutations transmitted in an X-linked or autosomal recessive pattern. Most (90%) CAH cases involve a deficiency of 21-hydroxylase. Testosterone production is normal, whereas cortisol and aldosterone production is depressed. Precocious virilization of males can occur, however, due to excessive adrenal drive (elevated corticotropin) and accumulation of androgenic steroid intermediates.

Infertility in males with 21-hydroxylase deficiency may result from testicular adrenal rest tumors (TART), derived from adrenocorticotrophic hormone-dependent pluripotent cells.[4,5] Impaired spermatogenesis and Leydig cell failure have been attributed to TART. Although

TART may be a manifestation of poorly-controlled CAH from suboptimal corticosteroid regimens, one study reported that even medically optimized patients can develop TART and infertility[5]. Indeed, resection of TART in CAH patients may not improve semen quality.[4]

Spermatogenesis in CAH varies with severity of CAH and how well the metabolic derangements are managed[6]. Excessive androgens can impair follicle stimulating hormone (FSH) and luteinizing hormone (LH) production, causing central hypogonadism and resulting in azoospermia. Alternatively, adrenal androgens can induce maturation of the hypothalamic-pituitary-gonadal axis prematurely and initiate spermatogenesis. However, fertility in CAH may be normal in well-managed patients. One group reported normal paternity rates and sperm concentrations in 18 of 20 men with CAH.[7] In summary, CAH is a metabolic disorder in which infertility is possible, but its likelihood depends on disease severity and the quality of medical management. If natural paternity is impossible due to impaired semen quality, then IUI or IVF, with or without ICSI, can be used to overcome most cases of infertility due to this condition.

Androgen Insensitivity Syndromes

In the androgen insensitivity syndromes, including Reifenstein and Rosewater syndromes, the androgen receptor (AR) gene is functionally altered or absent so that testosterone and dihydrotestosterone cannot activate target genes. There are hundreds of mutations described in the AR, occurring in clusters around the functional domains of the receptor [8]. The clinical picture ranges from phenotypic females with intraabdominal testes (complete androgen insensitivity) to healthy but infertile males. Investigators believe that up to 40% of men with low or no sperm counts may have subtle AR abnormalities.[9] Despite isolated reports of successful paternity through exogenous testosterone supplementation[10], most severe forms of androgen insensitivity syndrome-associated infertility require IVF-ICSI to allow conception with surgically retrieved testis sperm.

DISORDERS WITH INFERTILITY DUE TO TESTICULAR EFFECTS

Varicocele

Varicoceles noted in men presenting with infertility likely arise during adolescence. The high prevalence of varicoceles in the fertile population, however, suggests that varicocele is not solely a sufficient cause of infertility. Indeed, 80–82% of men with untreated varicoceles are fertile. This suggests that there might be genetic susceptibility to infertility associated with varicocele, and six such genes have been linked to varicocele, including *MT-ATP6*, *MT-ATP*, *CACNA1C*, *MT-CO1*, *MT-CO2*, and *MT-ND3* (reviewed by Matzuk and Lamb[8]). Individual predisposition to the adverse effects of varicocele on male infertility is complex and incompletely understood, which necessitates individualized patient decision-making when treatment is considered.

Indications for repair of adolescent varicoceles include testicular hypotrophy (>4 ml difference in testis sizes) and large (grade III) varicoceles, with the assumption that these findings indicate possible impairment of future fertility. Diamond et al. examined this relationship and concluded that varicocele size did not necessarily correlate with semenanalysis findings, but observed an association between sonographically determined testis volume differentials of >10% between normal and affected testes and decreased sperm concentrations. [11] In another series of boys with varicoceles, the presence of these lesions was associated with decreased motility and abnormal sperm morphology, but not with reduced sperm concentrations.[12] Bertolla et al. reported that although semenanalysis findings are similar between boys with and without varicoceles, rates of DNA fragmentation, which may be linked to infertility, is higher in boys with varicoceles.[13] The role of adjunctive testing to qualify patients for varicocele treatment

is also unclear. For example, although a GnRH stimulation test may identify varicocele patients with early testicular failure, this test features poor sensitivity and specificity.[14] After repair of varicocele, older adolescents reliably demonstrate improved semen parameters, with increases in total motile sperm counts and sperm motility.[15] The fertility status of non-operatively managed older adolescents with varicoceles has not been well-studied.

In conclusion, semen analysis, physical examination findings, and other tests do not accurately predict future fertility in boys with varicoceles. Moreover, there has been no prospective study of subsequent paternity in treated vs. untreated adolescent varicocele patients. If fertility is not achieved naturally in adult varicocele patients, the range of reproductive technologies, from IUI to IVF and ICSI, can overcome infertility.

Prune belly syndrome

Prune belly syndrome (PBS) is characterized by the triad of lax abdominal wall musculature, urinary tract dilation, and abdominal cryptorchidism. Fertility is impaired due to ejaculatory dysfunction from bladder neck incompetence and megalourethra, obstruction caused by an absent or atretic vas deferens, and cryptorchidism. To date, testis sperm has been found in 3 PBS patients and epididymal sperm found in two.[16] A live twin pregnancy has been reported in one case. However, one man had spontaneous descent of a solitary testis, which is inconsistent with classic PBS. Regardless, ART enables selected patients with PBS to achieve paternity.

Cryptorchidism

Cryptorchidism is the most common developmental male genitourinary defect. There are two phases of testicular descent, and mutations can impact either phase. The abdominal phase of testicular descent occurs as the testes descend from the mid abdomen to the internal inguinal ring. This is mediated by the gubernaculum, which pulls the testicles downwards, and is regulated by the signaling of insulin-like 3 (INSL3) through relaxin/insulin-like family peptide receptor 2 (RXFP2) in the gubernaculum [17]. Mutations can occur in both of these proteins, causing abdominal cryptorchidism. The second phase of testicular descent, movement of the testes into the scrotum, is mediated by the action of androgens on their receptors. Mutations in the AR or problems synthesizing androgens cause inguinal cryptorchidism.

In boys with uncorrected cryptorchidism, gonocytes begin disappearing by 6 months of age. This finding, as well as the rarity of spontaneous testicular descent after 4 months of life and the ease and safety of performing orchidopexy in the older infant, has led many surgeons to operate before 9 months of age. Whether early surgery translates into improved fertility, however, has not been established.

Performance of unilateral orchidopexy at younger ages does not seem to improve paternity rates.[18] This may be due to the fact that a difference in paternity rates in men with repaired unilateral cryptorchidism and control patients with normally descended testes is inherently small (89.7% and 93.7%, respectively). Preoperative testicular location does not predict subsequent fertility, and FSH, LH, testosterone, sperm density, motility and morphology are similar among men who have undergone unilateral orchidopexy versus men with normally descended testes [18].

The fertility of men with bilateral cryptorchidism is very different than that of men with unilateral disease. A meta-analysis reported that no men with untreated bilateral cryptorchidism had normal sperm density, whereas normal parameters were observed in 25% of men who had undergone bilateral orchidopexy.[19] Regardless of childhood age at treatment, patients with bilateral cryptorchidism have unchanged rates of azoospermia and oligospermia. However,

this study did not include men treated during infancy or early childhood. Overall, natural paternity rates range up to 33% in men with treated bilateral cryptorchidism.[20] Even with infertility due to azoospermia, testis sperm can be found in most patients with a history of prepubertal bilateral orchidopexy, and used for IVF-ICSI [21].

In conclusion, untreated, bilateral abdominal testes typically results in infertility, whereas repair of bilateral cryptorchidism may allow fertility in selected patients. However, men with treated unilateral cryptorchidism may not feature impaired natural paternity compared to normal men. In cases of severe male infertility, IUI and ART techniques are effective ways to overcome infertility from these conditions.

Testis tumors

Testis cancer is one of several malignancies that affect men during their reproductive years. Although typically sporadic, testis cancer susceptibility has been associated with 26 genes (reviewed in [8]). Examples include the RNase Dicer, which aids in microRNA synthesis [22] MicroRNAs bind to and suppress complementary mRNAs, preventing translation. Deletions of Dicer cause embryonic lethality, but postembryonic deletions have been implicated in infertility as well as male and female genitourinary cancers [23].

Testis cancer is associated with treatment- and cancer-related effects on fertility. Testis germ cell tumors are related to disorders and toxins that also affect fertility, including cryptorchidism, gonadal dysgenesis, and putative endocrine disruptors. However, cancer-related effects on fertility are difficult to study in prepubertal boys, given the lack of complete spermatogenesis and the ethics of obtaining ejaculated samples in these patients. Here we review adult studies of testis cancer and infertility to help inform issues regarding prepubertal testis cancer.

Testis cancer results in impaired semen parameters in 50% of affected patients. This was first described by Carroll et al. in 15 pre-orchietomy patients, 10 of whom featured poor motility, low sperm concentration, or low semen volume [24]. Testis cancer treatments can also result in infertility. Retroperitoneal lymph node dissection (RPLND) can result in ejaculatory dysfunction from disruption of sympathetic nerves. However, with nerve-sparing RPLND techniques, it is reported that 76% of men undergoing surgery for stage I non-seminomatous germ cell tumors successfully fathered children [25]. Infradiaphragmatic radiotherapy is often used to treat stage I or IIA seminoma. Although ionizing radiation can impair spermatogenesis through refraction despite gonadal shielding, Nalesnik et al. reported that 64% of men who had undergone infradiaphragmatic radiotherapy for seminoma and attempted paternity did so successfully without ART.[26] Like radiotherapy, chemotherapy also affects semen quality. Reversibility of chemotherapy-induced decreases in semen quality was demonstrated by Lampe et al. who reported improved sperm counts in 48% and 80% of patients at 2 and 5 years post-chemotherapy, respectively.[27] Moreover, the likelihood of normal sperm concentrations was 22% and 58% at 2 and 5 years, respectively. High pretreatment sperm concentrations appear to be associated with a higher likelihood of improved fertility post-chemotherapy. In men who are azoospermic before chemotherapy and who desire to bank sperm, testis sperm retrieval has been done at the time of orchietomy [28]. If patients are azoospermic after chemotherapy, the majority of these men will have testis sperm by fine needle mapping or testis sperm retrieval [29]. In post-chemotherapy patients, there is a decrease in fertilization rates and pregnancy rates after IVF ICSI cycles, and also decreased pregnancy rates compared to men with testis cancer who have not received chemotherapy.[30] Hence, sperm banking before chemotherapy should be offered to men who have testis cancer.

The value of sperm banking with testis cancer is supported by fertility studies. Among 67 couples referred for ART with male factor infertility following treatment of testicular cancer and other malignancies, 82% percent of men banked sperm before treatment and 58% of these

men subsequently used banked sperm for ART. In a study featuring 55 IUI's, 82 IVF-ICSI cycles, and 14 IVF-ICSI-frozen embryo replacements, the delivery rates were 11.1%, 30.5%, and 21%, respectively.[31] Another series reported pregnancy rates of 37%, 23%, and 7% with IVF-ICSI, IVF, and IUI, respectively, in couples with cancer-related male infertility.[32] In conclusion, men, and probably adolescent males, should be recommended to undergo sperm banking prior to initiating testis cancer treatment. Furthermore, through ART, many testis cancer patients can achieve paternity.

Gonadal dysgenesis

The testicular dysgenesis disorders are phenotypically pleiomorphic conditions that share a common feature: the presence of histologically disordered gonads. Children with these disorders often experience infertility as adults. A range of abnormal karyotypes and associated phenotypes has been observed in these patients, including 45 XO/46 XY mosaicism (typically mixed gonadal dysgenesis with one dysgenetic testicle and a contralateral streak gonad), and 46 XY testicular dysgenesis (bilateral streak gonads). In a series of 21 cases, most testes were undescended, and all were morphologically abnormal.[33] Testes that harbor seminiferous tubules typically develop to a moderately advanced state, macroscopically resembling normal testes, but microscopically are architecturally disorganized. Over time, the seminiferous tubules become hyalinized and atrophy. Notably, germ cells have never been found in the streak gonads of children older than 2 years. Streak gonads are removed to avert the subsequent development of gonadoblastoma, and intraabdominal testes that cannot be surgically placed in or near the scrotum are likewise excised to prevent testis cancer. Given the bleak histologic findings in patients with gonadal dysgenesis, as well as the surgical considerations in these children, ART-refractory, untreatable infertility is the rule.

5-alpha-reductase deficiency

Males with 5α-reductase deficiency, an autosomal recessive disorder, have incompletely virilized external genitalia but normal testes and internal genital ductal structures. In these patients, a deficiency in one of two described 5α-reductase enzymes results in the lack of conversion of testosterone to dihydrotestosterone (DHT). Typically, seminal vesicles are rudimentary, the prostate may be absent, and the testes are within the inguinal canal or labio-scrotal folds. Infertility results from the structural abnormalities of the genitalia, as well as from associated cryptorchidism. Natural paternity is rare but possible with this condition. Fertility with ART using either ejaculated or testis sperm should be considered in these patients, as they are similar to men with cryptorchidism.

DISORDERS WITH INFERTILITY DUE TO POST-TESTIS EFFECTS

Exstrophy/Epispadias Complex

Bladder exstrophy/epispadias complex results from aberrant development of the cloacal membrane and is phenotypically variable. Urethral reconstruction and closure of the abdominal wall and bony pelvis are performed during infancy and childhood but are associated with significant erectile and ejaculatory dysfunction in adulthood. Natural paternity is possible in the absence of bladder neck reconstruction, since normal physiological functions of this region during ejaculation are preserved. Despite sufficient erectile function and antegrade ejaculation, however, fertility is hampered in many patients due to impaired spermatogenesis.[34] Woodhouse reported that only half of the men with exstrophy who wished to have children were successful. [35] Fertility was better in boys who had had an early diversion rather than reconstruction. Men with exstrophy face many challenges to fathering children, including defective spermatogenesis and ejaculatory impairments. IUI or IVF with ICSI are excellent alternatives to overcome the infertility that results from these disorders.

Hypospadias

Hypospadias can be caused by at least 14 different genetic defects [8], or by partial androgen insensitivity. Men with severe hypospadias may suffer from infertility due to the inability to deliver sperm to the cervix, either because of a proximally located urethral meatus, urethral strictures or diverticulae after surgery, or an associated chordee preventing vaginal intromission. One study reported that 27 of 30 men with unrepaired penile or distal hypospadias fathered children, but only 4 of 17 men with more severe, unrepaired penoscrotal or perineal hypospadias achieved paternity.[36] Jugenburg and Kipikasa described 33 hypospadias patients who had undergone reconstruction followed by testicular biopsy during adolescence or early adulthood[37]. In this series, 48.8% of patients had normal findings, and the remaining patients had histological abnormalities. Although compelling, this data is complicated by the 24–25% rate of cryptorchidism among hypospadiac patients in these series. In brief, severe, untreated hypospadias can result in infertility due to mechanical problems, but fertility is complicated by associated cryptorchidism. Despite this, fertility can be improved with IUI or IVF-ICSI in most affected patients.

Spina bifida and tethered cord

Infertility is a common among men with spina bifida, and may be caused by erectile dysfunction, ejaculatory dysfunction, and spermatogenic defects. Furthermore, patients with spina bifida often have tethered cords, which can compound ejaculatory dysfunction. Men with a tethered cord can suffer from infertility due to retrograde ejaculation from bladder neck incompetency, or due to complete neurogenic failure of semen emission. Paternity has been achieved with treatment of retrograde ejaculation and rectal probe electroejaculation can overcome neurogenic failure [38]. Natural paternity rates in men with spina bifida range up to 72%, with lower lesions associated with a greater likelihood of paternity.[39] Decker showed that 87% of men who achieved paternity had L5 or sacral lesions.[39] In 10 men with spina bifida and related erectile dysfunction, rectal probe electroejaculation produced ejaculates in all patients.[40] In conclusion, men with spina bifida may be infertile due to impotence, disorders of ejaculation, and testicular dysfunction. However, these men are candidates for IUI or IVF-ICSI if sperm can be retrieved by electroejaculation or through surgical retrieval.

Posterior urethral valves

To our knowledge, only 11 men with treated posterior urethral valves (PUV) have achieved natural paternity.[41,42] Infertility in men with a history of PUV can be multifactorial. Retrograde ejaculation due to an open bladder neck is common.[42] Patients with PUV and renal failure can also suffer from erectile dysfunction and reduced libido.[41] A higher rate of cryptorchidism among patients with PUV may also compromise fertility. Semen analyses in a series of 5 PUV patients demonstrated prolonged liquefaction time (40% of men), greater numbers of immotile sperm (all men), abnormally viscous semen (40%), and abnormal sperm agglutination (80%) suggesting that abnormalities in seminal plasma are also common in these patients.[43][68] Two other patients in this series were anejaculatory. Clearly, infertility affects men with PUV and treatment should be directed at the underlying metabolic or physiologic cause. In addition, IUI and IVF can be valuable adjunctive treatment options.

FUTURE ADVANCES IN FERTILITY TREATMENTS

What does the future hold for fertility treatment in men with pediatric urological disorders? Most exciting are the findings from research in stem cell biology. Clearly embryonic stem cells can self-renew and can also differentiate into every cell type in the body. It is also clear that other cells besides embryonic stem cells may harbor this “pluripotency” potential, including genetically modified fibroblasts [44] and adult germ line (testis) cells. Both adult spermatogonial stem cells have been reprogrammed into multipotent stem cells [45]. This

technology could allow the production of embryonic-like stem cells without the ethical issues associated with the generation of germ line stem cells. Such cells could be used to reverse, restore or preserve fertility.

Another advance with fertility implications is gene therapy to cure pediatric urologic disorders. Promising research in the field of testis-mediated gene transfer and sperm-mediated gene transfer may enable men with pre-testicular and testicular infertility disorders to achieve paternity in the future. [46] Gene manipulation of mouse spermatogonial stem cells *in vitro* has been reported [47]. For this, a viral vectors are needed to transduce genetic material into stem cells in culture [48]. However, questions of safety and ethics may limit the overall applicability of such advances.

Another recent advance in infertility treatment is preimplantation genetic diagnosis (PGD). With this method, embryos produced via IVF or IVF-ICSI are genetically screened after embryo biopsy (blastomere biopsy) to identify genetic problems and healthy embryos can be selected for clinical use [49]. Newer embryo quality assessments, such as transcriptomic, proteomic or metabolomic assessments of embryos, are currently being developed and may have implications for treating genetic-based pediatric urologic disorders [50]. As with many emerging molecular or genetic treatments, ethical issues can loom large, especially with respect to the selection of genetic traits in offspring.

CONCLUSIONS

Largely enabled by advances in ART, fertility is now possible for many men with pediatric urologic conditions. Knowledge of these developments is critical to parents of affected children, for the contemporary care of affected men, and for counseling men regarding genetic consequences of ART in offspring. Combined with a careful fertility assessment, a working knowledge of rapidly advancing ART techniques can help tailor infertility treatment and maximize chances of paternity. Advances in adult stem cell and germ line stem cell biology offer the most potential to create new avenues of fertility for many affected patients with pediatric urological conditions that are currently untreatable.

List of abbreviations

IVF	in vitro fertilization
ICSI	intracytoplasmic sperm injection
IUI	intrauterine insemination

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