

Brief Report

**PATERNITY BY INTRAUTERINE
INSEMINATION WITH SPERM FROM
A MAN WITH 5 α -REDUCTASE-2
DEFICIENCY**

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MALE pseudohermaphroditism results from the abnormal differentiation of male external genitalia in a genotypic male. One cause of male pseudohermaphroditism is a deficiency of 5 α -reductase-2, the enzyme that converts testosterone to 5 α -dihydrotestosterone in specific androgen-dependent target tissues.¹⁻³

Males who are homozygous for the disorder usually present with pseudovaginal perineoscrotal hypospadias at birth. Most have distinct urethral and vaginal openings within a urogenital sinus and a clitoris-like phallus. Virilization occurs at puberty with phallic growth, rugation and pigmentation of the scrotum, and testicular descent.¹⁻³ At this time, the voice deepens and muscle mass increases, with the development of a male physique. For the many affected persons who were raised as females, the assumption of a male role occurs during or after puberty.^{1,3,7}

A number of male pseudohermaphrodites with 5 α -reductase-2 deficiency and decreased dihydrotestosterone production have married and expressed a desire to father a child. However, a deficiency in dihydrotestosterone production not only impairs differentiation of male external genitalia but also affects the development and secretory function of the prostate gland and seminal vesicles.^{1,2,8-15} Consequently, affected adults have a rudimentary prostate and underdeveloped seminal vesicles, resulting in highly viscous semen⁸ and an extremely low volume of ejaculate,^{8,16,17} although their sperm counts may be normal.^{8,18,19}

We describe the use of intrauterine insemination

with sperm from a man with this disorder and a history of infertility to achieve fertilization. The insemination was done because the man had both abnormalities in semen quality and several urethroscrotal (midshaft and penoscrotal) fistulas after correction of hypospadias, despite a satisfactory sperm count.

CASE REPORT

A 36-year-old Dominican male pseudohermaphrodite who was homozygous for a mutation in which thymidine was substituted for cytosine in exon 5 of the 5 α -reductase-2 gene wanted to father a child.^{1,20} This missense mutation, which affects a large kindred from the Dominican Republic with 5 α -reductase-2 deficiency, results in the substitution of tryptophan for arginine at position 246 (R246W) of the enzyme.²⁰ The product of the mutant gene has a markedly reduced ability to catalyze the conversion of testosterone to the more potent androgen dihydrotestosterone.²⁰

The subject was the fifth of seven children, another of whom was also affected.^{1,20} At birth, the subject had ambiguous genitalia and bilaterally descended testes. He was raised as a boy. At puberty, examination revealed a male body habitus, deepening of his voice, penile enlargement, and pseudovaginal perineoscrotal hypospadias. The length of the phallus was 3.5 cm (stretched); the volume of the testes was normal, 25 ml on the right and 20 ml on the left. The prostate gland was not palpable.⁸ At the age of 20 years, the subject underwent left testicular biopsy and surgery to correct chordee. At the age of 34 years, penile length (stretched) was 3.5 cm, with a circumference of 7 cm. He was treated once daily with 25 mg of dihydrotestosterone cream administered topically as 1/4 teaspoon of 2 percent dihydrotestosterone in cold-cream base just above the pubic area. After five months of this therapy, the penile length (stretched) was 6.5 cm, with a circumference of 6 cm. After treatment with two daily applications of dihydrotestosterone cream for three months, the stretched penile length was 7.5 cm and the circumference was 6.5 cm. Hair growth on the chin, upper lip, and abdomen along the linea alba increased progressively during the eight months of treatment. At the age of 35 years, the subject underwent surgery to correct the hypospadias, with resulting urethroscrotal fistulas. Despite attempts at repair, the fistulas persisted.

His unrelated 35-year-old Dominican wife of 13 years was healthy. Her sexual development and menstrual cycles were normal, and analysis of single-strand conformation polymorphisms and DNA-sequence analysis of her 5 α -reductase-2 gene revealed no abnormalities (Fig. 1). Despite years of unprotected intercourse, impregnation had not occurred. The couple sought help because they wanted to have children.

They were given genetic counseling in which the genetics of the condition were reviewed, including the possibility of having a carrier child or the unlikely possibility of having an affected child. The couple understood and wished to proceed. They were referred to the Center for Reproductive Medicine and Infertility at New York Hospital-Cornell University Medical College, where the genetics of inheritance were again reviewed.

METHODS

Plasma Androgen Concentrations

Plasma testosterone and dihydrotestosterone were separated by paper chromatography and measured by radioimmunoassay.⁴ Some results have been reported previously.⁸

Semen Collection and Analysis

Semen samples were obtained by masturbation after three days of sexual abstinence. The laboratory methods and normal values for semen volume and sperm concentration and motility as defined by the World Health Organization (WHO)²² were used. Motility was assessed by video microscopy. The morphologic features of the specimens obtained before the correction of hy-

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pospadias were analyzed according to the WHO criteria, and those obtained postoperatively were analyzed according to the criteria of Kruger et al.²³

Semen Preparation

Each semen sample used for intrauterine insemination was washed twice and then centrifuged for 20 to 30 minutes at 300×g through layers of increasing concentrations of Percoll (50, 70, and 95 percent). Artificial human tubal fluid containing penicillin G and streptomycin sulfate in combination with 6 percent (vol/vol) human plasma protein fraction (Plasmanate) was used to dilute the Percoll solution. After centrifugation, the pellet containing motile sperm, debris, and nonmotile cells was washed twice by resuspension in 1 ml of fresh medium and centrifugation at 300×g for three to five minutes. The final pellet was resuspended in 0.5 ml of fresh medium.

Intrauterine Insemination

Intrauterine insemination was performed during a natural menstrual cycle in the subject's wife 25 hours after the documentation of a surge in luteinizing hormone secretion and transvaginal ultrasonographic visualization of a dominant ovarian follicle. Three attempts were required to achieve the first pregnancy, and two for the second pregnancy. At each attempt, a speculum was placed in the vagina and the cervix cleansed, after which a catheter was inserted through the cervix into the uterus. A total of 0.2 to 0.3 ml of the semen suspension was placed in a 1-ml syringe and injected through the catheter into the uterine cavity. The wife then remained supine for 10 minutes.

Genetic Studies

Blood from the subject, his wife, and the three children who were born as a result of intrauterine insemination was drawn into tubes containing EDTA, and genomic DNA was isolated as previously described.²⁴ Exons 1 to 5 of the 5 α -reductase-2 gene were amplified by the polymerase chain reaction and sequenced as previously described.²⁴ Analysis of single-strand conformation polymorphisms was performed according to the method of Orita et al.²⁵ with modifications.²⁴

RESULTS

Plasma Androgen Concentrations and Testicular Biopsy

Between the ages of 20 and 36, the subject had normal as well as elevated plasma testosterone concentrations, low plasma dihydrotestosterone concentrations, high ratios of testosterone to dihydrotestosterone in plasma, and elevated ratios of 5 β to 5 α C19 and C21 steroid metabolites in urine (data not shown) — findings consistent with a diagnosis of 5 α -reductase-2 deficiency (Table 1).^{1,4} The testicular biopsy revealed decreased spermatogenesis, as reported previously.⁸

Semen Analyses, Intrauterine Insemination, and Pregnancies

The results of semen analyses before and after the correction of hypospadias are shown in Table 2. The analyses revealed low semen volumes with normal sperm concentrations and normal motility and morphology. All specimens were extremely viscous. The samples used in the two successful intrauterine inseminations had sperm concentrations of 23 million per milliliter and 43 million per milliliter after washing.

The first successful intrauterine insemination was

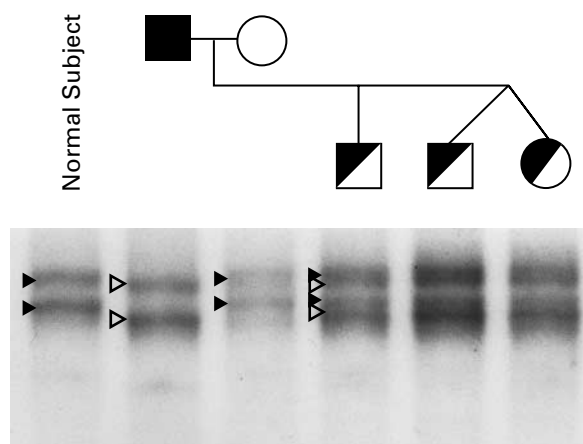


Figure 1. Single-Strand Conformation Polymorphism Analysis of Exon 5 of the 5 α -Reductase-2 Gene in a Man with 5 α -Reductase-2 Deficiency, His Wife, Their Three Children, and a Normal Subject.

Genomic DNA was extracted, and exon 5 of the 5 α -reductase-2 gene was radiolabeled and amplified by the polymerase chain reaction, denatured, and subjected to electrophoresis in a neutral gel (HydroLink MDE, J.T. Baker, Phillipsburg, N.J.) containing 10 percent glycerol at room temperature overnight. The gel was then dried and exposed to Kodak BioMAX film (Eastman Kodak, Rochester, N.Y.) at room temperature.²¹ Squares denote male subjects, circles female subjects, the solid symbol homozygosity for 5 α -reductase-2 deficiency, and half-solid symbols heterozygosity for an R246W mutation in exon 5. The pattern of the normal allele of the 5 α -reductase-2 gene is indicated by the solid arrowheads. The altered pattern in affected subjects is indicated by the open arrowheads. The pattern in heterozygotes combines normal and affected alleles and is indicated by alternating solid and open arrowheads.

TABLE 1. PLASMA ANDROGEN CONCENTRATIONS IN A MAN WITH 5 α -REDUCTASE-2 DEFICIENCY.*

CHARACTERISTIC	TESTOSTERONE	DIHYDRO-TESTOSTERONE	RATIO OF TESTOSTERONE TO DIHYDROTESTOSTERONE
	ng/dl		
Age			
20 yr	1102	29	38
34 yr	681	12	57
36 yr	669	10	67
Normal range	300-952	30-70	8-17

*To convert values for testosterone to picomoles per liter, multiply by 34.7. To convert values for dihydrotestosterone to picomoles per liter, multiply by 34.4.

TABLE 2. SEMEN ANALYSES IN A MAN WITH 5 α -REDUCTASE-2 DEFICIENCY.

VARIABLE	VOLUME		SPERM	
	ml	CONCENTRATION	MOTILE	NORMAL MORPHOLOGY
		millions/ml		percent
Before hypospadias repair*				
Specimen 1	0.2	321	56	71
Specimen 2	0.2	150	61	49
After hypospadias repair	0.5	350	55	5†
Pregnancy 1				
Before washing of sample	0.5	165	33	
After washing of sample	0.2	23	80	
Pregnancy 2				
Before washing of sample	0.5	65	66	
After washing of sample	0.3	43	93	
Normal value	>2	>20	>50	>60

*The specimens were analyzed in two different laboratories; the results for Specimen 1 have been reported previously.⁸

†Two percent had slight vacuolation (normal range, 5 to 15 percent).²²

performed one day after a spontaneous surge in the serum luteinizing hormone concentration was detected and a 14-mm ovarian follicle was seen on transvaginal ultrasonography. A pregnancy ensued, and the couple declined amniocentesis. Premature rupture of the membranes occurred 33 weeks after intrauterine insemination. Cesarean section resulted in the delivery of a healthy boy with normal genitalia.

Nine months later, a second attempt at intrauterine insemination was also successful. On this occasion transvaginal ultrasonography showed follicles of 13.5 mm and 10.5 mm in the left ovary. Transvaginal ultrasonography after insemination revealed a twin (nonidentical) pregnancy. Premature rupture of the membranes occurred 32 weeks after intrauterine insemination. Cesarean section was again performed, and healthy male and female twins were delivered.

Genetic Studies of Offspring

All three of the subject's children were found to be heterozygous for the C→T mutation in exon 5 of the 5 α -reductase-2 gene on the basis of single-strand conformation polymorphism analysis (Fig. 1) and DNA-sequence analysis.²⁰

DISCUSSION

Male pseudohermaphrodites with 5 α -reductase-2 deficiency, despite the severe ambiguity of the genitalia at birth, undergo a male puberty. Among those raised as females, many later choose to live as men.^{1,3-7} The achievement of biologic fatherhood by intra-

uterine insemination with sperm from our subject demonstrates that this facet of male reproductive function is possible in men with this genetic disorder. Most men with a 5 α -reductase-2 deficiency are, however, infertile¹⁹ due to a number of factors. Many have azoospermia or oligospermia associated with undescended testes.^{8,19} In addition, perineoscrotal hypospadias or complications of genitourinary surgery such as urethroscrotal fistulas, which occurred in this man, can interfere with insemination.^{4,5,8} Even in affected men with adequate spermatogenesis who have undergone surgical correction of the genitalia, the characteristic very low semen volume and increased viscosity can preclude natural insemination.

In normal men, prostatic and seminal-vesicle secretions provide over 80 percent of semen volume, with the former contributing 15 to 30 percent and the latter 50 to 60 percent.⁸ Men with 5 α -reductase-2 deficiency (and decreased dihydrotestosterone production) have rudimentary prostate glands and small seminal vesicles and therefore low semen volumes and highly viscous semen.^{8-11,13,15,19}

The use of intrauterine insemination in this couple circumvented the difficulties with insemination consequent to the husband's urethroscrotal fistulas and abnormal semen quality and resulted in two successful pregnancies. Thus, intrauterine insemination can be used in men with 5 α -reductase-2 deficiency who wish to father children and who have adequate sperm counts and motility. Furthermore, this method is feasible regardless of whether adequate corrective genital surgery has been done. It may even be successful in affected men with low sperm counts and diminished motility, because washing the semen before insemination rids the sample of many of the abnormal sperm.

The diagnosis of 5 α -reductase-2 deficiency should be made in infancy with the use of biochemical²⁶ and molecular genetic^{20,21,25} techniques. Early correction of cryptorchidism and hypospadias is crucial to prevent damage to the seminiferous tubules and preserve spermatogenesis and future fertility.⁷ Dihydrotestosterone cream should be used to enlarge the phallus before reconstructive surgery and facilitate the correction of hypospadias.^{7,27} In men who were not treated in childhood, topical dihydrotestosterone therapy may also stimulate penile growth after puberty.^{7,19}

In summary, paternity by intrauterine insemination is feasible in men with 5 α -reductase-2 deficiency, affirming their full reproductive potential and providing further support for raising them as males.^{3,5-8}

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