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First Successful Conception Induced by a Male Cystinosis Patient

Koenraad R. Veys • Kathleen W. D'Hauwers •
Angelique J. C. M. van Dongen • Mirian C. Janssen •
Martine T. P. Besouw • Ellen Goossens •
Lambert P. van den Heuvel • Alex A. M. M. Wetzels •
Elena N. Levchenko

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Abstract Cystinosis is a rare autosomal recessive lysosomal storage disease characterized by multi-organ cystine accumulation, leading to renal failure and extra-renal organ dysfunction. Azoospermia of unknown origin is the main cause of infertility in all male cystinosis patients. Although spermatogenesis has shown to be intact at the testicular level in some patients, no male cystinosis patient has been reported yet to have successfully induced conception.

We present the first successful conception ever reported,

induced by a 27-year-old male renal transplant infantile nephropathic cystinosis patient through percutaneous epididymal sperm aspiration (PESA) followed by intracytoplasmic sperm injection (ICSI). After 36 weeks and 6 days of an uncomplicated pregnancy, a dichorial diamniotic (DCDA) twin was born with an appropriate weight for gestational age and in an apparently healthy status. Moreover, we demonstrate that the sperm of epididymal origin in selected male cystinosis patients can be viable for inducing successful conception.

Our observation opens a new perspective in life for many male cystinosis patients whom nowadays have become adults, by showing that despite azoospermia fathering a child can be realized. In addition, our findings raise questions about the possibility of sperm cryopreservation at a young age in these patients.

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K. R. Veys (✉) • E. N. Levchenko
Department of Pediatric Nephrology, University Hospitals Leuven,
Herestraat 49, 3000 Leuven, Belgium
e-mail: koenraad.veys@uzleuven.be

K. R. Veys • M. T. P. Besouw • L. P. van den Heuvel •
E. N. Levchenko
Department of Growth and Regeneration, Unit of Organ Systems, KU
Leuven, Herestraat 49, 3000 Leuven, Belgium

K. W. D'Hauwers
Department of Urology, Radboud University Medical Center, Philips
van Leydenlaan 15, 6562 EX Nijmegen, The Netherlands

A. J. C. M. van Dongen
Department of Gynaecology, Radboud University Medical Center,
Philips van Leydenlaan 15, 6562 EX Nijmegen, The Netherlands

M. C. Janssen
Department of Internal Medicine, Unit of Metabolic Diseases,
Radboud University Medical Center, Philips van Leydenlaan 15, 6562
EX Nijmegen, The Netherlands

E. Goossens
Department of Reproduction, Biology of the Testis, Genetics and
Regenerative Medicine, Vrije Universiteit Brussel, Laarbeeklaan 103,
1090 Brussels, Belgium

A. A. M. M. Wetzels
Department of Fertility, Radboud University Medical Center, Philips
van Leydenlaan 15, 6562 EX Nijmegen, The Netherlands

Introduction

Cystinosis (OMIM #219800) is a rare, autosomal recessive lysosomal storage disease, caused by mutations in the *CTNS* gene encoding cystinosin, a lysosomal proton-cystine cotransporter (Town et al. 1998). It is characterized by lysosomal cystine accumulation and crystal formation in all organs and tissues (Town et al. 1998; Gahl et al. 2002).

The disease initially affects the kidneys, causing renal Fanconi syndrome in early childhood, and progresses to end-stage renal disease in puberty or early adulthood. However, during the first decades of life, various endocrine organs can also be affected (Chan et al. 1970; Fivush et al. 1987, 1988). In addition to primary hypothyroidism,

growth retardation, and pancreatic insufficiency, hypergonadotropic hypogonadism has been reported as a frequent finding in male cystinosis patients (Chik et al. 1993). As of yet, in contrast to a few female patients who have given birth, no male cystinosis patient is known to have induced pregnancy. More recently, it was shown that this infertility in male cystinosis patients is due to azoospermia of yet unknown origin (Besouw et al. 2010).

Treatment with the cystine-depleting drug cysteamine delays the onset of end-stage renal disease and prevents multi-organ damage (Markello et al. 1993; Brodin-Sartorius et al. 2012). While first described in 1976 (Thoene et al. 1976), the use of cysteamine in cystinosis patients only became widespread in the early 1990s with the availability of the commercial preparation of cysteamine bitartrate (Cystagon®) (Schneider et al. 1995). Currently, a growing population of cystinosis patients who were treated with cysteamine starting from infancy or early childhood is reaching young adulthood. This prolonged life expectancy raises novel quality of life issues such as male infertility. Here, for the first time ever, we report a successful conception induced by epididymal sperm of a male cystinosis patient through assisted reproductive technology (ART).

Case Report

A 27-year-old male nephropathic cystinosis patient and his wife were consulted at the fertility clinic for realizing their wish of having children.

The patient was formerly diagnosed with nephropathic cystinosis at the age of 4 years when he presented with renal Fanconi syndrome and photophobia. Slit lamp examination revealed the presence of corneal cystine crystals. The diagnosis of cystinosis was confirmed by

genetic analysis of the *CTNS* gene showing a compound heterozygous mutation (the common 57 kb deletion on one allele, and a point mutation in the lariat branch site of intron 4: c.141-24T>C on the other allele). The latter mutation results in skipping of exon 5 during transcription (Taranta et al. 2010). The leucocyte cystine level at the moment of diagnosis was 7.8 nmol 1/2 cystine/mg protein. Ever since the initiation of treatment, the total dose of cysteamine has been between 1.3 and 1.9 g/m²/day. End-stage renal disease was developed by the age of 10 years requiring renal replacement therapy (RRT) in the form of peritoneal dialysis. Deceased donor kidney transplantation was followed at the age of 11. The immunosuppressive regimen consisted of ciclosporin A, azathioprine, and low-dose prednisone. Both growth and pubertal development were delayed. At the age of 15, the Tanner stages of puberty were A1P2G2. Height was 153.5 cm, which is below -2 standard deviations (SD), and bone age lagged behind 2 years in comparison to calendar age. Recombinant human growth hormone (rhGH) therapy was initiated after kidney transplantation and resulted in a catch-up growth and a final height of 176 cm. At the age of 18 years, the patient reached a full sexual maturation with Tanner stage 5 and a final bilateral testes volume of 18 mL with a normal consistence. In addition, thyroid function tests always remained within the normal range. Until now, no signs of renal transplant rejection have ever occurred and current eGFR is 110 mL/min/1.73 m².

A previous fertility status evaluation at the age of 21 years showed normal levels of FSH, LH, testosterone, and inhibin B (Table 1, first column). However, no sperm was present in the ejaculate obtained by masturbation, which led to the diagnosis of azoospermia.

Following the consultation at the fertility clinic, the pituitary-testicular axis hormonal status was reassessed

Table 1 Hormonal levels of the pituitary-testicular axis in the reported male cystinosis patient

Age (years)	Ref. value	21					
		21	22	22	23	26	27
FSH (U/L)	1.5–11.0	7.79	5.5	6.3	7.4	8.3	8.4
Inhibin B (pg/L)	150–400	210					174
LH (U/L)	1.4–8.5	7.44	4.6	7.4	10.0 ^a	7.4	7.3
Testosterone (nmol/L)	11–45	16	9.5	21.6	30.3	15	17
Creatinine (μmol/L)	60–110	83	96	93	100	109	115
Leucocyte cystine level (nmol 1/2 cystine/mg protein)	<1	0.96	2.9	2.42	1.42	2.14	0.78
Cysteamine dose		1,050 mg qid					

Results are reported from the first fertility assessment at the age of 21 years (first column; reported as patient 2 in Besouw et al. (2010)), until the assessment at the age of 27 years prior to the first percutaneous epididymal aspiration (PESA) procedure (last column)

^a Above the upper normal limit. Based on these results, no primary hypogonadism can be observed. The ejaculate showed azoospermia at the age of 21 and 27 years (data not shown)

(Table 1, last column). Because of proven azoospermia, and in order to avoid complications related to testicular sperm extraction (TESE), a percutaneous epididymal sperm aspiration (PESA) was performed. The funiculus was anesthetized with prilocaine hydrochloride (Citanest[®]) and 0.25 mL of epididymal fluid was aspirated on the left side, and 0.5 mL on the right side. This procedure revealed the bilateral presence of mature spermatozoa. In total, from the left epididymis 0.2×10^6 spermatozoa were harvested ($0.80 \times 10^6/\text{mL}$); the right epididymis yielded 2.5×10^6 spermatozoa ($5.0 \times 10^6/\text{mL}$). In both sides, 1% of the spermatozoa showed progressive motility, and 8% showed nonprogressive motility. The sperm was mixed with Test Yolk Buffer (Irvine Scientific, USA) and cryopreserved in abundance of an intracytoplasmic sperm injection (ICSI) procedure.

In the first ICSI attempt, two oocytes (metaphase II) were obtained, of which one was fertilized. In the second attempt, six out of seven oocytes were fertilized. Single embryo transfer did not result into pregnancy and cryopreservation of the remaining embryos was not possible due to insufficient quality.

A new PESA procedure was performed before the third ICSI attempt. Fifty thousand spermatozoa were obtained from the right epididymis (aspirated total epididymal fluid: 0.5 mL; concentration of spermatozoa: $0.10 \times 10^6/\text{mL}$), with 12% progressive and 14% nonprogressive motility. The semen was again cryopreserved. In the third attempt, eight oocytes could be obtained of which six were fertilized. Double embryo transfer was followed on day 3. One embryo was suitable for cryopreservation on day 5 (blastocyst). Two weeks later, a pregnancy test resulted positive, and at 7 weeks of gestation an intact dichorial diamniotic (DCDA) twin was identified by ultrasound. Pregnancy was monitored in a regional hospital and at a gestational age of 36 weeks and 6 days the DCDA twin was born via an uncomplicated caesarean section. Both newborns were apparently healthy with respective birth weights of 2,910 g (son) and 2,884 g (daughter). Subsequently, DNA analysis of the cord blood showed the heterozygous c.141-24T>C mutation in the *CTNS* gene in both offspring, confirming the paternity of our cystinosis patient.

Discussion

Over the last few decades, the advent and progress in cystine-depleting treatment and RRT have significantly improved the prognosis of cystinosis patients (Gahl et al. 2002; Markello et al. 1993; Brodin-Sartorius et al. 2012). As a result of earlier diagnosis, rapid initiation of adequate cysteamine therapy, and improved therapeutic monitoring,

the life expectancy of cystinosis patients has been greatly extended, allowing them nowadays to survive into adulthood.

This favorable evolution has created a new perspective, raising specific adult-related issues. Hence, being one of the essential human needs and major determinants of quality of life, having children has become a matter of concern to adult cystinosis patients and their families.

Here, for the first time, we report a successful conception induced by a male cystinosis patient through ART with PESA followed by ICSI. The paternity of our patient could be confirmed as both offspring inherited the rare heterozygous mutation in the *CTNS* gene, which has only been described in the index case and his sister thus far (Taranta et al. 2010). In addition to the previous report of Besouw et al. which has shown intact spermatogenesis at the testicular level (Besouw et al. 2010), we demonstrate for the first time that the sperm of epididymal origin can be viable in selected male cystinosis patients and can be used for inducing conception successfully.

To this date, the exact pathophysiology of the primary hypogonadism and azoospermia observed in patients with cystinosis is not yet fully understood. In a first organized report on reproductive function in male infantile nephropathic cystinosis patients after renal transplantation published in 1993, hypergonadotropic hypogonadism was shown to be present in the majority of patients (70%) (Chik et al. 1993). This finding could neither be explained by the effect of the previous chronic renal insufficiency nor by renal transplantation and immunosuppressive agents used in the established regimens. In a more recent study, the pituitary-testicular axis remained within normal limits in a subset of male patients although azoospermia was present in all of them (Besouw et al. 2010). Surprisingly, a testicular biopsy in one of these patients showed an intact spermatogenesis (Johnsen score 8–9) (Besouw et al. 2010; Johnsen 1970). As azoospermia was also present in patients treated with cysteamine starting from early age, it remained uncertain whether cysteamine had no therapeutic effect on the pathophysiology of azoospermia, or the drug itself would be involved in causing infertility. In addition, as azoospermia was documented in some patients in the presence of a normal renal function (eGFR), altered renal function cannot be regarded as a major determinant of fertility status (Besouw et al. 2010).

Some hypothetical causes for this azoospermia in nephropathic cystinosis patients with a normal pituitary-testicular axis and renal function can be suggested. First, as spermatogenesis at the testicular level has shown to be intact, it could be hypothesized that epididymal sperm maturation is altered. Epididymal sperm maturation is a process in which spermatozoa acquire their motility and fertilizing capacity during transit from caput to cauda

epididymis (Dacheux and Dacheux 2013). This is a crucial step in the establishment of male fertility. Herein, the composition and changing biochemical properties (water, sodium, potassium, bicarbonate, pH, calcium, and osmolality) of the epididymal luminal fluid play a vital role (Dacheux and Dacheux 2013; Pholpramool et al. 2011; Pastor-Soler et al. 2005; Weissgerber et al. 2011). The renal proximal tubule and the epididymis share the same embryonic precursor – the intermediate mesoderm – both are highly metabolically active and serve a shared goal of maintaining electrolyte and acid–base homeostasis through similar transepithelial transport processes. Therefore, although – in contrast to the renal proximal tubule – the sensitivity of epididymal tubular epithelial cells to cystinosis dysfunction is unknown, one could speculate that epididymal transepithelial electrolyte transport in cystinosis is impaired. Indeed, altered acidification of the epididymal luminal fluid milieu has been recognized as a cause of infertility (Pholpramool et al. 2011; Pastor-Soler et al. 2005). In addition, although the exact role of the epididymal luminal Ca^{2+} is not fully clear, the motility and viability of sperm of the cauda epididymis was markedly reduced in a vanilloid type transient receptor potential cation channel 6 knock-out (*trpv6^{-/-}*) murine model, in the presence of abnormal high calcium concentrations in the intraluminal fluid of the cauda epididymis (Weissgerber et al. 2011).

Second, *in vivo* and *in vitro* data on cysteamine, being the only available disease-modifying drug, suggest an indirect local as well as a systemic endocrine effect, the latter through actions of ghrelin. Via inhibition of somatostatin secretion, cysteamine increases plasma ghrelin levels (Fukuhara et al. 2005). Ghrelin has shown to exert a central inhibitory effect on FSH and LH secretion, as well as a local inhibitory effect on Leydig cell proliferation and testosterone secretion (Comninou et al. 2014). In addition, cysteamine can potentially induce alterations of posttranslational modifications in epididymal sperm (Sutovsky 2014). Furthermore, a spermicide effect of cysteamine has been documented by a moderate reversible inhibition of human acrosin, a protease which is released from the acrosome of the spermatozoa in the acrosome reaction, which is crucial to the penetration of the zona pellucida (Anderson et al. 1998). However, whether cysteamine penetrates the blood–testes barrier and which concentrations can be reached in the human sperm is currently unknown.

Cystine crystal deposits and signs of increased fibrosis have been previously demonstrated in testes in an infantile nephropathic cystinosis patient under cysteamine therapy (Chik et al. 1993). However, in this case, the patient had clear signs of primary hypogonadism, sufficient to explain his infertility. As several studies demonstrated recently that cystinosis is involved in numerous cellular processes, one

can argue whether merely the lysosomal cystine accumulation should be considered as the only pathophysiological mechanism involved in infertility (Raggi et al. 2014; Giade Chevronnay et al. 2015; Ivanova et al. 2015, 2016; Rega et al. 2016). In this regard, it is of note that the cystinosin-LKG isoform, of which its localization is not limited to the lysosomal membrane, has the highest expression in the testes compared to other organs (Taranta et al. 2008). Obviously, further research is needed in order to unravel the underlying mechanism of the azoospermia observed in male cystinosis patients. In the meanwhile, ART can help these patients to realize their wish of having children.

As an important subset of male cystinosis patients have been described to evolve into a primary hypogonadism as early as the second decade of life, sperm cryopreservation in pubertal male cystinosis patients could be considered to maximize their chances on fathering a child. PESA offers the advantage of avoiding complications related to sperm retrieval techniques at the testicular level. Moreover, the success rate of a surgical sperm retrieval technique, like PESA, followed by ICSI, is non-inferior in comparison to the regular *in vitro* fertilization (IVF) with ejaculated semen (Gozlan et al. 2007). It remains uncertain however, whether the ejaculate of pubertal boys with cystinosis will contain sperm cells that can be used for cryopreservation at all, since this has never been tested before. Obviously, the optimal timing at which cryopreservation would have to be performed will raise ethical dilemmas and may also verge on the borders of current scientific and practical feasibility. Because of the vulnerability of this age group, the potential emotional impact of fertility preservation and the ethical concerns, psychological counseling should be a regular part of a multidisciplinary team, experienced in guiding patients through the cryopreservation and ART procedures. Ultimately, the most important ethical justification for considering fertility preservation techniques in cystinosis patients is to serve their best interests and universal needs in life.

Taken together, this first successful conception induced by a male cystinosis patient may be considered as a milestone and the dawn of a new era for the entire cystinosis community.

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Take-Home Message

The first successful conception induced by a male cystinosis patient, whom are known to have azoospermia of

unknown origin, introduces the dawn of a new era for all patients with this previously fatal metabolic disease.

Details of the Contributions of Individual Authors

The Abstract, Introduction, and Discussion sections were drafted by K. Veys and carefully revised by M. Besouw, E. Goossens, B. van den Heuvel, and E. Levtchenko. The Case Report section was drafted by K. D'Hauwers, A. Van Dongen, M. Janssen, and A. Wetzels, and revised by E. Goossens. Sequencing of the CTNS mutation of the reported patient was performed by B. van den Heuvel. The urologic procedures (PESA) in the reported patient were performed by K. D'Hauwers. The fertilization procedures were coordinated by A. Wetzels. The follow-up of the pregnancy and delivery were managed by A. Van Dongen. M. Janssen and E. Levtchenko are the treating physicians of the cystinosis patient.

Competing Interest Statement

None to declare.

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Details of Ethical Approval

The procedure was performed as part of regular patient care.

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