Congenital bilateral absence of the vas deferens, cystic fibrosis mutation analysis and intracytoplasmic sperm injection

G.T.M. Phillipson1,3, O.M. Petrucco2 and C.D. Matthews2

1 The Fertility Centre, Private Bag 4711, Christchurch Women's Hospital, Christchurch, New Zealand and 2 Reproductive Medicine Unit, Department of Obstetrics and Gynaecology, University of Adelaide, The Queen Elizabeth Hospital, Woodville, Australia
3 To whom correspondence should be addressed

The aim of this study was to assess the outcome of intracytoplasmic sperm injection (ICSI) with fresh and frozen–thawed surgically retrieved spermatozoa from men diagnosed with congenital bilateral absence of the vas deferens (CBAVD). Twenty-seven azoospermic men with their partners were treated [25 with CBAVD and two with clinical cystic fibrosis (CF)]. CF gene mutation analysis and genetic counselling was provided. Spermatozoa were aspirated by microsurgical epididymal sperm aspiration (MESA), percutaneous epididymal sperm aspiration (PESA) or open testis biopsy. Of the men with CBAVD, 60% carried a single mutation, 20% were compound heterozygotes, and 20% had no CF mutation identified. Of the 28 sperm aspiration procedures, 86% had supplementary spermatozoa for cryopreservation with 83% of those samples assessed as satisfactory when thawed. Of 29 cycles with fresh spermatozoa a fertilization rate of 76% of oocytes injected and 17% embryo implantation rate occurred. Twenty-four cycles in which cryopreserved spermatozoa were used resulted in an oocyte fertilization rate of 69% and embryo implantation rate of 20%. Eighteen clinical pregnancies occurred with 14 live births without congenital anomaly. Two pregnancies were achieved following pre-implantation genetic diagnosis. It is concluded that the presence of CF mutations in the male partner does not compromise in-vitro fertilization treatment outcomes or the opportunity for healthy live births.

Key words: congenital bilateral absence of the vas deferens/cystic fibrosis/genetic counselling/male infertility/obstructive azoospermia

Introduction

In 1987, Silber reported the first pregnancy for a couple in whom the man had congenital bilateral absence of the vas deferens (CBAVD) (Silber et al., 1987). The initial in-vitro fertilization (IVF) cycles were notable for poor oocyte fertilization rates with only 15.8% of oocytes inseminated reaching cleavage stage (Mathieu et al., 1992). Since 1993, couples have been offered intracytoplasmic sperm injection (ICSI) with a considerably improved outlook. An increased frequency of cystic fibrosis (CF) gene mutations was reported for men diagnosed with CBAVD (Anguiano et al., 1992). Several series have observed that more than 50% of men with CBAVD carry a single mutation, and approximately 20% are compound heterozygotes (Chillon et al., 1995). Should the female partner also carry a mutation, there is an increased chance of a child with CF for such couples. Here we document our general experience with such couples and discuss the clinical advice to couples in the light of increased knowledge of the genotype–phenotype relationship. Of the 27 couples treated, 15 conceived a total of 18 clinical pregnancies and 14 livebirths have resulted.

Materials and methods

Patients

From 1993 to 1997, 25 couples with primary infertility in whom the male partner was usually admitted on the day of oocyte retrieval.

Analysis of CF gene mutations prior to treatment

Both partners provided a blood specimen, from which the leucocytes were separated and analysed, for a series of CF gene mutations (ΔF508, ΔI506/7, G551D, G542X, R117H, R117C, R553X, and W1282X). The analysis was performed by Biochemical Genetics, Department of Chemical Pathology, Women’s and Children's Hospital, North Adelaide, South Australia, based on the methods originally described to identify the CF gene (Kerem et al., 1989). This group of mutations would be expected to identify about 80% of those mutations expected in the CF population. The identification of the poly T variant associated with cystic fibrosis mutations and discussion of the significance for potential parents required further analysis of the stored specimens for this variant from 1995 onward (Chillon et al., 1995). Once the analyses were complete, clinical genetic counselling was provided.

Surgical sperm aspiration

The male partner was usually admitted on the day of oocyte retrieval. During the time course of this series, two surgical methods were
employed for retrieval of spermatozoa. (i) Microsurgical epididymal sperm aspiration (MESA) under general anaesthesia with the use of an operating microscope was employed for the majority of cases (Oates et al., 1992). On one occasion, because no spermatozoa were harvested from either epididymis an open biopsy of the testicle was taken. (ii) Satisfactory reports of percutaneous epididymal sperm aspiration (PESA) (Shrivastav et al., 1994) led to the introduction of PESA in 1996. PESA was offered as an alternative for those men preferring a less invasive procedure. A 21-gauge scalp vein needle was used to aspirate fluid directly from the epididymis under general or local anaesthesia. The patient recovery after this procedure involved bed rest for only 30 min if performed under local anaesthesia or 4 h if performed under general anaesthesia.

Preparation of spermatozoa
The samples were diluted with human tubal fluid medium (HTFM) containing 5% serum, centrifuged, and resuspended in HTFM before being incubated at 37°C in 5% CO₂ until required. Whenever possible, supplementary spermatozoa were cryopreserved in several microstraws. A trial thaw of a representative straw was performed to assess if spermatozoa exhibiting some motility could be recovered. If satisfactory motile spermatozoa were recovered, the frozen–thawed spermatozoa were used for subsequent treatment cycles. For a few couples, the sperm aspiration was performed electively prior to the treatment cycle or at the time of initial assessment and scrotal exploration, resulting in the use of cryopreserved spermatozoa in the initial IVF cycle.

Ovarian stimulation and embryology
The female partner underwent ovarian stimulation utilizing gonadotrophin-releasing hormone agonist (Lucrin®, Abbott Australasia, Kurnell, NSW, Australia) and human menopausal gonadotrophin (Pergonal® or Metrodin®; Serono, Frenchs Forest, NSW, Australia; Humegon®; Organon, Lane Cove, NSW, Australia) for ovarian stimulation. Ovarian response was monitored with serum oestradiol and transvaginal sonography, with transvaginal oocyte retrieval performed under ultrasonographic guidance. These protocols have been previously documented (Sathanandan et al., 1989). The procedure of ICSI has also been previously described and does not differ from that used for ejaculated spermatozoa (Payne, 1995).

Embryos were transferred to the uterus on the second or third day after oocyte retrieval at the 2- to 8-cell stage. Supplementary embryos were considered for cryopreservation according to the usual laboratory criteria. Luteal support with intramuscular human chorionic gonadotrophin (HCG; Pregnyl®, Organon or Profasi®, Serono) injections following oocyte recovery. A serum pregnancy test was performed 16 days after the oocyte retrieval. Only clinical pregnancies defined by sonographic visualization of the gestational sac are included in this review. All pregnancies were followed up with the attending obstetrician providing a report of obstetric outcome and neonatal outcome.

Statistics
The results have been analysed using GraphPad InStat software, Version 2.04a, 1993, GraphPad Software with calculation of 95% confidence intervals and Fisher’s exact test P values where appropriate.

Results
Of the 25 men with CBAVD, without clinical CF, 15 (60%) carried a single mutation, five (20%) were compound heterozygotes and five (20%) had no mutation identified. These results are listed in Table I. All the men had satisfactory motile spermatozoa recovered at the time of the surgical sperm aspiration. Where possible, further spermatozoa were cryopreserved and a trial thaw analysis performed. Table II lists the results of the sperm aspiration procedures and cryopreservation of spermatozoa. In total, 28 aspiration procedures were performed and of those 20 (71%) had satisfactory spermatozoa frozen for future treatment cycles. This compares favourably with our experience with surgical aspiration of spermatozoa from the epididymis from men with other causes of obstructive azoospermia. Cryopreserved spermatozoa were used for 14 couples for a total of 24 cycles with 264 oocytes available for ICSI. Three of the 14 couples electively used spermatozoa collected at earlier scrotal exploration unrelated to an IVF cycle. There were no significant differences between the observed oocyte fertilization rate, pregnancy rate or implantation rate following the use of fresh compared to cryopreserved spermatozoa. Table III documents the clinical cycle results.

Including both fresh and frozen procedures, 15 of the 27 couples (55%) conceived 18 pregnancies. From the fresh embryo transfers, 15 couples conceived a total of 17 clinical pregnancies with 13 livebirths (eight boys, five girls) from eight singleton and three twin pregnancies. Six couples had miscarriages (one triplet, one twin, and four singleton pregnancies). Of the 70 frozen embryos stored supplementary to the fresh treatment cycle, 33 embryos have been thawed, and 17 (52) were suitable for transfer. One clinical pregnancy occurred from the 12 embryo transfer procedures with the satisfactory livebirth of a girl.

Two pregnancies resulted from cycles in which pre-implantation genetic diagnosis (PGD) by blastomere biopsy was performed to identify the cystic fibrosis mutation ΔF508 prior to embryo transfer. One couple were ΔF508 heterozygotes and

<table>
<thead>
<tr>
<th>CFTR</th>
<th>Thymidine alleles</th>
<th>Number of men</th>
</tr>
</thead>
<tbody>
<tr>
<td>No mutation</td>
<td>7T:7T</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>7T:9T</td>
<td>1</td>
</tr>
<tr>
<td>R117H only</td>
<td>7T:7T</td>
<td>1</td>
</tr>
<tr>
<td>R117C only</td>
<td>9T:9T</td>
<td>1</td>
</tr>
<tr>
<td>ΔF508 only</td>
<td>9T:7T</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>9T:5T</td>
<td>7</td>
</tr>
<tr>
<td>ΔF508:R117H</td>
<td>9T:7T</td>
<td>5</td>
</tr>
<tr>
<td>ΔF508:ΔF508a</td>
<td>9T:9T</td>
<td>1</td>
</tr>
<tr>
<td>ΔF508:W1282X²</td>
<td>9T:7T</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>27</td>
</tr>
</tbody>
</table>

*Denotes patient with CF.

<table>
<thead>
<tr>
<th>Table II. Epididymal sperm cryopreservation</th>
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<tr>
<td>Procedures (n)</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>PESA</td>
</tr>
<tr>
<td>MESA</td>
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<tr>
<td>Total n (%)</td>
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CBAVD, CF mutations and ICSI

Table III. Clinical cycle results

<table>
<thead>
<tr>
<th>Male cystic fibrosis genotype detected allele1:allele2</th>
<th>Nil:nil</th>
<th>Nil:R117H or nil:R117C</th>
<th>AF508:nil</th>
<th>AF508:R117H</th>
<th>AF508: W1282X or AF508: AF508</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Couples (n)</td>
<td>5</td>
<td>2</td>
<td>13</td>
<td>5</td>
<td>2</td>
<td>27</td>
</tr>
<tr>
<td>Mean age female partner (years)</td>
<td>33</td>
<td>29</td>
<td>31</td>
<td>34</td>
<td>26</td>
<td>31</td>
</tr>
<tr>
<td>Oocyte retrieval cycles (n)</td>
<td>7</td>
<td>9</td>
<td>22</td>
<td>12</td>
<td>3</td>
<td>53</td>
</tr>
<tr>
<td>Oocytes injected (n)</td>
<td>69</td>
<td>134</td>
<td>199</td>
<td>113</td>
<td>43</td>
<td>558</td>
</tr>
<tr>
<td>Oocytes fertilized (n)</td>
<td>46</td>
<td>102</td>
<td>144</td>
<td>85</td>
<td>29</td>
<td>406</td>
</tr>
<tr>
<td>Fertilization rate (%) (ET) (n)</td>
<td>5</td>
<td>(76)</td>
<td>(72)</td>
<td>(75)</td>
<td>(67)</td>
<td>(73)</td>
</tr>
<tr>
<td>Embryos transferred (n)</td>
<td>13</td>
<td>25</td>
<td>52</td>
<td>29</td>
<td>6</td>
<td>125</td>
</tr>
<tr>
<td>(mean per ET)</td>
<td>(2.6)</td>
<td>(2.8)</td>
<td>(2.4)</td>
<td>(2.4)</td>
<td>(2.0)</td>
<td>(2.5)</td>
</tr>
<tr>
<td>Clinical pregnancies (n)</td>
<td>3</td>
<td>0</td>
<td>9</td>
<td>3</td>
<td>2</td>
<td>17</td>
</tr>
<tr>
<td>(mean per ET)</td>
<td>(2.6)</td>
<td>(2.8)</td>
<td>(2.4)</td>
<td>(2.4)</td>
<td>(2.0)</td>
<td>(2.5)</td>
</tr>
<tr>
<td>Embryo implantation rate (%)</td>
<td>23</td>
<td>0</td>
<td>21</td>
<td>21</td>
<td>33</td>
<td>18</td>
</tr>
<tr>
<td>Embryos frozen (n)</td>
<td>16</td>
<td>9</td>
<td>34</td>
<td>21</td>
<td>6</td>
<td>86</td>
</tr>
<tr>
<td>Cycles with embryos frozen (n)(% of cycles)</td>
<td>4</td>
<td>44</td>
<td>10</td>
<td>5</td>
<td>2</td>
<td>25</td>
</tr>
<tr>
<td>(57)</td>
<td>(44)</td>
<td>(45)</td>
<td>(42)</td>
<td>(47)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*One cycle had a single oocyte which failed to fertilize, and one cycle had all embryos frozen.

ET = embryo transfer.

the other included a female partner who was identified as a compound heterozygote (see below). A first trimester spontaneous abortion and a live birth resulted. Analysis of the products of conception and blood from the neonate confirmed the genotype expected from the pre-implantation diagnostic procedure (Cui et al., 1995).

At the request of the parents the CF genotypes of two children derived from fathers who were ΔF508 heterozygotes were tested and found to be heterozygotes. Both children are healthy, consistent with their heterozygous genotype.

Discussion

CBAVD and CF gene mutations

CBAVD occurs in 1 in 1000 men in the population and represents 1–2% of infertile males. Testicular function is usually not compromised with normal testicular size and consistency, normal concentrations of serum FSH with normal spermatogenesis. Whereas almost all men with cystic fibrosis have CBAVD, only a small proportion of men with CBAVD have a medical history of pancreatic insufficiency, respiratory disease or chronic sinusitis and it is therefore considered a mild phenotype of cystic fibrosis (Oates and Amos, 1993). Fetal anomaly in the seventh week of gestation during Wolffian duct differentiation results in the characteristic pathology.

The Wolffian duct abnormalities evident in CF and CBAVD indicate a common genetic link confirmed with the identification of CF gene mutations in both conditions. A review of 420 published cases of CBAVD indicated 19% had two mutations, 47% carried a single mutation, and in 34% no mutation could be identified (Lissens et al., 1996). Our experience supports these findings with a similar proportion of heterozygotes (60%), compound heterozygotes (20%) and 20% without a mutation. There is an increased prevalence of the R117H mutation within the CBAVD population. In this series, of the men with CBAVD (excluding the two with CF), six of the 25 (24%) of the CF chromosomes identified were R117H. This compares with less than 0.3% incidence of R117H in those patients with classic CF and is similar to the 22% initially reported in CBAVD (Gervais et al., 1993).

Men with CBAVD but without CF gene mutations have a high incidence of urinary tract malformations (Dork et al., 1997). This group with urinary tract anomalies represent a separate clinical entity not related to CF and with a different embryological pathogenesis. In our study the five men without a CF mutation underwent ultrasonic assessment of their renal tract and two of them had unilateral kidney agenesis.

The protein encoded by the CF gene is designated CF transmembrane conductance regulator (CFTR). In normal individuals, five, seven or nine thymidine (T) nucleotides can behave CBAVD, only a small proportion of men with CBAVD occur in 1 in 1000 men in the population and represents 1–2% of infertile males. Testicular function is usually not compromised with normal testicular size and consistency, normal concentrations of serum FSH with normal spermatogenesis. Whereas almost all men with cystic fibrosis have CBAVD, only a small proportion of men with CBAVD have a medical history of pancreatic insufficiency, respiratory disease or chronic sinusitis and it is therefore considered a mild phenotype of cystic fibrosis (Oates and Amos, 1993). Fetal anomaly in the seventh week of gestation during Wolffian duct differentiation results in the characteristic pathology.

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Genetic counselling

Genetic counselling prior to conception provides an estimate of the likelihood of inheriting a CF mutation from each parent. The probable phenotype of the child is calculated based upon the female partner’s genotype, the severity of the mutation identified in the male partner, and the presence of intron 8 splice site variant. Even if CF mutation screening is negative there remains a small possibility that the individual carries an undetected mutation. For couples in whom the risk of CF is unacceptably high, prenatal diagnosis or pre-implantation genetic diagnosis can be offered. Pre-implantation genetic diagnosis has been successfully used for \( \Delta F508 \) and W1282X but not for the less common mutations.

Female heterozygotes carry a single CF gene mutation and have no signs of cystic fibrosis. No female equivalent of CBAVD exists due to the different embryological origin of the definitive female reproductive tract. One female compound heterozygote was identified. Her genotype (G551D/R117H) comprised a severe CF mutation (G551D) combined with R117H and the 7T allele identified on both chromosomes. She reported no past history of CF symptoms, and the assumption could be made that the R117H was present on a chromosome with an efficient intron 8–exon 9 acceptor splice site. As her partner was a \( \Delta F508 \) heterozygote, pre-implantation genetic diagnosis by blastomere biopsy with PCR amplification was performed to avoid the transfer of a \( \Delta F508 \) compound heterozygote embryo. Fifty per cent of such embryos would be \( \Delta F508/\ G551D \) and likely to result in a child with CF. The remaining \( \Delta F508 \) compound heterozygote embryos would be \( \Delta F508/\ R117H \) compound heterozygotes that would be expected to confer a mild phenotype to the child. Transfer of embryos without the \( \Delta F508 \) mutation would confer a G551D or R117H heterozygote genotype. The presence of an unidentified mutation together with \( \Delta F508 \) in the male partner was a remote possibility which could also result in a compound heterozygote. Despite establishment of a pregnancy, spontaneous miscarriage occurred. Analysis of the products of conception confirmed the expected genotype with absence of \( \Delta F508 \). The second couple for whom pre-implantation diagnosis was performed were \( \Delta F508 \) heterozygotes. Transfer of homozygous normal embryos resulted in a livebirth of a girl with the genotype confirmed from cord blood.

ICSI outcomes

Earlier studies indicating poor oocyte fertilization rate and embryo implantation rate are in contrast to this series. Our results are comparable to those with ejaculated spermatozoa, and the implantation rates are higher than those achieved with routine IVF in our clinic (Payne et al., 1994).

For the two couples in whom the male partner were carriers of either the R117H or R117C CF mutation, pregnancy did not occur despite the transfer of 25 embryos over nine cycles. Embryos derived from males with compound heterozygote \( \Delta F508/R117H \) genotypes demonstrated a satisfactory embryo implantation rate. Larger studies will be necessary to establish if there is an association between outcome and specific CF mutations.

No significant differences were observed between the use of fresh and cryopreserved spermatozoa, however previously reported smaller series have noted a possible adverse effect of cryopreservation (Devroey et al., 1995; Nagy et al., 1995). Our own concurrent larger series of treatment cycles including other causes of obstructive azospermia has not demonstrated a significant reduction in oocyte fertilization rate and embryo implantation rate. Couples are now advised to consider cryopreservation of spermatozoa prior to treatment provided that the post thaw analysis has confirmed motile sperm recovery.

Only one of the 17 frozen thawed embryos transferred has resulted in a live birth. However, substantially larger numbers of embryos will need to be transferred before analysis of outcomes will be possible.

The clinical examination and follow-up of children born to couples with CBAVD or CF is essential to understand the variable phenotypic expression of CF gene mutations. For most couples in whom the male has CBAVD, the male is a carrier of a severe CF gene mutation and the female tests negative for the group of mutations screened. Therefore the offspring are expected to be asymptomatic (homozygous negative or asymptomatic heterozygotes). No significant congenital anomalies have been identified. The eight boys born to CBAVD fathers were examined and found to have a palpable vas deferens as expected.

For the two couples in whom the male had been diagnosed with cystic fibrosis, three oocyte retrieval cycles were completed. Sperm recovery, sperm cryopreservation, and the ICSI treatment cycle results were all comparable to the CBAVD men without clinical cystic fibrosis. These couples both conceived; however, one resulted in a miscarriage late in the first trimester, the other pregnancy resulted in the birth of a healthy infant. Historically a few reports of fertile CF males have been published. The demonstration of satisfactory spermatogenesis and this successful delivery of a healthy infant adds to the evidence confirming the ability of males with CF to father children.

It is concluded that the advice and treatment options available for infertile couples in whom men have a diagnosis of CBAVD have changed substantially in the course of this series. Analysis of both partners’ medical and family history, cystic fibrosis genotype, and genetic counselling is required before initiating treatment. The presence of cystic fibrosis mutations is an important part of the assessment to permit adequate genetic counselling and the use of pre-implantation genetic diagnosis to reduce the likelihood of a child with cystic fibrosis may need to be considered. Surgical sperm aspiration and the use of fresh or frozen spermatozoa provide outcomes similar to those achieved with ejaculated spermatozoa. Cystic fibrosis mutations in the male partner do not appear to compromise oocyte fertilization, embryo implantation rates, or the opportunity for healthy live births. However the total number of live births reported remains small and continued study will be required to provide sufficient information to counsel couples contemplating treatment.

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