Reproductive Biology Section

Three singleton deliveries with healthy children from one couple after Cryo-TESE and ICSI

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Summary

We report on a couple who delivered three healthy babies in three deliveries after cryo-TESE combined with ICSI. The male patient suffers from congenital bilateral absence of the vas deferens (CBAVD). *Methods:* Three testicular sperm extraction (TESE) operations were performed in the male accompanied by six stimulated ICSI cycles in the female patient. Altogether, 59 oocytes were retrieved. Fifty-one oocytes (86%) were in metaphase II and 38 fertilized regularly (75%). Sixteen embryos, in the 3-6 cell stage, were transferred to the uterus. *Results:* The first, fifth and sixth embryo transfers of fresh embryos led to intact intrauterine singleton pregnancies. The pregnancy and implantation rates with fresh embryos were 50% and 20%, respectively. *Conclusions:* TESE or microscopic epididymal sperm aspiration in patients with CBAVD in combination with a healthy female partner is likely to yield very good results in ICSI/ET. As azoospermia can be caused by cystic fibrosis and cystic fibrous transmembrane conductance regulator gene mutation range varies dramatically in patients of different ethnic groups.

Key words: Azoospermia; Cystic fibrosis; ICSI; Pregnancy; TESE.

Introduction

Azoospermia is a common problem that occurs in about 5% of all investigated infertile couples [1] and in 10-20% of infertile men with abnormal seminal fluid [2]. In azoospermia non-obstructive and obstructive cases are distinguished.

With testicular sperm extraction (TESE), first introduced in 1993 by Craft [3] and Schoysman [4], it is possible to treat patients who have either obstructive or non-obstructive azoospermia [5, 6] by performing intracytoplasmic sperm injection (ICSI). The delivery rate with TESE is generally higher in patients with obstructive azoospermia [7] than with non-obstructive azoospermia, but similar to patients treated with microscopic epididymal sperm aspiration (MESA) [8].

Congenital bilateral absence of the vas deferens (CBAVD) is found in 2% of men who present with infertility [9] and in 10% of men with obstructive azoospermia [10]. CBAVD is diagnosed intraoperatively or on the basis of otherwise unexplained obstructive azoospermia. In a large proportion of cases, CBAVD is thought to be a probable consequence of a mutation in the cystic fibrosis transmembrane conductance regulator gene (CFTR) (for review see [11, 12] and to represent a monosymptomatic form of cystic fibrosis (CF).

To date, more than 1,000 CFTR gene mutations have been discovered. Forty to 83% of patients with CBAVD were shown to have at least one known CFTR gene mutation and 10% to 66.7% to have two mutations, according to the applied method and population differences. In 21.6 to 60% of patients with CBAVD no CFTR mutations were found [12-16]. The reasons for such differences in the data available may be related to some heterogeneity in the etiology of CBAVD [17, 18], patient group selection, the method applied and origin of the patients.

Therefore, it is important to keep in mind that the combination TESE-ICSI or MESA/ICSI contains the risk of transmitting genetic disorders to offspring who otherwise would not have been born.

Materials and Methods

Medical history

A Palestinian couple was first admitted to our Division of Reproductive Medicine in 1997. The female partner, born in 1979, healthy and with no history of serious diseases in her family, had had a five-day cycle (every 24-27 days) from the age of 12, took no oral contraceptives and had no previous pregnancies. She had a latent hypothyreosis with the highest TSH level of 5.4 mU/l which was treated with L-thyroxin and KJ to the lower the TSH level 3.5 mU/l before the first TESE-ICSI cycle began.

The male partner, born in 1964, had normal renal anatomy and no report of chronic respiratory complaints or pancreatic failure in his personal or family history. Repeatedly, no sperm was found in his ejaculate. The histology of both testicles showed the same picture of obstructive azoospermia: big calibre canaliculi and full formation of mature spermatids in reduced numbers. Intraoperational diagnosis suggested congenital bilateral absence of the vas deferens (CBAVD).

Genetic counselling

Genetic counselling took place and both partners underwent a chromosomal examination and CFTR test.

The 36 mutation panel used

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Table 1. — *Stimulation and results.*

Date of tratment	Stimulation	Fertilized oocytes	Transfer (fresh)[1]	Success
07/1997	Decapeptyl/Menogon 75 (3-2 Amp.)	3 of 8	3 Embryos	04/1998 spontaneous delivery, healthy boy 3,090 g/52 cm, 39 weeks + 2
03/1999	Synarela/Fertinorm 75 (3-1 Amp.)	12 of 15	3 Embryos	
06/1999	Synarela/Puregon 100 (2-1 Amp.)	2 of 4	2 Embryos	
10/1999	Synarela/Menogon 75 (2-4 Amp.)	6 of 8	3 Embryos	
01/2001	Enantone/Menogon 75 (2 Amp.)	4 of 10	2 Embryos	10/2001 spontaneous delivery, healthy girl 3,550 g/56 cm, 39 weeks
02/2004	Decapeptyl/Gonal F 150 (1 Amp.)	5 of 6	2 Embryos	09/2004 spontaneous delivery, healthy boy 4,400 g/52 cm
1997-2004		32 of 51	15 Embryos	3 births (PR 50%, IR 20%)

[1]One cryopreserved embryo was transferred out of the 2[nd] cycle.

(F508del, G542X, ***N1303K, W1282X,*** *G551D, 1717-1G->A, R553X, CFTRdele2,3 (21kb), I507del, 711+1G->T, 3272-26A->G, 3905 ins T, R560T, 8098+1G->A, 5125IN, I148T, 3199del6, 3120+1G->A, Q552X;*

621+1G->T, 3849+10kbC->T, ***2183AA->G,*** *394delTT, 2789+5G-A, R1162X, 3659delC, R117H, R334W, R347P,* ***G85E,*** *1078delT, A455E, 2143delT, E60X, 2184delA, 711+5G->A; intron 8: 7T/9T/5T)*

uncovers 85% of the known CFTR gene mutations in Caucasians and included five of eight mutations shown to account for 90% of CFTR mutations in Israeli/Arab patients (DF508, N1303K, W1282X, 3120+1Kbdel8.6Kb, G85E, R75X, 2183AA>G, and del (exon2) [19]. No abnormalities were found. The couple was informed that the test performed uncovers 85% of the known CFTR gene mutations but there was a possibility of having one of more than 900 mutations that were not tested.

The male patient underwent three TESE operations with cryopreservation as our group does not perform MESA. From the first two operations a sufficient amount of vital motile sperms was obtained for five ICSI treatments. The last operation in 2003 resulted in preservation of individual immotile avital sperms which were used for the sixth ICSI. No hormone substitution was required.

Stimulation and intracytoplasmic sperm injection

Since July 1997 the female patient had undergone six controlled ovarian hyperstimulations using long GnRH agonist stimulation protocols. Stimulation was performed with starting dosages of 150-225 IU of FSH (Table 1). Ovulation was triggered with 10000 IU of HCG when the diameter of the majority of follicles was 16 mm or more. Follicular puncture was performed 34-35 hours after HCG injection vaginally, under ultrasound control, on day 12-15 of stimulation. The luteal phase was supported daily with a vaginal application of progesterone and one HCG injection with a dosage dependent on the E2 level on day 5 after embryo transfer. The pregnancy test was performed two weeks after embryo transfer. Early pregnancy was monitored at three, four and five weeks after embryo transfer with transvaginal ultrasound and HCG.

One day before ICSI the testicular tissue was thawed and rinsed in IVF universal culture medium (Medicult, Denmark), dissected mechanically into pieces and incubated overnight at 37°C in an atmosphere of 5% CO_2. On the day of ICSI the tissue was centrifuged for 10 min at 300 g. The sediment was diluted in 200 µl of IVF medium and cultivated for six hours until ICSI. ICSI was performed in the usual way.

Results

In six follicular punctures 59 oocytes were retrieved (10 oocytes/FP). Fifty-one oocytes (86%) were in metaphase II, 32 of them fertilized regularly (63%) (Table 1). Sixteen embryos, in the 3-6 cell stage, (15 fresh and 1 embryo after pronuclear stage cryopreservation) were transferred to the uterus two days after oocyte retrieval in seven embryo transfers (ET) (2-3 embryos in every fresh transfer). The first, fifth and sixth ET with fresh embryos led to intact intrauterine single pregnancies. The pregnancy and implantation rates with fresh embryos were 3/6 = 50% and 3/15 = 20%, respectively.

The first baby, a healthy boy, 3,090 g/52 cm, was spontaneously born in April 1998 after 39 weeks + 2 of gestation after the first ICSI. The second baby, a healthy girl, 3,550 g/56 cm, was spontaneously born in October 2001 after 39 weeks of gestation after the fifth ICSI (the sixth embryo transfer) in January 2001. The latest TESE-ICSI cycle was performed in February 2004. Two of six mature oocytes were injected with mature sperms and four with sperms that contained cytoplasm fragments. Five oocytes fertilized normally. The transfer of two good quality embryos in the 4-cell stage resulted in the development of an intact singleton pregnancy. The patient delivered her third child, a healthy boy, 4,400 g/52 cm, at term in November 2004.

Discussion

The fertilization rate in patients with obstructive azoospermia with frozen-thawed spermatozoa in a TESE-ICSI cycle is reported to be 62.7%, and the pregnancy rate 21.7%-26% [20, 21]. In our couple the fertilization rate was 75% and the pregnancy rate was 50%. Both lie well above the reported data.

The pregnancy rate and live birth rate strongly depend on the age of the female patient, but not on the sperm quality. Therefore, women between 20-29 years of age have significantly higher pregnancy rates with TESE-ICSI than women over 34 [22]. Wives of azoospermic men who were in their 20s had a 46% live delivery rate per cycle whereas wives who were over 40 had a rate of only 3% [23]. The 50% pregnancy rate in our patient, who was between 18 and 24 years at the time of treatment, is in compliance with this data, even under the strict conditions of the German embryo protection law, prohibiting cultiva-

tion of more than three embryos. The average pregnancy rate per cycle after ICSI in Germany is 26% [24].

Generally, the first TESE procedure results in a sufficient amount of mature spermatozoa to perform ICSI [23]. Repeated TESE operations can also be successful if the testicle tissue is homogeneous in CBAVD and there is no necessity for hormone substitution in the men. Mildly impaired spermatogenesis in patients with CBAVD in combination with the beneficial female partner's age factor is likely to yield very good results in TESE-ICSI. Repeated pregnancies and births are possible in these couples.

The combination TESE-ICSI is difficult, large-scale and expensive but apart from MESA it is the only possibility for men with azoospermia to become a genetic father. It has been shown in the past that testicular spermatozoa results in higher abortion rates than epididymal spermatozoa [25], but as this technique is not standard for our team we performed TESE. Our concern lies in the fact that CBAVD patients, in whom no mutations in the CFTR gene are found, are still at high risk of having offspring with CF. Accumulated data show the incidence of different CFTR mutation-types in different ethnic groups. Mutation panels which are presently used for CFTR screening detect only 7-12% of the CFTR mutations of tested alleles in Turkish males with CBAVD. Direct sequencing reveals pathogenic alterations in 72.5% of the tested alleles [16].

Although the DF508 mutation accounts for 67% of all CFTR mutations in the European population, it uncovers only 7.4% of mutations in Arabs [26]. In our clinical practice we include mutation panels F508del, N1303K, W1282X, 2183AA->g and G85E as a routine method. They are reported to detect five of the eight most common CF mutations in Israeli/Arab patients [9].

Because lung disease may develop later in the life of young CFTR mutation carriers seeking assisted reproduction, careful genetic counselling and testing are necessary. As the CFTR gene mutation range varies dramatically between populations, genetic counselling and test application must take into consideration the patient's ancestry. For this purpose, future studies in patients of different ethnic groups are required. Preimplantation genetic diagnosis should be discussed with all male patients testing positively for a CFTR mutation.

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