

Surgical sperm retrieval and intracytoplasmic sperm injection as treatment of obstructive azoospermia

G.R.Dohle^{1,3}, L.Ramos², M.H.E.C.Pieters²,
D.D.M.Braat² and R.F.A.Weber¹

¹Department of Andrology and ²Obstetrics and Gynaecology,
University Hospital Rotterdam, The Netherlands

³To whom correspondence should be addressed

Male genital tract obstructions may result from infections, previous inguinal and scrotal surgery (vasectomy) and congenital bilateral absence of the vas deferens (CBAVD). Microsurgery can sometimes be successful in treating the obstruction. In other cases and in cases of failed surgical intervention, the patient can be treated by microsurgical or percutaneous epididymal sperm aspiration (MESA, PESA) or testicular sperm extraction (TESE) and intracytoplasmic sperm injection (ICSI). We present the results of 39 ICSI procedures for obstructive azoospermia in 24 couples. The aetiology of the obstruction was failed microsurgery in 11 patients, CBAVD in nine and genital infections in four. Sperm retrieval was accomplished via MESA in four cases, PESA in 18 cases and via TESE in 11 cases. TESE was only applied when PESA failed to produce enough spermatozoa for simultaneous ICSI. In six patients, the ICSI procedure was performed with cryopreserved spermatozoa after an initial PESA procedure. Fertilization occurred in 47% of the metaphase II oocytes; embryo transfer was performed in 92% of procedures and resulted in a clinical pregnancy in 13/39 procedures. Ongoing pregnancy was achieved in 10/39 procedures. One pregnancy was terminated early after prenatal investigation showed a cytogenetic abnormality (47,XX+18, Edwards syndrome). The other nine pregnancies resulted in the live birth of 10 children, without any congenital abnormalities. Epididymal and testicular retrieved spermatozoa were successfully used for ICSI to treat obstructive azoospermia, and resulted in an ongoing pregnancy in 10 of 24 couples (41.6%) after 39 ICSI procedures, a success rate of 25.6% per treatment cycle and of 27.7% per embryo transfer.

Key words: ICSI/MESA/obstructive azoospermia/PESA/TESE

Introduction

Azoospermia is found in 10% of male infertility cases and is caused by a testicular insufficiency in the majority of patients. In 20% of cases, a bilateral obstruction of the male genital tract is responsible for the azoospermia (Hendry, 1994). In the presence of a history of previous scrotal or inguinal surgery or recurrent genital infection, an obstruction can be suspected. These patients are characterized by normal testis volume and

normal concentrations of gonadotrophins [luteinizing hormone (LH)/follicular stimulating hormone (FSH)]. In 1–2% of the infertile male population, congenital bilateral absence of the vas deferens (CBAVD) is found (Anguiano and Oates, 1992). The definitive diagnosis of obstructive azoospermia is made by performing a testicular biopsy, showing normal spermatogenesis.

Microsurgical repair of vas deferens obstructions or epididymal blockage can result in spontaneous pregnancies in 27–56% of cases (Belker *et al.*, 1991; Jarow *et al.*, 1997). The results are determined by several factors, including site and duration of the obstruction, epididymal function, recurrent genital tract infections and sperm antibodies. In cases of poor sperm quality after the operation, assisted reproductive techniques (ART) have been applied successfully. Recently, intracytoplasmic sperm injection (ICSI) has become available for treatment of severe oligospermia (Palermo *et al.*, 1992). This technique has also been applied to treat azoospermia if viable spermatozoa could be retrieved from the epididymis or the testis, giving results similar to those of ICSI for oligospermia (Devroey *et al.*, 1994; Silber *et al.*, 1994).

We present the results of 39 ICSI procedures for obstructive azoospermia in combination with microsurgical epididymal sperm aspiration (MESA), percutaneous epididymal sperm aspiration (PESA) or testicular sperm extraction (TESE).

Materials and methods

Patients

The combination of ICSI and sperm retrieval was offered to couples with male factor infertility due to bilateral genital obstruction. The mean age of the treated males was 38.1 years (SD 6.3), the mean age of the treated females was 34.1 years (SD 4.2). All males had normal testicular volume, normal serum FSH concentration and, on testicular biopsy, a normal sperm count according to the Johnson scoring system (Johnson, 1970). Azoospermia was found on at least two occasions. In men with CBAVD genetic analysis of common cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations were performed on the patient and his spouse. These couples were counselled for genetic risks concerning cystic fibrosis inheritance before the procedure. In cases of pregnancy, all patients were offered prenatal screening, amniocentesis and ultrasound examination. Informed consent was obtained from all couples after explanation of the experimental nature of the treatment and its known and unknown medical and genetic risks.

Ovulation induction and oocyte retrieval

Pituitary down-regulation was achieved by administering a gonadotrophin-releasing hormone analogue (GnRHa), triptoreline (Decapeptyl[®], Ferring, Hoofddorp, The Netherlands) subcutaneously

once a day. Multiple follicle development was induced with human menopausal gonadotrophin (HMG) (Humegon®, Organon, Oss, The Netherlands). Depending on the size of the follicles and the serum oestradiol concentration, a single injection of 10000 IU of human chorionic gonadotrophin (HCG) (Pregnyl®, Organon) was administered and a transvaginal ultrasound-guided follicular aspiration was performed 34–36 h later.

Oocyte processing

Oocyte preparation was performed according to the protocol as described by Palermo *et al.* (1992), with the modification of a maximum of 3 min hyaluronidase treatment. Only intact oocytes showing a polar body were microinjected.

Sperm retrieval

The MESA procedure was performed under general anaesthesia as an outpatient procedure. After exposure a dilated tubule of the epididymis was microsurgically opened and its fluid examined for the presence of motile spermatozoa. After four MESA procedures had been performed, the procedure was abandoned in favour of PESA in all subsequent cases. Percutaneous aspiration of the epididymal head (Tsirigotis *et al.*, 1995) was performed under local anaesthesia of the spermatic cord (Figure 1). In the case of an unsuccessful PESA procedure, a testicular excisional biopsy (TESE) was performed for extraction of viable spermatozoa (Silber *et al.*, 1995).

Sperm processing

Following MESA/PESA, the epididymal aspirates were diluted in 10 ml in-vitro fertilization (IVF) medium and centrifuged for 10 min at 1500 g. The supernatant was removed and the pellet was resuspended in 10 ml IVF medium and the same centrifugation procedure was performed. After removal of the supernatant, the cell pellet was incubated until the start of the ICSI procedure. Approximately 1 µl of this suspension was added to 50 µl of polyvinylpyrrolidone (PVP) solution or diluted in 50 µl IVF medium in case of very poor motility.

During the TESE procedure, the testicular biopsies were performed under local or general anaesthesia. After obtaining a testicular sample, its adequacy was immediately assessed: depending on the outcome of the first sample (i.e. the presence or absence of spermatozoa), more testicular tissue might be necessary. In the laboratory, the tissue sample was shredded by means of two sterile glass slides and a suspension of the cells was made in IVF medium. During an incubation period of at least 2 h, spermatozoa were allowed to gain motility. After the incubation period, the suspension was centrifuged for 5 min at 300 g and the supernatant was removed and recentrifuged for 10 min at 1500 g. Shortly before the injection procedure, ~1 µl of the cell pellet suspension was added to 50 µl of IVF medium.

During the MESA/PESA and TESE laboratory procedures, none of the suspensions or washing media was discarded. If spermatozoa were absent in the pellet, they might still be found in one of the other suspensions.

ICSI procedure

Commercially available injection pipettes (Gynotec®, Malden, The Netherlands) were used with an outer and inner diameter of 7 and 5 µm respectively. The oocyte holding pipette had an outer and inner diameter of 90 and 10 µm respectively. One drop of sperm–PVP solution was placed in the centre of a Petri dish, surrounded by 5 drops of IVF medium containing metaphase II oocytes. All drops were covered by paraffin oil. The ICSI procedure was performed under a ×400 magnification, using hydraulic micromanipulators and microinjectors. A single spermatozoon was isolated, immobilized and aspirated tail-first into the injection pipette. The oocyte was fixed by

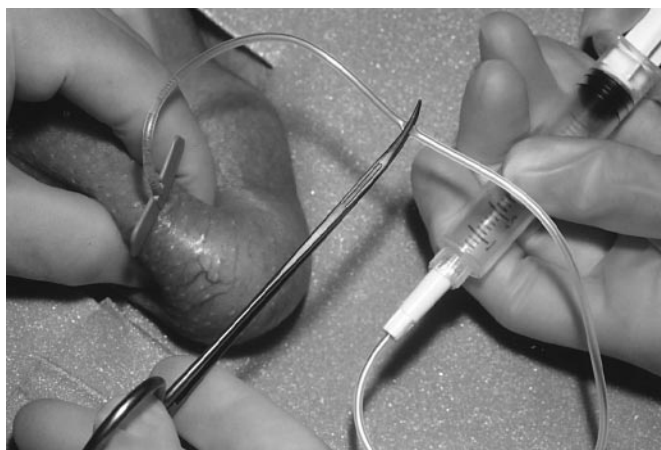


Figure 1. Percutaneous epididymal sperm aspiration (PESA). A 19 gauge butterfly needle is inserted into the epididymal head. A mild vacuum is applied and the butterfly needle is moved gently within the epididymal head. Clear epididymal fluid is aspirated into the collecting tube for microscopical examination.

the holding pipette and the injection pipette was pushed through the zona at the opposite side. Breakage of the ooplasmic membrane was initiated by gentle suction with the injection pipette, and one spermatozoon was injected immediately after membrane breakage was observed.

Fertilization

At 16–18 h after microinjection, the oocytes were microscopically examined. Normal fertilization was defined by the presence of two pronuclei and a second polar body. Depending on the day of embryo transfer, embryo cleavage was judged 3, 4 and 5 days after the ICSI procedure. A maximum of two embryos was transferred into the uterine cavity and supernumerary embryos of good morphological quality were cryopreserved. Pregnancy was confirmed 18 days after oocyte retrieval by a positive pregnancy test. A clinical pregnancy was defined as the presence of at least one gestational sac with a fetal heart beat. An ongoing pregnancy was defined as a clinical pregnancy beyond 12 weeks of gestation.

Results

The combined sperm retrieval–ICSI procedure included 39 treatment cycles in 24 couples. Azoospermia was present in all men in at least two semen samples. CBAVD was present in nine patients, a history of recurrent infections in four and a previous surgical intervention in the scrotum or the inguinal region, including failed microsurgery, in 11.

MESA was performed in the first four patients only, and was successful in all. Subsequently, PESA was introduced as the first approach for epididymal sperm retrieval for future cases. PESA was successful in 18/29 (62%) procedures. In 11 cases of failed epididymal sperm retrieval an excisional testicular biopsy was performed under local or general anaesthesia in the same procedure, and viable spermatozoa were found in 9/11 (82%) biopsies. In one patient, a repeated biopsy had to be performed on the same day, which yielded viable spermatozoa which were successfully used for ICSI. Only in one case of TESE could no viable spermatozoa be found after incubation. Sperm cryopreservation was performed only after epididymal

Table I. Results of 39 ICSI cycles with epididymal (MESA/PESA), testicular (TESE) retrieved spermatozoa and spermatozoa which were cryopreserved (CRYO) after initial PESA procedure

	Number of cycles	Oocytes	MII oocytes	Fertilization (%)	Embryo transfer (%)	Pregnancy (%)	Ongoing pregnancy (%)
MESA/PESA	22	275	186	101/186 (54)	21/22 (95)	9/22 (41)	6/22 (27)
TESE	11	127	90	32/90 (36)	9/11 (82)	4/11 (36)	4/11 (36)
CRYO (after PESA)	6	52	27	9/27 (33)	6/6 (100)	0/6 (0)	
Total	39	454	303	142/303 (47)	36/39 (92)	13/39 (33)	10/39 (25.6)

Table II. Pregnancy and delivery results of 39 ICSI procedures combined with surgical or percutaneous sperm retrieval

Patient number	Prenatal cytogenetics	Delivery term	M/F	Birth weight (g)	Percentile (P) (Kloosterman <i>et al.</i> , 1970)
1	46,XX	38 weeks	F	3555	P90
2	46,XY	41 weeks	M	4110	P95
3	46,XY	37 weeks	M	2435	P15
4	46,XY	36 weeks	M	2660	P50
5 (twin)	46,XX	35 weeks	F	2270	P40
	46,XX	35 weeks	F	2370	P50
6	47,XX+18 (trisomy 18)	early termination	F		
7	46,XX	42 weeks	F	4160	P95
8	–	41 weeks	F	3990	P90
9	–	41 weeks	F	4190	P90
10	46,XY	41 weeks	M	3760	P75

retrieval and was successfully used in six patients as a sperm source where the first combined cycle had failed.

Table I summarizes the results of the 39 treatment cycles. Oocyte retrieval resulted in a total of 454 oocytes, of which 66.7% had extruded the first polar body (metaphase II oocytes). Fertilization occurred in 47% of the metaphase II oocytes after the ICSI procedure. The fertilization rate and embryo transfer after MESA/PESA and TESE was similar. Transfer of two embryos into the uterine cavity was performed in 36/39 (92%) cycles, resulting in a clinical pregnancy in 13/39 (33.3%). In eight cycles, 26 supernumerary embryos of good morphological quality were cryopreserved. Thawing of the embryos was performed for five patients and embryos were transferred in two couples. This, however, did not result in clinical pregnancies. MESA/PESA procedures produced 27% ongoing pregnancies and TESE procedures produced 36% ongoing pregnancies. Ten ongoing pregnancies occurred in 24 couples (41.7%), a pregnancy rate of 25.6% per treatment cycle and 27.7% per embryo transfer. Frozen-thawed epididymal spermatozoa were successfully used for a second ICSI procedure in six couples. Unfortunately, no pregnancy occurred in these patients. Amniocentesis was performed in eight out of 10 pregnancies, and gave normal karyograms in seven pregnancies. One pregnancy was terminated early for trisomy 18 (47XX+18, Edwards syndrome). Nine deliveries occurred at term, resulting in 10 babies with no obvious congenital malformations. Birth weight was low in two children (<P50; Kloosterman, 1970), one singleton and one twin baby (Table II).

Discussion

Ductal obstruction occurs in 20% of azoospermic patients. Acquired ductal obstructions account for most of the cases of obstructive azoospermia and can sometimes successfully be

treated by microsurgery. Infections of the ductal system and surgery of the scrotum and the groin are the main causes of this type of obstruction.

Surgical sperm retrieval has been introduced as an alternative for microsurgery and is used in cases of failed vasectomy reversal. Initial attempts to perform IVF with epididymal sperm showed limited results, due to poor sperm quality after retrieval (Pryor *et al.*, 1984; Temple-Smith *et al.*, 1985). In 1988, Silber introduced a microsurgical technique for sperm aspiration, the MESA procedure, which was successful in 10 of 32 cases of CBAVD (Silber *et al.*, 1988). The combined MESA/IVF procedure gave a pregnancy rate of 10–14% (The Sperm Microaspiration Retrieval Techniques Study Group, 1994). Recently, the ICSI procedure has been introduced for the treatment of severe male factor infertility (Palermo *et al.*, 1992). Combining the ICSI procedure with the MESA procedure has resulted in high fertilization rates and pregnancies in 31–34% of treatment cycles (Devroey *et al.*, 1994; Tournaye *et al.*, 1994).

We have performed the ICSI procedure with surgically or percutaneously retrieved spermatozoa from the epididymis (MESA/PESA) or the testis (TESE) in 39 treatment cycles. PESA was introduced as a minimal invasive technique of sperm retrieval in an outpatient clinic setting under local anaesthesia, and was successful in 62% of cases (Figure 1). Several reports have shown PESA to be successful in the majority of cases of obstructive azoospermia (Craft *et al.*, 1995; Tsirigotis *et al.*, 1995; Collins *et al.*, 1996). The advantages of PESA are: minimal discomfort for the patient, low complication rate compared to open surgery, repeatability and the production of clear aspirated fluid with usually minimal blood contamination and less debris. PESA does not require microsurgical skills, is easy to learn and can be performed as an outpatient clinic procedure. In a recent report, PESA was

shown to be as effective as MESA with comparable pregnancy rates (Meniru *et al.*, 1997).

In a series of 47 men, PESA was unsuccessful in 11 cases and a subsequent MESA procedure was performed, which gave viable spermatozoa in only two cases (Tsirigotis *et al.*, 1995). In cases of non-obstructive azoospermia, MESA and PESA were usually unsuccessful and a testicular biopsy was performed.

Testicular biopsy may be an alternative to PESA as a source of spermatozoa. Successful harvesting of spermatozoa has been reported with TESE using both the open biopsy technique and more recently the testicular fine needle aspiration (TEFNA), although needle biopsies were less effective as compared to open biopsies (Friedler *et al.*, 1997). Late complications have been described after testicular sperm retrieval techniques, including inflammation, haematoma and even devascularization of the testis (Schlegel and Su, 1997). Therefore we believe that PESA should be the first technique to be performed in cases of obstructive azoospermia.

We have chosen to treat only cases of obstructive azoospermia because of the unknown medical and genetic risks of treating azoospermia due to testicular insufficiency. It has been shown that non-obstructive azoospermia may be associated with cytogenetic abnormalities, including a high degree of sex chromosome aneuploidy (Yoshida *et al.*, 1995; Persson *et al.*, 1996) and (micro)deletions of the Y chromosome (Reijo *et al.*, 1995).

In this study, nine cases of CBAVD were selected for this treatment. CBAVD is found in 2% of infertile men and has recently been described as a primary genital form of cystic fibrosis. CBAVD is highly associated with mutations of the cystic fibrosis transmembrane conductance regulator gene (CFTR; Oates and Amos, 1994). Since CBAVD is accompanied by the absence of the seminal vesicles, these men consistently produce a low semen volume with a low pH. Spermatogenesis in CBAVD appears normal, but spermatozoa derived from the caput epididymis appear to have a low fertilizing capacity, because of their short passage through the epididymis (Silber *et al.*, 1988). In couples with CBAVD-related infertility, genetic screening of the patient and his partner is mandatory before MESA/ICSI procedures are performed. The risk of inheritance of cystic fibrosis (CF) or CBAVD is mainly determined by the presence of a CFTR-gene mutation in the partner (1:25): if she also carries a mutation, there is a 25–50% chance of a form of CF (severe, mild, CBAVD) in the offspring (Anguiano and Oates, 1992). In all cases treated in the present report, no common mutations of the CFTR gene were found in the female partners.

In conclusion, ICSI has been shown to give a high fertilization rate with ejaculated, epididymal and testicular spermatozoa. We have combined ICSI with surgical and percutaneous sperm retrieval in men with bilateral obstruction of the genital tract, which resulted in an ongoing pregnancy rate of 25.6% per treatment cycle. Non-obstructed cases of azoospermia were excluded from this treatment due to their potential genetic risks.

References

Anguiano, A. and Oates, R. (1992) Congenital bilateral absence of the vas deferens, a primarily genital form of cystic fibrosis. *JAMA*, **267**, 1794–1797.

- Belker, A.M., Thomas, A.J., Fuchs, E.F. *et al.* (1991) Results of 1469 microsurgical vasectomy reversals by the vasovasotomy study group. *J. Urol.*, **145**, 505–511.
- Collins, G.N., Critchlow, J.D., Lau, M.M.W. *et al.* (1996) Open versus closed epididymal sperm retrieval in men with secondarily obstructed vasal systems. *Br. J. Urol.*, **78**, 437–439.
- Craft, I.L., Khalifa, Y., Boulos, A. *et al.* (1995) Factors influencing the outcome of in-vitro fertilisation with percutaneous aspirated epididymal spermatozoa and intracytoplasmic sperm injection in azoospermic men. *Hum. Reprod.*, **10**, 1791–1794.
- Devroey, P., Liu, J., Nagy, Z. *et al.* (1994) Normal fertilisation of human oocytes after testicular sperm extraction and intracytoplasmic sperm injection (TESE and ICSI). *Fertil. Steril.*, **62**, 639–641.
- Friedler, S., Raziell, A., Strassburger, D. *et al.* (1997) Testicular sperm retrieval by percutaneous fine needle aspiration compared with testicular sperm extraction by open biopsy in men with non-obstructive azoospermia. *Hum. Reprod.*, **12**, 1488–1493.
- Hendry, W.F. (1994) Azoospermia and surgery for testicular obstruction. In Hargreave, T.B. (ed.), *Male Infertility*, Springer Verlag, London, pp. 337–363.
- Jarow, J.P., Oates R.D., Buch J.P. *et al.* (1997) Effect of level of anastomosis and quality of intraepididymal sperm on the outcome of end-to-side epididymovasostomy. *Urology*, **49**, 590–595.
- Johnson, S.G. (1970) Testicular biopsy score count. A method for registration of spermatogenesis in human testis: normal values and results in 335 hypogonadal males. *Hormones*, **1**, 1–24.
- Kloosterman, G.J. (1970) On intrauterine growth. *Int. J. Gynaecol. Obstet.*, **8**, 895–912.
- Meniru, G.I., Forman, R.G. and Craft, I.L. (1997) Utility of percutaneous epididymal sperm aspiration in situations of unexpected obstructive azoospermia. *Hum. Reprod.*, **12**, 1013–1014.
- Oates, R.D. and Amos, J.A. (1994) The genetic basis of congenital absence of the vas deferens and cystic fibrosis. *J. Androl.*, **15**, 1–8.
- Palermo, G., Joris, H., Devroey, P. *et al.* (1992) Pregnancy after intracytoplasmic injection of a single spermatozoon into an oocyte. *Lancet*, **340**, 17–18.
- Persson, J., Peters, G. and Saunders D. (1996) Is ICSI associated with risks of genetic disease? Implications for counselling, practice and research. *Hum. Reprod.*, **11**, 921–924.
- Pryor, J., Parson, J. and Goswamy, R. (1984) In vitro fertilisation for men with obstructive azoospermia. *Lancet*, **2**, 762.
- Reijo, R., Lee, T., Salo, P. *et al.* (1995) Diverse spermatogenic defects in humans caused by Y chromosome deletions encompassing a novel RNA-binding protein gene. *Nat. Genet.*, **10**, 383–393.
- Schlegel, P.N. and Su, L.I. (1997) Physiological consequences of testicular sperm extraction. *Hum. Reprod.*, **12**, 1688–1692.
- Silber, S.J., Balmaceda, J., Asch R. *et al.* (1988) Pregnancy with sperm aspiration from the proximal head of the epididymis: a new treatment for congenital absence of the vas deferens. *Fertil. Steril.*, **50**, 525–528.
- Silber, S.J., Nagy, Z., Liu, J. *et al.* (1994) Conventional in-vitro fertilization versus intracytoplasmic sperm injection for patients requiring microsurgical sperm aspiration. *Hum. Reprod.*, **9**, 1705–1709.
- Silber, S.J., Van Steirteghem, A.C., Liu, J. *et al.* (1995) High fertilization and pregnancy rate after intracytoplasmic sperm injection with spermatozoa obtained from testicle biopsy. *Hum. Reprod.*, **10**, 148–152.
- Temple-Smith, P.D., Southwick, G.J., Yates, C.A. *et al.* (1985) Human pregnancy by *in vitro* fertilisation (IVF) using sperm aspirated from the epididymis. *J. In Vitro Fertil. Embryo Transfer*, **2**, 119.
- The Sperm Microaspiration Retrieval Techniques Study Group (1994) Results in the United States with sperm microaspiration retrieval techniques and assisted reproductive technologies. *J. Urol.*, **151**, 1255–1259.
- Tournay, H., Devroey, P., Liu, J. *et al.* (1994) Microsurgical epididymal sperm aspiration and ICSI: a new effective approach to infertility as a result of congenital bilateral absence of the vas deferens. *Fertil. Steril.*, **62**, 644–647.
- Tsirigotis, M., Pelakanos, M., Yazdani, N. *et al.* (1995) Simplified sperm retrieval and intracytoplasmic sperm injection in patients with azoospermia. *Br. J. Urol.*, **76**, 765–768.
- Yoshida, A., Tamayama, T., Nagao, K. *et al.* (1995) A cytogenetic survey of 1,007 infertile males. *Contracept. Fertil. Sex.*, **23**, S23.

Received on August 18, 1997; accepted on December 2, 1997