

Efficacy of intracytoplasmic sperm injection using intentionally cryopreserved epididymal spermatozoa

Robert D.Oates^{1,4}, Susan M.Lobel², Doria H.Harris², Samuel Pang³, Colleen M.Burgess³ and Ronald S.Carson³

¹Department of Urology, Boston University School of Medicine, Boston, MA 02118, ²Department of Obstetrics and Gynecology, Beth Israel Hospital and Boston IVF, Brookline, MA 02146 and ³IVF America Boston, Deaconess-Waltham Hospital, Waltham, MA 02254, USA

⁴To whom correspondence should be addressed at: DOB Suite 606, 720 Harrison Avenue, Boston, MA 02118, USA

Microsurgical epididymal sperm aspiration was a great advance in the therapy of patients with non-reconstructable, obstructive azoospermia, most notably congenital bilateral absence of the vas deferens. Using conventional in-vitro fertilization, pregnancies were rarely achieved because the rate of oocyte fertilization was extremely poor. However, the use of retrieved spermatozoa in conjunction with intracytoplasmic sperm injection (ICSI) has dramatically increased the likelihood of embryo formation. Typically, sperm and oocyte harvesting are performed simultaneously. We have investigated whether frozen-thawed spermatozoa work as well as fresh spermatozoa. When we had concluded from our own population of patients (groups I and II) that they did, we adopted a policy of aspirating spermatozoa, primarily cryopreserving them and using them for ICSI at a later date. We found the fertilization rates of this latter cohort of patients (group III) to be excellent (37% per oocyte), and the ongoing pregnancy rate is quite satisfactory (40% per couple, 29% per cycle). We offer this approach as an alternative to the traditional scheme because it markedly eases the burden of partner scheduling on both the couple and the clinicians involved. In addition, assurance of the availability of male partner spermatozoa can be attained prior to beginning ovulation induction.

Key words: cryopreservation/epididymal spermatozoa/ICSI/MESA/pregnancy

Introduction

As first described by Temple-Smith *et al.* (1985) and Silber *et al.* (1988), microsurgical epididymal sperm aspiration (MESA) allows for the retrieval of spermatozoa from the proximal epididymis and/or efferent ducts in those men with obstructive azoospermia which is not surgically correctable (Jequier *et al.*, 1990; Oates *et al.*, 1992; Hovatta and von Smitten, 1993). Testicular tissue may also be harvested (by testicular sperm aspiration) and individual spermatozoa may be extracted from

the parenchyma (Schoysman *et al.*, 1993; Devroey *et al.*, 1994). Before the development of intracytoplasmic sperm injection (ICSI), the fertilization and pregnancy rates combining MESA with in-vitro fertilization (IVF) and embryo transfer were poor, at ~7% per oocyte and ~5% per cycle respectively (Silber *et al.*, 1994; Sperm Micro-aspiration Retrieval Techniques Study Group, 1994). It was logical to explore the use of MESA in conjunction with ICSI to evaluate whether this technique could enhance fertilization and increase the ultimate chance of pregnancy. Indeed, initial results were extremely encouraging, and most clinicians performing MESA have adopted this combined approach (Silber *et al.*, 1994; Tournaye *et al.*, 1994).

The aim of the MESA/IVF and embryo transfer scheme is to collect both oocytes and spermatozoa on the same day to maximize the inherent potential that these spermatozoa have vis-a-vis motility, forward progression and fertilizing ability (Oates, 1992). Even though consideration was given to the cryopreservation of excess spermatozoa, the prediction for success in a subsequent IVF cycle using the thawed spermatozoa was poor. However, ICSI has radically altered our thinking about the quality and quantity of spermatozoa that may be used successfully (Payne *et al.*, 1994). Theoretically, cryopreserved spermatozoa should be quite capable of embryo generation if ICSI is employed. We sought to test this hypothesis by comparing the fertilization rate of freshly aspirated spermatozoa with that of cryopreserved 'excess' spermatozoa obtained during a prior simultaneous MESA/ICSI attempt. The fertilization rates were equivalent for the two groups, a finding supported by the recent data of Devroey *et al.* (1995). Using this information, we subsequently altered and formalized a novel approach of intentionally harvesting spermatozoa on a day far removed from oocyte retrieval. The collected sperm aliquot was subdivided into several individual vials and cryopreserved. At a later date, a single vial was thawed and used for ICSI. This allowed us to temporally dissociate the male sperm and female oocyte retrievals. During one procedure, enough spermatozoa were obtained for multiple attempts at ICSI. This approach affords the couple much greater flexibility in their own scheduling and offers them the opportunity to have spermatozoa aspirated in one facility and ICSI performed in another.

Materials and methods

Patients

Group I

A total of 14 men with a mean age of 38 years (range 27–64) underwent 18 cycles of MESA on the day of oocyte retrieval, followed

by ICSI. Diagnoses included congenital bilateral absence of the vas deferens (CBAVD; $n = 9$), clinical cystic fibrosis ($n = 1$), distal ductal dysfunction or removal ($n = 2$) and failed vasoepididymostomy ($n = 2$). The mean age of the spouses was 32 years (range 22–38).

Group II

In all, 10 men (a subgroup of group I) with a mean age of 37 years (range 27–48), all of whom had undergone a previous simultaneous MESA/ICSI cycle, used their excess spermatozoa that were cryopreserved at the time of the original aspiration for a subsequent ICSI attempt. A total of 11 cycles were carried out. Diagnoses of this subgroup included CBAVD ($n = 7$), distal ductal dysfunction or removal ($n = 1$) and failed vasoepididymostomy ($n = 2$). The mean age of the spouses was 32 years (range 22–38).

Group III

Five men with a mean age of 31 years (range 26–37) underwent MESA with intentional and immediate cryopreservation of all harvested spermatozoa into several different cryostraws. Seven cycles of subsequent ICSI using thawed spermatozoa were accomplished (four patients, one cycle each; one patient, three cycles). Diagnoses included CBAVD ($n = 4$) and distal ductal dysfunction or removal ($n = 1$). Only one patient had undergone a previous simultaneous MESA/ICSI attempt. The mean age of the spouses was 30 years (range 22–38). Group III comprised the first five patients who required MESA/ICSI after the decision had been made to apply intentional cryopreservation. This decision was reached on the basis of the results accumulated by looking at those patients who had 'excess' frozen epididymal spermatozoa used (group II) and noting the reasonable fertilization rates.

MESA

Bupivacaine was injected into the spermatic cord at the level of the pubic tubercle and into the scrotal skin to serve as the anaesthetic block. After opening the scrotum, the testis and epididymis were delivered. Beginning distally, a 1 cm incision was made in the epididymal tunic to expose the underlying epididymal tubule. A small nick was then made in the anterior surface and the exuding fluid was aspirated into 3 ml syringes with attached angiocath tips. A microscopical survey revealed the presence/absence of motile spermatozoa. If no motile spermatozoa were visualized, an incision was carried out more proximally in 1 cm increments. In many patients, motile spermatozoa were encountered only from the efferent ducts, which were accessed by incising the bridge of tunical tissue spanning the caput epididymis and the testis. Released fluid was aspirated and checked microscopically. External testicular compression augmented the flow of entrapped sperm-rich fluid from the rete testis. Continually rotating only three syringes, all of the aspirated fluid that contained motile spermatozoa was discharged into a 12 ml conical tube containing 0.5 ml of either Test yolk buffer (TYB; Irvine Scientific, Santa Ana, CA, USA) or human tubal fluid (HTF; Irvine Scientific). All opened tubules were microsurgically reapproximated with a 10-0 nylon suture.

Cryopreservation of epididymal aspirates

Epididymal aspirates diluted with TYB, as described above, were transported to the laboratory at room temperature and adjusted to a total volume of 2.0 ml and a final concentration of 10% v/v glycerol (Sigma Chemical Co., St Louis, MO, USA). Aliquots of 500 μ l were transferred aseptically to sterile, labelled polypropylene tubes (Intermed Nunc, Denmark). The temperature of each tube was lowered to -7°C at $-2^{\circ}\text{C}/\text{min}$ before ice crystal formation was induced, and then lowered further to -30°C at $-1^{\circ}\text{C}/\text{min}$, at which point the tubes were transferred directly to liquid nitrogen. Freezing was performed

using a computer-controlled unit (model Kryo 10; Planar Pty Ltd, Sunbury-on-Thames, UK). Those collected in HTF supplemented with human plasma proteins (HTF:PL; Plasmanate[®]; 10% v/v; Miles Laboratories, Elkhart, Indiana, USA) were frozen in an alternative fashion. A 1:1 ratio of TYB was added to the samples after collection. The samples were divided into 1 ml aliquots and frozen by suspension in liquid nitrogen vapours for 3 h. Then they were plunged directly into liquid nitrogen and stored, pending the isolation of oocytes from the female partner.

Ovarian hyperstimulation and oocyte retrieval

Pituitary suppression was achieved using daily s.c. injections of leuprolide acetate (Lupron[®]; TAP Pharmaceuticals Inc., Deerfield, IL, USA), starting either in the luteal phase of the antecedent cycle (1.0 mg) or on day 1 of menstruation (0.5 mg). To obtain ovarian hyperstimulation, human menopausal gonadotrophin (HMG; Pergonal[®]; Serono Laboratories Inc., Norwell, MA, USA) and/or pure follicle stimulating hormone (FSH; Metrodin[®]; Serono) was used. Human chorionic gonadotrophin (HCG; Profasi[®]; Serono), 10 000 IU, was administered when a cohort of follicles between 15 and 20 mm in diameter was obtained. Transvaginal ultrasound-guided aspiration of the ovarian follicles was performed 36 h after the administration of HCG.

ICSI

Oocytes were isolated immediately from the follicular aspirates. Adherent cumulus cells were removed by exposure to bovine testis hyaluronidase (Sigma; 80 IU/ml). Oocytes were judged as mature (MII) if a second polar body had been extruded. All oocytes were cultured in 30 μ l drops of HTF containing 15% (v/v) protein covered by mineral oil in an incubator with 5% O_2 , 5% CO_2 and 90% N_2 at 37°C .

A vial of epididymal aspirate cryopreserved previously was thawed at 20°C for 30 min, and 250 μ l of the original aliquot were layered on a discontinuous density gradient (Percoll[®]; Sigma) and centrifuged at 1800 g for 20 min. The most dense fraction of the discontinuous gradient was removed, diluted with bovine serum albumin (HTF:BSA; Sigma; 3% w/v) or Plasmanate[®] (15% v/v) in synthetic HTF, and centrifuged at 1800 g for 20 min. The supernatant was removed and the precipitate resuspended in 200 μ l HTF:BSA or HTF:PL. Spermatozoa were transferred to a 5.0 μ l drop of BSA (0.5% w/v) in Dulbecco's phosphate-buffered saline (PBS:BSA), or to a 5.0 μ l drop of HTF:PL under mineral oil, and immobilized before aspiration into a glass injection pipette (5–6 μ m internal diameter). The spermatozoa were immobilized by the addition of polyvinylpyrrolidone (Medicult, Copenhagen, Denmark). Mature (MII) oocytes, as described above, were transferred to separate drops of PBS:BSA or HTF:PL under mineral oil and a single spermatozoon was injected into each oocyte. The entire procedure was carried out under direct visualization with phase-contrast optics and the assistance of electro-mechanical micromanipulators (Narishige USA, Sea Cliff, NY, USA).

Assessment of fertilization and embryo development in vitro

Injected oocytes were maintained in drops of HTF supplemented with human plasma proteins (Plasmanate; 6% v/v; Miles Laboratories) under mineral oil and examined for the formation of a second pronucleus at 16, 18 and 20 h after ICSI. Oocytes forming a second pronucleus were regarded as fertilized and were allowed to undergo further development over the ensuing 48 h. Embryos were examined using phase-contrast optics and ranked within each cohort on the basis of the number of blastomeres, the degree of fragmentation, the uniformity of blastomere size and the distribution of cytoplasmic organelles. The zonae of the three or four uppermost embryos

Table I. Sperm parameters and fertilization results using freshly aspirated spermatozoa

Patient	Fate of retrieved spermatozoa	Aetiology	Site	Unilateral (U)/ Bilateral (B) ^a	Volume (ml)	Count ($\times 10^6$ /ml)	Motility (%)	Forward progression	No. of vials cryopreserved	No. of oocytes	No. of embryos	Fertilization rate (%)	Pregnancy
1	Used fresh	CBAVD	ED	B	1.3	20	0.25	1		8	3	38	No
	Cryopreserved				0.9	10	0.0	–	2				
1	Used fresh	CBAVD	ED	U	1.0	10	10	2		7	2	29	No
2	Used fresh	CBAVD	ED	U	1.5	8.1	1.0	1		7	3	43	No
2	Used fresh	CBAVD	ED	U	0.4	59	1.7	1		10	0	0	No
	Cryopreserved				0.2	59	1.7	1	2				
3	Used fresh	CBAVD	ED	U	0.5	68	35	2		6	1	17	No
	Cryopreserved				1.0	25	24	2	2				
			Post-thaw		–	11	2.0	1					
4	Used fresh	CBAVD	Caput	U	1.0	29	31	2		3	0	0	No
	Cryopreserved				1.0	27	19	2	3				
5	Used fresh	CBAVD	ED	U	1.5	58	14	2		5	2	40	No
	Cryopreserved				1.0	13	1.0	1	1				
6	Used fresh	CBAVD	ED	U	2.5	79	13	2		3	1	33	No
	Cryopreserved				0.8	106	11	2	2				
			Post-thaw			15	1.0	1					
7	Used fresh	CBAVD	ED	B	1.4	29	17	2		5	0	0	No
	Cryopreserved				0.5	42	1.0	2	1				
8	Used fresh	Failed VE	ED	B	0.6	26	9.0	1		9	4	44	No
	Cryopreserved				2.1	16	10	1	3				
9	Used fresh	CBAVD	ED	U	1.0	16	5.0	2		11	2	18	No
	Cryopreserved				1.0	25	25	2	3				
10	Used fresh	CF	ED	U	1.5	47	29	2		10	0	0	No
	Cryopreserved				1.0	38	28	2	3				
10	Used fresh	CF	ED	U	1.5	75	5.0	2		8	0	0	No
	Cryopreserved				0.5	18.8	5.0	1	4				
			Post-thaw		0.5	20	1.0	1					
11	Used fresh	CBAVD	ED	U	2.3	87	8.0	2		10	1	10	No
	Cryopreserved				0.5	1.3	45	2	4				
12	Fresh	DD	Corpus	U	1.0	60	30	2		9	2	22	No
	Cryopreserved				0.5	29	33	2	1				
			Post-thaw			25	8.0	2					
12	Fresh	DD	Caput	U	1.8	28	3.0	2		5	0	0	No
	Cryopreserved				0.5	4.5	20	2	4				
			Post-thaw			4	1.0	1					
13	Fresh	DD	Vas	B	1.1	30	5.0	1		17	8	47	No
14	Fresh	Failed VE	Caput	U	2.5	30	20	2		11	2	18	No
	Cryopreserved				0.5	30	20	2	4				

CBAVD = congenital bilateral absence of the vas deferens; CF = cystic fibrosis; DD = ductal dysfunction; ED = efferent ducts; VE = vasoepididymostomy.

^aUnilateral or bilateral surgery.

within the cohort were perforated immediately prior to transcervical placement in the uterine lumen.

Embryo transfer and pregnancy assessment

Embryos were transferred at ~72 h following oocyte retrieval. The first pregnancy test was performed 14–16 days after oocyte recovery using a quantitative assay for the β -HCG subunit. Transvaginal ultrasound was used to confirm the intrauterine location of the pregnancy and the number of viable gestational sacs present at ~5 weeks after oocyte collection.

Results

Group I

A total of 14 couples underwent 18 cycles of simultaneous MESA/ICSI in which the spermatozoa used for ICSI were freshly harvested on the same morning as oocyte retrieval. The sperm parameters of both the samples used immediately and those that were 'excess' and cryopreserved are listed in Table I. Certain cryopreserved specimens were able to be divided into several separate cryovials and a test thaw was

performed ('post-thaw'). Sperm motility of the fresh samples averaged 13.2% (range 0.25–45.00%). Whether the procedure was unilateral or bilateral and the level of aspiration are also detailed in Table I. In all, 13 procedures were at the level of the efferent ducts, while three were from the caput, one from the corpus and one from the vas. A total of 14 procedures involved unilateral surgery only, while four were bilateral. Volume and density parameters reflect not only sperm quantity but also how much each sample was diluted with medium. If sufficient spermatozoa were obtained for ICSI, any 'excess' that may have been collected in a separate conical tube was cryopreserved. Table I also lists the number of cryovials stored and whether any were subsequently used (group II cycles). Table I also displays the number of oocytes employed in ICSI, the number of resultant embryos and the fertilization rate. No pregnancies occurred in group I. The mean number of eggs per patient was 8.1, while the mean number of embryos was 1.7 (2.6 in those couples with any level of fertilization). The mean rate of fertilization was 20%. Of the 14 couples, 67% achieved embryos for transfer.

Table II. Results of intracytoplasmic sperm injection (ICSI) using cryopreserved spermatozoa obtained in a prior micro-epididymal sperm aspiration/ICSI cycle (see Table I)

Patient	Aetiology	No. of oocytes	No. of embryos	Fertilization rate (%)	No. of cryopreserved vials remaining	ICSI cycle no.	Pregnancy
2	CBAVD	11	0	0	1	3	No
3	CBAVD	8	2	25	0	2	No
4	CBAVD	4	1	25	1	2	No
5	CBAVD	10	5	50	0	2	No
6	CBAVD	5	2	40	1	2	No
8*	Failed VE	13	7	54	2	2	Yes
9	CBAVD	20	1	5	2	2	No
11	CBAVD	11	2	18	3	2	No
12	DD	7	2	29	0	3	No
13	DD	7	5	71	3	4	No
14	Failed VE	7	1	14	2	2	No

CBAVD = congenital bilateral absence of the vas deferens; DD = ductal dysfunction; VE = vasocpididymostomy.

*Two embryos cryopreserved; tubal embryo transfer.

Table III. Parameters of primarily cryopreserved spermatozoa used for intracytoplasmic sperm injection at a later date

Patient	Aetiology	Site	Unilateral (U)/ Bilateral (B)*	Volume (ml)	Count ($\times 10^6$ /ml)	Motility (%)	Forward progression	Sperm morphology	No. of vials cryopreserved
A	CBAVD	ED	U	5.0	33	15	2	72 WHO	10
B	CBAVD	ED	B	5.0	31	10	2	—	10
		Post-thaw		0.5	28	1	1		
C	CBAVD	ED	B	2.0	15	7	2	3	3
D	CBAVD	ED	B	1.0	132	3	2	4	6
E	DD	ED	B	1.7	0.9	5	2	28 WHO	3

CBAVD = congenital bilateral absence of the vas deferens; DD = ductal dysfunction; ED = efferent ducts; WHO = World Health Organization criteria.

*Unilateral or bilateral surgery.

Table IV. Results of intracytoplasmic sperm injection (ICSI) using spermatozoa primarily cryopreserved

Patient	Aetiology	No. of oocytes for ICSI	No. of embryos for ICSI	ICSI fertilization rate (%)	ICSI cycle no.	Pregnancy
A	CBAVD	5	3	60	1	Yes
B	CBAVD	8	3	38	1	No
B	CBAVD	16	4	25	2	No
B	CBAVD	17	8	47	3	No
C	CBAVD	12	3	25	1	No
D	CBAVD	14	4	29	1	Yes
E	DD	19	8	42	1	Yes

CBAVD = congenital bilateral absence of the vas deferens; DD = ductal dysfunction.

Group II

A total of 10 patients underwent 11 cycles of ICSI using cryopreserved spermatozoa obtained during a prior simultaneous MESA/ICSI attempt. Data in Table I correlate with the sperm samples used in each of the cycles listed in Table II. Table II details the number of oocytes subjected to ICSI (mean 9.4 per cycle), the number of resultant embryos (mean 2.6 per cycle) and the fertilization rate (mean 27%). Embryo transfer occurred in 91% of cycles and there was one singleton pregnancy (10% per couple, 9% per cycle).

Group III

Five patients underwent seven cycles of ICSI using spermatozoa that were intentionally cryopreserved at an earlier date completely separate from any oocyte harvesting and ICSI.

This MESA was purposefully not timed to be coincident with an ICSI cycle. Table III lists the aetiology, site of aspiration (efferent ducts in all patients), whether the procedure was unilateral or bilateral (one and four patients respectively) and the various sperm parameters of the aspirated specimen, as well as those of the subsequently thawed specimen actually used for ICSI. The number of individual vials ranged from a minimum of three to an arbitrarily set upper limit of 10.

Table IV details the number of oocytes subjected to ICSI (mean 12.9 per cycle), the number of resultant embryos (mean 4.7 per cycle) and the fertilization rate (mean 37% per cycle). All of the couples achieved embryo transfer. Three pregnancies occurred, resulting in five fetuses (one triplet and two singleton gestations). Pregnancies were confirmed with ultrasound at 5–6 weeks following embryo transfer. The initial pregnancy rate

was 60% per couple, 43% per cycle and 43% per embryo transfer. One couple experienced a late first trimester miscarriage. Therefore, the present ongoing pregnancy rate is 40% per couple, 29% per cycle and 29% per embryo transfer. The embryo implantation rate was 15% (5/33).

Discussion

ICSI has proved itself to be an advance in the therapy of severe male factor infertility (Van Steirteghem *et al.*, 1993). Some spermatozoa that are incapable of normal oocyte fertilization are nevertheless capable of undergoing post-fertilization events quite normally once they are mechanically placed within the confines of the oocyte cytoplasm (Nagy *et al.*, 1994). In classic terms, 'maturation' of the sperm population takes place as the spermatozoa wind their way along the length of the epididymal tubule, with an increase in sperm motility and forward progression and an augmentation in the fertilizing capacity of each spermatozoon. It is unclear whether this is a time-dependent phenomenon, an effect of the epididymal epithelium or a combination of the two (Bedford, 1994). However, testicular and proximal epididymal spermatozoa, with little natural competence for either of these functions (Silber, 1988; Jow *et al.*, 1993), are capable of contributing to embryo formation if placed within the cytoplasm of the egg during ICSI (Devroey *et al.*, 1994; Tournaye *et al.*, 1994). Therefore, nuclear maturation appears to temporally precede membrane/acrosomal (fertilization) and axonemal (movement) development.

Cryopreservation is known to impair sperm motility and decrease the fertilization rate by detrimental effects on the acrosomal structure and acrosin activity (Critser *et al.*, 1987; Cross and Hanks, 1991). The consequences of cryopreservation on the integrity of the sperm nucleus and centrosome (critical for 'pronuclear cleavage') are less clear but probably not as significant as those on the acrosome and axoneme. It appears that ICSI obviates the requirement for sperm motility and acrosome function, demanding only a normal nucleus and centrosome. Spermatozoa obtained during MESA have little capacity for motility and fertilization, as shown by the poor movement characteristics (percentage motility and forward progression) and extremely low of rate fertilization during MESA/IVF and embryo transfer (Silber *et al.*, 1994; Sperm Micro-aspiration Retrieval Techniques Study Group, 1994). Because cryopreservation reduces the values of these parameters even further, it would be fruitless to attempt IVF and embryo transfer using cryopreserved epididymal spermatozoa. However, ICSI makes this concept appealing. Indeed, the fertilization and cleavage rates using frozen-thawed spermatozoa retrieved during MESA, when combined with ICSI, are as efficacious as those from freshly harvested spermatozoa. This can be seen clearly by comparing groups I and II overall, as well as when certain patients serve as their own controls. This finding has been confirmed recently by Devroey *et al.* (1995), who have also shown that excess epididymal spermatozoa can be cryopreserved and used with success at a later date. It was logical to adopt our present policy of intentional and immediate cryopreservation of all harvested spermatozoa

at a time distinct from oocyte retrieval and ICSI with the planned use of those spermatozoa at a subsequent time convenient for the couple.

Using this scheme (group III) fertilization rates remained good at a value of 37%, and compared well with those of fresh (group I; 20%) and cryopreserved 'excess' (group II; 27%) spermatozoa. The ongoing pregnancy rate in the five couples is 40% (2/5) and in the seven cycles is 29% (2/7). This is actually an improvement on groups I and II, and more than likely reflects the continued refinement of the ICSI procedure at both institutions where the pregnancy rate for all couples needing ICSI, regardless of the reason, has risen steadily since the initiation of ICSI as a clinical service. It is less likely, although possible, that the cryopreservation process enhances the ability of the spermatozoa to undergo the events required for fertilization or embryo and fetal development.

We feel that spermatozoa retrieved during MESA can be intentionally cryopreserved and used at a later date for ICSI with great efficacy. This approach dissociates MESA and ICSI, allowing the spermatozoa to be collected at a time completely separate from oocyte harvesting and ICSI. If the aspirated sperm sample is collected in one conical tube during surgery, the embryology laboratory can subdivide it into multiple vials for cryopreservation. If each vial is normalized to a standardized number of motile spermatozoa, e.g. $1 \times 10^6/\text{ml}$, motile spermatozoa will most likely be found upon thawing and can be chosen for ICSI.

This 'one-time procedure' approach, especially when performed unilaterally and with minimal anaesthesia, provides enough spermatozoa for numerous ICSI attempts with little morbidity. The frozen spermatozoa can be used at any time or anywhere by the couple. We prefer to have the specimen manipulated and frozen by the embryology staff at the facility where ICSI will eventually be carried out. Thus the couple no longer needs to wait for the urological surgeon and the IVF programme to coordinate events and orchestrate a combined 'cycle' when both partners will undergo simultaneous gamete harvesting. This makes it much simpler from the couple's viewpoint because the time constraints and limitations imposed upon them are eliminated. The male partner can arrange his surgery at a convenient time, while the female partner may choose the cycle she feels is most appropriate given her own circumstances. An obvious peripheral benefit is that all couples who begin the arduous process of ovarian hyperstimulation are already assured of sperm availability.

In conclusion, we feel that this approach greatly simplifies the overall MESA/ICSI scheme for both patients and clinicians, without compromising the overall chances of pregnancy.

Note added in proof

After 27 cycles of ICSI employing internationally cryopreserved epididymal spermatozoa, the pregnancy rate per cycle is 33% with an ongoing/delivered rate of 22% per cycle.

References

- Bedford, J.M. (1994) The status and state of the human epididymis. *Hum. Reprod. Update*, 9, 2187-2199.

- Critser, J.K., Arneson, B.W., Aaker, D.V., Huse-Benda, A.R. and Ball, G.D. (1987) Cryopreservation of human spermatozoa II: post-thaw chronology of motility and of zona-free hamster ova penetration. *Fertil. Steril.*, **47**, 980–984.
- Cross, N.L. and Hanks, S.E. (1991) Effects of cryopreservation on human sperm acrosomes. *Hum. Reprod.*, **6**, 1279–1283.
- Devroey, P., Liu, J., Nagy, Z., Tournaye, H., Silber, S.J. and Van Steirteghem, A.C. (1994) Normal fertilization of human oocytes after testicular sperm extraction and intracytoplasmic sperm injection. *Fertil. Steril.*, **62**, 639–641.
- Devroey, P., Silber, S., Nagy, Z., Liu, J., Tournaye, H. *et al.* (1995) Ongoing pregnancies and birth after intracytoplasmic sperm injection with frozen-thawed epididymal spermatozoa. *Hum. Reprod.*, **10**, 903–906.
- Hovatta, O. and von Smitten, K. (1993) Sperm aspiration from vas deferens and in-vitro fertilization in cases of non-treatable anejaculation. *Hum. Reprod.*, **8**, 1689–1691.
- Jequier, A.M., Cummins, J.M. and Gearon, C. (1990) A pregnancy achieved using sperm from the epididymal caput in idiopathic obstructive azoospermia. *Fertil. Steril.*, **53**, 1104–1107.
- Jow, W.W., Steckel, J., Schlegel, P.N., Magid, M.S. and Goldstein, M. (1993) Motile sperm in human testis biopsy specimens. *J. Androl.*, **14**, 194–198.
- Nagy, Z.P., Liu, J., Joris, H., Devroey, P. and Van Steirteghem, A. (1994) Time-course of oocyte activation, pronucleus formation and cleavage in human oocyte fertilized by intracytoplasmic sperm injection. *Hum. Reprod.*, **9**, 1743–1748.
- Oates, R.D. (1992) Microscopic epididymal sperm aspiration (MESA) in conjunction with advanced reproductive techniques. *Rep. Urol. Techn.*, **2**, 88–90.
- Oates, R.D., Honig, S., Berger, M.J. and Harris, D. (1992) Microscopic epididymal sperm aspiration (MESA): a new option for the treatment of the obstructive azoospermia associated with cystic fibrosis. *J. Assist. Reprod. Genet.*, **9**, 36–40.
- Payne, D., Flaherty, S.P., Jeffrey, R., Warnes, G.M. and Matthews, C.D. (1994) Successful treatment of severe male factor infertility in 100 consecutive cycles using intracytoplasmic sperm injection. *Hum. Reprod.*, **9**, 2051–2057.
- Schoysman, R., Vanderzwalmen, P., Nijs, M., Segal-Bertin, G. and van de Casseye, M. (1993) Successful fertilization by testicular spermatozoa in an in-vitro fertilization programme. *Hum. Reprod.*, **8**, 1339–1340.
- Silber, S.J. (1988) Pregnancy caused by sperm from vasa efferentia. *Fertil. Steril.*, **49**, 373–375.
- Silber, S.J., Balmaceda, J., Borrero, C., Ord, T. and Asch, R. (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.P., Liu, J., Godoy, H., Devroey, P. and Van Steirteghem, A.C. (1994) Conventional IVF versus ICSI (intracytoplasmic sperm injection) for patients requiring microsurgical sperm aspiration (MESA). *Hum. Reprod.*, **9**, 1705–1709.
- Sperm Micro-aspiration Retrieval Techniques Study Group (1994) Results in the United States with sperm micro-aspiration retrieval techniques and assisted reproductive technologies. *J. Urol.*, **151**, 1255–1259.
- Temple-Smith, P.D., Southwick, G.J. and Yates, C.A. (1985) Human pregnancy by in vitro fertilization (IVF) using sperm aspirated from the epididymis. *J. In Vitro Fertil. Embryo Transf.*, **4**, 298–303.
- Tournaye, H., Devroey, P., Liu, J., Nagy, Z.P., Lissens, W. and Van Steirteghem, A.C. (1994) Microsurgical epididymal sperm aspiration and intracytoplasmic sperm injection: a new effective approach to infertility as a result of congenital bilateral absence of the vas deferens. *Fertil. Steril.*, **61**, 1045–1051.
- Van Steirteghem, A.C., Nagy, Z.P., Joris, H., Liu, J., Staessen, C. *et al.* (1993) High fertilization and implantation rates after intracytoplasmic sperm injection. *Hum. Reprod.*, **8**, 1061–1066.

Received on July 24, 1995; accepted on September 28, 1995