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No differences in outcome after intracytoplasmic sperm injection with fresh or with frozen–thawed epididymal spermatozoa

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This retrospective consecutive case series aimed at comparing the results of intracytoplasmic sperm injection (ICSI) with fresh and with frozen–thawed epididymal spermatozoa obtained after microsurgical epididymal sperm aspiration (MESA) in 162 couples. These couples were suffering from infertility because of congenital bilateral absence of the vas deferens (n = 109), failed microsurgical reversal for vasectomy or postinfectious epididymal obstruction (n = 44), irreparable epididymal obstruction (n = 4), ejaculatory duct obstruction (n = 2) or anejaculation (n = 3). Overall, 176 MESA procedures were performed in the husbands, followed by 275 ICSI procedures with either fresh (n = 157) or frozen–thawed (n = 118) epididymal spermatozoa. No significant differences were observed in the parameters of spermatozoa used either freshly or frozen–thawed. In the fresh epididymal sperm group 59.4% of all the injected oocytes fertilized normally as compared to 56.2% of all injected oocytes in the frozen–thawed epididymal sperm group, and embryonic development was comparable between the two groups. A total of 245 transfers were performed: 145 after the use of fresh epididymal spermatozoa and 100 after the use of frozen–thawed spermatozoa. The overall pregnancy rate per ICSI cycle was significantly lower when frozen–thawed epididymal spermatozoa were used (26.3 versus 39.5%). However, no significant differences were found either in clinical and ongoing pregnancy rates or in implantation rates. There were no differences in pregnancy outcome. In patients suspected of having obstructive azoospermia with no work-up or an incomplete one, MESA is the preferred method for sperm recovery because a full scrotal exploration can be performed and, whenever indicated, a vasoepididymostomy may be performed concomitantly. Recovery of epididymal spermatozoa for cryopreservation during a diagnostic procedure is certainly a valid option in these patients since ICSI may be performed later or even in another centre using the frozen–thawed epididymal spermatozoa without jeopardizing the ICSI success rate.

Key words: azoospermia/cryopreservation/epididymis/ICSI/MESA

Introduction

Since the report by Temple-Smith *et al*. (1985) on the use of microsurgically aspirated epididymal spermatozoa in in-vitro fertilization (IVF), an alternative fertility treatment for patients suffering from infertility because of irreparable obstruction has become possible. The combination of microsurgical epididymal sperm aspiration (MESA) and IVF–embryo transfer has been shown to offer men suffering from virtually untreatable disorders, e.g. congenital bilateral absence of the vas deferens (CBAVD), a chance to have their own genetic children (Silber *et al*., 1987). With the introduction of intracytoplasmic sperm injection (ICSI), high fertilization and pregnancy rates have been obtained with epididymal spermatozoa (Tournaye *et al*., 1994) and the combination of MESA and ICSI has proven more successful than MESA and IVF (Silber *et al*., 1994). In 1993, testicular sperm recovery was introduced as an alternative to MESA where recovery of epididymal spermatozoa had failed (Schoysman *et al*. 1993). Although less invasive percutaneous epididymal sperm recovery procedures have been introduced (Craft *et al*., 1995), high fertilization rates and implantation rates after testicular sperm recovery and ICSI (Tournaye *et al*., 1996, 1997) may render the use of epididymal spermatozoa obsolete in the near future for patients with normal spermatogenesis.

Although the use of frozen–thawed testicular spermatozoa for ICSI has been reported (Hovatta *et al*., 1995; Fischer *et al*., 1996; Gil-Salom *et al*., 1996; Podsiadly *et al*., 1996; Romero *et al*., 1996; Khalifeh *et al*., 1997), sperm numbers retrieved after testicular biopsies tend to be limited as compared with those recovered after MESA, which makes cryopreservation far easier in the latter case. Pregnancies have been reported after ICSI with frozen–thawed epididymal spermatozoa (Devroey *et al*., 1995; Oates *et al*., 1996; Holden *et al*., 1997). However, results after ICSI using frozen–thawed epididymal spermatozoa have never been compared to those using fresh epididymal spermatozoa and the question arises as to whether freezing epididymal spermatozoa may be considered generally useful.

In the present study we compared the results of ICSI with either fresh or frozen–thawed epididymal spermatozoa in a large retrospective case series.

Materials and methods

Patients

From January 1992 until December 1996 a total of 162 couples underwent MESA followed by ICSI. In 109 couples MESA–ICSI was performed because of CBAVD (67.3%) while 44 couples underwent MESA–ICSI because microsurgery for reversal of vasectomy or

postinfectious obstruction had failed (27.2%). Another nine couples had MESA combined with ICSI for other indications: irreparable postinfectious epididymal obstruction ($n = 4$), ejaculatory duct obstruction ($n = 2$) and anejaculation with failed electroejaculation ($n = 3$). In CBAVD couples, cystic fibrosis carrier status was evaluated in both partners (Lissens *et al*., 1996).

Microsurgical epididymal sperm aspiration (MESA)

A total of 176 MESA procedures were performed, 18 of which were carried out during a preliminary scrotal exploration with cryopreservation of the epididymal spermatozoa and 158 of which were carried out on the day of ovum retrieval for ICSI or the day before in a few patients. In the latter subgroup, 135 men had a single MESA procedure, 10 men had two procedures, five of which involved freezing of epididymal spermatozoa which turned out to be 100% immotile after thawing. In one couple the husband even underwent three MESA procedures.

The MESA procedure was performed under general anaesthesia. Using an operating microscope, the epididymis was carefully dissected and after haemostasis, using bipolar coagulation, an epididymal tubule was longitudinally opened by micro-scissors through a small opening in the serosa. In line with the findings of Silber *et al*. (1990), the proximal corporal or distal head region of the epididymis was first opened. The epididymal fluid was aspirated by means of a disposable tip from an intravenous cannula (Medicut, Sherwood Medical, Tullamore, Ireland) mounted on a 1 ml syringe (Plastipak; Becton Dickinson, Aalst, Belgium) filled with 0.1 ml HEPES-buffered Earle's medium supplemented with 0.4% human serum albumin (Belgian Red Cross, Brussels, Belgium). In a few MESA cycles, spermatozoa were aspirated by means of a mouth-controlled sterile hand-pulled glass pipette with a diameter of 200 μm. The aspirated epididymal fluid was then transferred into a Falcon test tube, filled with 0.9 ml of this Earle's medium. When motile spermatozoa, as assessed by peroperative microscopical examination of the aspirates, were recovered, no further epididymal incision was made and a maximum of fluid was aspirated in order to allow cryopreservation of as many epididymal spermatozoa as possible. The tubular incision was then closed by 9–0 nylon sutures. If microscopical assessment did not reveal any motile spermatozoa, a more proximal incision was made until motile spermatozoa were found. Sperm analysis was performed according to the World Health Organization (1992) guidelines taking dilution by Earle's medium into account, but for morphology assessment, the strict Tygerberg criteria were applied (Kruger *et al*., 1986). After analysis, the aspirated epididymal fluid was layered on a two-layer Percoll gradient (95 and 47.5%) in order not to rescue too many senescent spermatozoa. After washing, the spermatozoa were used for ICSI.

Cryopreservation of epididymal spermatozoa

The epididymal spermatozoa from 148 patients (91.3%) were cryopreserved. After Percoll preparation, one fraction of the epididymal aspirate was diluted to $\leqslant 2\times10^6$/ml spermatozoa in order to be able to freeze a maximum number of straws. The cryopreservation medium used contained glycerol at a concentration of 15%, egg yolk, citrate, glycine, glucose and antibiotics (Verheyen *et al*., 1993). After equilibration in a 37°C water bath for 10 min, the mixture was aspirated into 0.25 ml straws [Instruments de Médecine Véterinaire (IMV), l'Aigle, France]. The straws were then rapidly frozen in liquid nitrogen vapour by placing them horizontally 8–10 cm above the liquid nitrogen surface for 10 min (room temperature to –80°C; rate –10°C/min). The straws were then plunged directly into the liquid nitrogen (–196°C). For thawing, the straws were removed from the liquid nitrogen and placed at 37°C for 10 min. In order to remove the cryoprotectant, thawed specimens were put on a two-layer Percoll gradient (95–47.5%) and centrifuged for 20 min at 300 *g*. Afterwards, the 95% fraction was washed by centrifugation with Earle's medium at 1800 *g* for 5 min and the pellet was washed again and centrifuged in a 1.5 ml Eppendorf tube (1800 *g*, 5 min) in order to concentrate the spermatozoa. This step is necessary in order to collect most spermatozoa into a small microdroplet ready for injection.

Intracytoplasmic sperm injection (ICSI)

All female partners received ovarian stimulation treatment by a combination of gonadotrophin-releasing hormone agonist (GnRHa) and human menopausal gonadotrophins (HMG). When the patient had at least three follicles with a diameter of 17 mm and serum oestradiol concentrations of 1000 ng/l, administration of both GnRHa and HMG were discontinued and ovulation was induced with 10 000 IU human chorionic gonadotrophin (HCG).

All patients had a transvaginal ultrasound-guided ovum aspiration ~36 h after HCG injection under local anaesthesia. After recovery, oocytes were denuded from the surrounding granulosa cells and metaphase-II oocytes were microinjected as described previously (Van Steirteghem *et al*., 1995). Injected oocytes were cultured individually in 25 μl droplets of B2 medium (Bio-Mérieux, Brussels, Belgium) under oil. At 16–18 h after injection, oocytes were examined under an inverted microscope for the presence of two polar bodies and two distinct pronuclei, as evidence of normal fertilization. After an additional 24–30 h of in-vitro culture, embryos were examined under the microscope to assess their developmental stage and quality on the basis of their morphological aspects. Excellent embryos contained no anucleate fragments and showed equal or unequal blastomeres. Good embryos contained $<$20% anucleate fragments while fair embryos contained between 20 and 50% of anucleate fragments. A maximum of three cleaving embryos were then transferred into the uterine cavity, except in patients $>$40 years old where, on some occasions, more than three embryos were transferred. Supernumerary embryos were cryopreserved at the 4–8-cell stage using dimethylsulphoxide as a cryoprotectant (Van Steirteghem *et al*., 1994). The luteal phase was supplemented by vaginal administration of 200 mg micronized progesterone three times daily (Utrogestan; Laboratoires Piette, Brussels, Belgium).

Pregnancy was diagnosed at least 10 days after transfer by rising HCG concentrations of $\geqslant$20 IU/ml in serum on two occasions. Preclinical abortion describes pregnancies where no gestational sac was detected and/or where hormone concentrations were falling. Clinical pregnancies were confirmed by the presence of a gestational sac containing a fetus with heart activity detected by transvaginal ultrasonography 4–6 weeks after transfer. Implantation rate was defined as the ratio of the number of gestational sacs containing a fetus with heart activity and the number of transferred embryos. Clinical pregnancies reaching 20 weeks of gestation were considered ongoing.

Statistical analysis

Outcome measures are expressed as means with 95% confidence intervals (CI). Whenever indicated, the χ^2-test was applied at the 5% level of significance. Statistical analysis was performed with the Medcalc statistical software package (Medcalc, Gent, Belgium),

Results

In the present study, 275 consecutive MESA–ICSI cycles in 162 couples were analysed retrospectively. In 157 cycles, fresh epididymal spermatozoa were used for ICSI while in 118 cycles frozen–thawed epididymal spermatozoa were used. The

Table I. Intracytoplasmic sperm injection (ICSI) with fresh and frozen–thawed epididymal spermatozoa

	Fresh	Frozen–thawed
ICSI procedures	157	118
No. of OCC retrieved[a]	2193	1456
mean (95% CI)[b]	14.0 (12.7–15.3)	12.3 (10.9–13.7)
range	1–42	1–41
No. of oocytes injected	1821	1255
mean (95% CI)	11.6 (10.5–12.7)	10.6 (9.4–11.9)
range	0–37	0–37
Fertilization rate[c]		
mean (95% CI)	61.8 (57.8–65.9)	54.5 (49.8–59.3)
range	0.0–61.8	0.0–100
Cleavage rate[d]		
mean (95% CI)	73.2 (68.7–77.7)	67.0 (61.0–73.0)
range	0.0–100	0.0–100

[a]Oocyte–cumulus complexes.
[b]95% confidence interval of the mean.
[c]Mean of percentages of injected oocytes showing two pronuclei per ICSI cycle.
[d]Mean of percentages of oocytes with two pronuclei developing into an embryo with ⩽50% anucleate fragments per ICSI cycle.

Table II. Outcome of intracytoplasmic sperm injection procedures with with fresh and frozen–thawed epididymal spermatozoa

	Fresh	Frozen–thawed
No. of embryo transfers	145	100
No. of embryos replaced	420	278
mean (range)	2.9 (1– 7)	2.8 (1– 8)
No. of pregnancies (+ HCG)[a]	62	31
Pregnancy (%)/cycle	39.5	26.3
No. of clinical pregnancies	46	28
Clinical pregnancy (%)/cycle	29.3	23.7
No. of fetuses with heart activity	62	34
Implantation (%)/embryo	14.8	12.2
No. of ongoing pregnancies	42	25
Ongoing pregnancy (%)/cycle	26.7	21.2

Differences not significant except for [a]$P < 0.03$ (χ^2-test).
HCG = human chorionic gonadotrophin.

duration of infertility ranged from 1 to 15 years. In the group in which fresh epididymal spermatozoa were used the average duration was 6.4 years (95% CI 5.7–7.0) and in the group of those undergoing ICSI with cryopreserved epididymal spermatozoa the average duration was 6.4 years (95% CI 5.5–7.3). The rank of trial ranged from one to six in both groups with an average rank of trial of 1.2 (95% CI 1.1–1.4) in the group in which fresh spermatozoa were used and 2.2 (95% CI 2.1–2.4) in the group in which frozen spermatozoa were used. The mean ages of the female and male partners undergoing ICSI with fresh epididymal spermatozoa were respectively 33.9 years (range 23–45, 95% CI 33.1–34.8) and 39.1 years (range 24–63, 95% CI 37.6–40.6). In ICSI cycles with frozen–thawed epididymal spermatozoa the mean female age was 34.7 years (range 24–44, 95% CI 33.4–35.9) while the age of the men was 40.3 years on average (range 24–63, 95% CI 37.9–42.5).

No significant differences were observed in parameters of fresh or frozen–thawed epididymal spermatozoa used for ICSI. After correction for dilution, the mean density was 9.8×10^6 per ml (95% CI 7.6–12.1) versus 10.8×10^6 per ml (95% CI 2.3–19.3) for fresh and frozen–thawed spermatozoa respectively. Overall motility was respectively 15.1% (95% CI 12.1–18.1) versus 10.6% (95% CI 4.5–16.7). A mean of 13.0% of fresh spermatozoa had a normal morphology (95% CI 10.8–15.1) compared to a mean of 9.5% of frozen–thawed spermatozoa (95% CI 4.1–15.0).

Table I shows the ICSI results. No significant differences in oocyte recovery were observed between the two groups. A total of 3649 oocyte–cumulus complexes (OCC) were aspirated, 279 of which showed a germinal vesicle and 134 of which were in metaphase I. A total of 3076 metaphase-II oocytes were injected, 292 of which were damaged (9.5%).

In the fresh epididymal sperm group, 59.4% of all the injected oocytes fertilized normally compared with 56.2% of all injected oocytes in the frozen–thawed epididymal sperm group and there was no significant difference in mean fertilization rate per cycle. Embryonic development was comparable across the two groups: 71.7% of all the pronuclear stage oocytes developed into embryos with <50% of their volume filled with anucleate fragments (776/1082) in the group in which fresh epididymal spermatozoa was used versus 67.3% (475/706) in the group using frozen–thawed epididymal spermatozoa. The mean cleavage rate per cycle was not statistically different between the two groups and neither was there any difference in quality distribution of the embryos (see Table I).

As may be seen from Table II, a total of 245 transfers were performed. In the group with fresh epididymal spermatozoa the transfer rate was 92.3 versus 84.7% in the group with frozen–thawed spermatozoa ($P < 0.03$ by χ^2-test). In the first group, 12 patients did not have embryos transferred because either no fertilization occurred ($n = 6$) or their fertilized oocytes did not develop any further ($n = 6$). In the second group, nine patients had fertilization failure while another nine patients had no cleaving embryos after normal fertilization (difference not significant). A total of 698 cleaving embryos were transferred. The overall pregnancy rate per ICSI cycle was significantly lower when frozen–thawed epididymal spermatozoa were used. However, no significant differences were found either in clinical and ongoing pregnancy rates or in implantation rates. In the fresh epididymal sperm group 69 embryonic sacs were observed, including five triplet pregnancies and 13 twin pregnancies. In the group in which frozen–thawed epididymal spermatozoa were used, one triplet and seven twin pregnancies were observed. In the group with fresh epididymal spermatozoa, 62 viable fetuses were observed at ultrasound (implantation rate of 14.8% per embryo transferred) and 42 pregnancies were ongoing (26.7% per ICSI cycle). The implantation rate was 12.2% in the group with frozen–thawed epididymal spermatozoa (34 viable fetuses) and 25 pregnancies were ongoing (21.2% per ICSI cycle). These differences were not significant.

Table III shows the pregnancy outcome in both groups. In the group with fresh epididymal spermatozoa, 42 pregnancies evolved beyond 20 weeks of gestation. However, a complete stillbirth occurred in two twin pregnancies while an incomplete stillbirth was observed in another twin pregnancy. Finally, 53 babies were born in this group, three of which had a major

Table III. Pregnancy outcome after intracytoplasmic sperm injection (ICSI) procedures with fresh and frozen–thawed epididymal spermatozoa[a]

	Fresh	Frozen–thawed
No. of pregnancies	62	31
Biochemical pregnancies	16 (25.8)	3 (9.7)
Clinical miscarriages (%)	4 (6.5)	2 (6.5)
Vanishing twin	3	1
Vanishing triplet	1	0
Therapeutic reduction (3 to >2)	1	0
No. of ongoing pregnancies (%)	42 (67.7)	26 (83.9)
Late miscarriages[b]	2	1
Children born	53	31
singleton	30	19
sets of twins	7	6
sets of triplets	3	0
Congenital malformations		
minor	0	2
major	3	0

[a]Differences not significant.
[b]Miscarriages after 20 weeks.

Table IV. Comparison of intracytoplasmic sperm injection (ICSI) with fresh and frozen–thawed epididymal spermatozoa in 67 couples

	Fresh	Frozen–thawed
ICSI procedures	77	88
Mean rank of trial (95% CI)[a]	1.4 (1.2–1.7)	2.4 (2.2–2.6)
No. of OCC retrieved[b]	1008	1076
mean (95% CI)	12.9 (11.2–14.7)	12.2 (10.6–13.8)
No. of oocytes injected	864	1255
Mean (%) fertilization rate[c] (95% CI)	60.1 (54.6–65.5)	53.0 (47.5–58.6)
Complete fertilization failures (%)	3 (3.8)	8 (9.1)
No. of embryo transfers	72	72
No. of pregnancies (+ HCG)	25	31
Pregnancy (%)/cycle	32.1	35.2
Fetuses with heart activity	21	34
Implantation (%)/embryo	10.3	12.2
No. of deliveries	14	18
Delivery (%)/cycle	17.9	20.5

[a]95% confidence interval of the mean.
[b]Oocyte–cumulus complexes.
[c]Mean of percentages of injected oocytes showing two pronuclei per ICSI cycle.
HCG = human chorionic gonadotrophin.

congenital malformation (all singletons). One child had a preaxial polydactyly, one had a proximal focal femoral deficiency (hip–leg malformation) and another one had a transposition of the great vessels.

In the group in which frozen–thawed epididymal spermatozoa were used, 26 pregnancies were ongoing. One premature delivery occurred in a triplet pregnancy at 24 weeks. Thirty-one babies were born, two of whom presented with minor congenital malformations, i.e. an inguinal hernia and a sacral dimple. No major congenital malformations were observed.

Table IV shows the subset of patients who had ICSI both with fresh and frozen–thawed epididymal spermatozoa. This comparison did not reveal any significant differences in fertilization rates, fertilization failure rate, cleavage rates or implantation rates.

Discussion

There is a great deal of controversy as to which technique should be preferred for recovering spermatozoa from infertile men with incurable obstructive azoospermia. These patients may be treated by ICSI with spermatozoa retrieved either by MESA, by percutaneous epididymal sperm aspiration (PESA), or by testicular sperm extraction (TESE) or aspiration (TESA). Each approach has its advantages and disadvantages. MESA, being the most invasive and elaborate technique, has the major benefit of allowing a full scrotal exploration. Whenever indicated, a vasoepididymostomy may then be performed concomitantly. Furthermore, the number of spermatozoa retrieved is usually high, facilitating cryopreservation for later ICSI. A limited number of ICSI cycles using frozen–thawed epididymal spermatozoa have been reported (Devroey *et al*., 1995: $n = 7$; Oates *et al*., 1996: $n = 11$; Holden *et al*., 1997: $n = 59$). Often the results after ICSI with frozen–thawed spermatozoa have been compared with retrospective controls from the literature (Devroey *et al*., 1995) or with those after ICSI using ejaculated spermatozoa (Holden *et al*., 1997). Only the study by Oates *et al*. (1996) compared ICSI with fresh and frozen–thawed spermatozoa and found no differences between the use of fresh or frozen–thawed epididymal spermatozoa in a very small series.

In the present study we analysed retrospectively a larger consecutive case series of couples undergoing ICSI with epididymal spermatozoa, either fresh or frozen–thawed, in order to assess whether the freezing and thawing of epididymal spermatozoa might jeopardize the outcome after ICSI.

Although the freezing–thawing process may impair the quality and especially the motility of the epididymal spermatozoa, we observed no significant differences in quality between fresh and frozen–thawed epididymal spermatozoa. This surprising finding results from the retrospective character of this study. Patients who had their epididymal spermatozoa thawed and in whom all spermatozoa were found immotile either had a repeat MESA (five such patients are included in this series) or a testicular sperm recovery on the day of the ovum retrieval.

Yet this happened only in patients who were scheduled for a second or third ICSI attempt with cryopreserved epididymal spermatozoa. This means that they had at least one ICSI cycle with frozen–thawed epididymal spermatozoa. Another option for such patients with only immotile spermatozoa after thawing might have been to select immotile but vital spermatozoa by the hypo-osmotic swelling test as has been suggested for ejaculated spermatozoa (Casper *et al*., 1996; Tsai *et al*., 1997; Verheyen *et al*., 1997). However, this test may not be reliable because of freezing-thawing damage to the cell membrane of the epididymal spermatozoa (Esteves *et al*., 1996).

A second prerequisite for adopting cryopreservation of epididymal spermatozoa as a valid alternative to repeat surgery or another sperm recovery technique, is that the outcome after ICSI with frozen–thawed epididymal spermatozoa has to be comparable to that of ICSI with fresh epididymal spermatozoa. Although we found no differences in fertilization rates, we observed a higher proportion of ICSI cycles with complete fertilization failure after using frozen–thawed spermatozoa than

after using fresh epididymal spermatozoa. We also observed a slightly greater number of cycles with no embryonic development after the use of frozen–thawed epididymal spermatozoa. The causes of these differences, which were not significant, are not clear.

Although the overall pregnancy rate (+ HCG) was lower when frozen–thawed spermatozoa were used, the clinical pregnancy rate per ICSI cycle was comparable. This is the result of a higher proportion of biochemical pregnancies in the group with fresh epididymal spermatozoa. It may be that the freezing–thawing process eliminates senescent spermatozoa that might otherwise be used as fresh spermatozoa for ICSI.

As for all pregnancies after ICSI, patients were asked to participate in the prospective follow-up study on ICSI (Bonduelle *et al*., 1996). In this small series of pregnancies after MESA-ICSI we observed no karyotype anomaly. In three babies born after ICSI with fresh epididymal spermatozoa, major congenital anomalies were observed. In the group in which frozen–thawed spermatozoa were used, only two minor congenital anomalies were observed. These figures underline the need for further prospective follow-up of babies born after the use of epididymal spermatozoa.

In another smaller study, the general outcome after ICSI with frozen–thawed spermatozoa was found comparable to that for ICSI with fresh epididymal spermatozoa (Oates *et al*., 1996). Others have compared ICSI with frozen–thawed epididymal spermatozoa to ICSI with ejaculated spermatozoa retrospectively and they too observed no difference in outcome (Holden *et al*., 1997).

Since the clinical pregnancy rates and implantation rates are comparable to those of ICSI with fresh epididymal spermatozoa, cryopreserving epididymal spermatozoa during any MESA procedure is advocated. This MESA procedure may be performed either during a scrotal exploration or at some other point by an andrological microsurgeon who has no direct access to ICSI. Since the outcome after microsurgical vasoepididymostomy is limited (Berardinucci *et al*., 1998), cryopreservation of epididymal spermatozoa for future ICSI may be performed whenever possible as a back-up whenever this procedure fails to restore fertility.

MESA may also be proposed as the primary sperm recovery technique in patients with obstructive azoospermia. Considering that other less invasive, less expensive and even simpler techniques are available to retrieve spermatozoa in such patients, e.g. PESA, we propose MESA as the first-line approach in those patients who may benefit from a scrotal exploration. Patients in whom the feasibility for reconstructive microsurgery has not been evaluated are a target group for MESA. Patients wishing to store a maximum of epididymal spermatozoa may be good MESA candidates too because at present no reliable data can be found in the literature concerning the possibilities of cryopreserving and using frozen–thawed spermatozoa obtained after PESA. Although pregnancies have been reported after ICSI with frozen–thawed testicular spermatozoa from patients with obstructive azoospermia, at present these data too are limited and patients may prefer MESA with cryopreservation as the method of choice by which to avoid repeat surgery.

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