Sperm Retrieval, Fertilization, and Pregnancy Outcome in Repeated Testicular Sperm Aspiration

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Purpose: To report the outcome of sperm retrieval and results after ICSI in up to six repeated testicular sperm aspiration procedures.

Methods: Twenty-two men with obstructive and thirty-four men with nonobstructive azoospermia underwent 50 and 91 needle aspirations, respectively. Sufficiency of spermatozoa for ICSI and cryopreservation, fertilization rate, and pregnancy outcome was analyzed retrospectively.

Results: No major differences were found in sperm recovery or pregnancy outcome in the repeated cycles. Testicular aspirate containing motile spermatozoa with maintained fertilizing capacity was obtained in up to six repeated procedures in the nonobstructive group. No postoperative complications were reported for any of the participants.

Conclusions: Testicular sperm aspiration is a simple and effective method of sperm retrieval, which can be performed from the same testis up to several times with good recovery of motile spermatozoa for ICSI and maintaining high fertilization and pregnancy rates, in men with both obstructive and nonobstructive azoospermia.

KEY WORDS: Azoospermia; fertilization; intracytoplasmic sperm injection; pregnancy outcome; testicular sperm aspiration.

INTRODUCTION

Several techniques for sperm recovery have recently been introduced to help azoospermic men to father

their own genetic children. The first pregnancy resulting from in vitro fertilization (IVF) using spermatozoa recovered from the epididymis was reported by Temple-Smith in 1985 (1). The fertilization rate was low but it has improved after the introduction of intracytoplasmic sperm injection (ICSI) (2). In patients with obstructive azoospermia, the fertilization and pregnancy rates from surgically recovered spermatozoa from the epididymis or testis are comparable to results where ejaculated spermatozoa have been used (3,4). In the nonobstructive cases, some studies have reported lower fertilization, pregnancy, and "live births per ET" rates (4,5).

Microsurgical epididymal sperm aspiration (MESA) was the first surgical sperm retrieval method involving the epididymis (1). It requires open surgery, and in combination with ICSI, it has become a common procedure for treating men with obstructive azoospermia (6,7). Percutaneous epididymal sperm aspiration (PESA), a less invasive procedure for retrieval of epididymal spermatozoa, was introduced by Craft and Shirastaw (8). PESA is simpler compared to MESA and can be performed under local anesthesia. If PESA fails, then testicular sperm aspiration (TESA) (9) has also been shown to be an efficient minimally invasive method. In all obstructive cases, spermatozoa can be successfully recovered by PESA or TESA (10). In the non-obstructive cases, the successful sperm recovery rate is lower, 40–70% (4,10–12).

There are few published studies concerning possible risks associated with sperm retrieval procedures. Schlegel *et al.* (13) found an increased risk of permanent devascularization of the testis after TESE. They also reported an increased rate of sperm recovery failure if the interval between TESE procedures was less than 6 months. Ron-El *et al.* (14) demonstrated,

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with ultrasonography, focal testicular lesions in 54% of men 6 months after TESE. Whether these findings have long-term consequences remains to be evaluated. A recently published study by Rosenlund *et al.* (15) evaluated the sperm recovery outcome and fertilization rate in repeated PESA among obstructive azoospermic men. Sufficient motile spermatozoa for ICSI were found during at least three repeated punctures on the same unilateral epididymis, and similar fertilization rates were achieved.

So far no studies have been published about possible risks after repeated aspiration from the testis. The aim of this retrospective study was therefore to compare the recovery of spermatozoa and fertilization and pregnancy rates in repeated TESA cycles.

MATERIALS AND METHODS

Between January 1995 and September 1999, 56 azoospermic men underwent repeated TESA procedures in three different IVF centers (Center for Reproductive Medicine, Sahlgrenska University Hospital, Göteborg; Fertility Center Scandinavia, Göteborg; and Department of Obstetrics and Gynecology, Huddinge University Hospital, Huddinge). Twenty-two men suffered from obstructive azoospermia, and 34 men suffered from nonobstructive azoospermia. All men underwent either a diagnostic pretreatment trial followed by treatment cycles or a treatment cycle followed by subsequent treatment cycles. All repeated procedures were performed on the same unilateral testis. Patients in whom different sides were used in repeated sperm retrieval procedures or in whom there was any doubt as to which side was used were excluded from the study. Patient characteristics are shown in Table I. Additional female factors were few, and most women were healthy in both groups.

In the first procedure, 10 out of 22 in the obstructive group and 10 out of 34 in the nonobstructive group were diagnostic pretreatment trials. All the 22 obstructive men underwent a second TESA procedure, and 6 of them also had a third TESA procedure carried out. In the nonobstructive group, all 34 men underwent two, 14 had three, 5 had four, 3 had five, and 1 patient had a sixth TESA carried out. The median time intervals between procedures for both groups are described in Table IV.

TESA was performed under local anesthesia. 0.5 mg alfentanil (Rapifen®, Jansen-Cilag, Beerse, Belgium) was given intravenously followed by infiltration of 7–8 ml lidocaine (Xylocaine®, Astra,

Table I. Characteristics of the Study Population

	Obstructive	Nonobstructive
No. of patients	22	34
No. of TESA recoveries	50	91
S-FSH (IU/l)		
Mean $\pm$ SD	3.7 $\pm$ 2.3 ^a	16.6 $\pm$ 10.8 ^b
Range	1–9	2.1–37
Testicular volume (ml)		
Mean $\pm$ SD	19.4 $\pm$ 4.2 ^c	15.2 $\pm$ 5.0 ^d
Range	15–25	10–25
Age (years)		
Mean $\pm$ SD	34.8 $\pm$ 5.1	35.0 $\pm$ 4.2
Range	27–48	28–47
Female partner's age (years)		
Mean $\pm$ SD	31.8 $\pm$ 3.6	31.6 $\pm$ 4.5
Range	23–38	23–39
Female factors detected (patients)		
None	19	29
Tubal pathology	1	2
Endometriosis	1	2
PCOS		1
Myoma uteri	1	

Note. ^aversus ^b $p = 0.0001$ (Mann-Whitney U-test) ^cversus ^d $p = 0.0038$ (Mann-Whitney U-test).

Södertälje, Sweden) around the spermatic cord. Under sterile conditions, a 19- or 21-gauge butterfly needle was passed through the scrotal skin. Suction was applied with a 20-ml syringe, and the negative pressure was maintained by clamping the distal end of the needle tubing with two pairs of forceps. The 20-ml syringe was then replaced by a 1-ml syringe containing 0.2 ml gamete culture medium (IVF-50, Scandinavian IVF Science, Göteborg, Sweden). The needle was pushed 4–6 times in different directions with quick thrusting movements into the testicular tissue. The needle was then slowly removed from the testis and the scrotal skin while the back pressure was maintained by the clamped tubing. The assistant used two pairs of fine tweezers to pick up the small tubules recovered by the needle. The clamps were then removed, and the needle was flushed with culture medium and its content was expelled into a sterile tube containing the culture medium. The procedure was repeated 1–5 times, covering the ventral surface of the testis. The aspirated testicular tissue was subsequently dissected, incubated in the culture medium, and examined 1, 4, and 24 h after puncture in the laboratory.

The size of the butterfly needle used for sperm retrieval was 19 gauge in the majority of cases. The 21-gauge needle was mainly used in the beginning of the study.

The treatment cycles included down-regulation and ovarian stimulation with follicle stimulating hormone

Table II. Outcome of Testicular Sperm Aspiration (TESA) Regarding Sperm Retrieval, Sufficiency of Spermatozoa for ICSI, and Sufficiency for Cryopreservation in the Obstructive Group

TESA	<i>n</i>	Insufficient number of spermatozoa for ICSI (%)	Sufficient for ICSI but not for all recovered oocytes (%)	Sufficient for ICSI for all recovered oocytes (%)	Sufficient for ICSI for all recovered oocytes and for cryopreservation (%)
1st	22	0	0	16 (73) ^a	6 (27)
2nd	22	0	1 (4)	18 (82)	3 (14)
3rd	6	0	1 (17)	4 (66)	1 (17)

^aincluding 10 pre-treatment trials.

(FSH), following protocols used for conventional IVF/ICSI. ICSI was performed essentially according to Hamberger *et al.* (16). In treatment cycles, the TESA procedure was performed either on the day of ovum pick-up (OPU) or 24 h before OPU, based on spermatozoa quality in cases where a diagnostic procedure had earlier been performed. In cases where sperm motility was improved after 24 h, TESA was performed 24 h before OPU. Depending on the outcome of TESA, the material was arbitrarily divided into four groups: 1). No spermatozoa were found or the yield from the aspirate was poor making ICSI impossible. 2). Sufficient motile or immotile spermatozoa for ICSI were found but not for all recovered oocytes. 3). Sufficient motile or immotile spermatozoa for ICSI were found for all recovered oocytes. 4). A sufficient number of spermatozoa were found for both ICSI of all recovered oocytes and cryopreservation.

In the treatment cycles, we studied the number of oocytes injected and the fertilization, pregnancy, and ongoing/delivery rates. Testicular volumes were estimated with an orchidometer.

Hormone Analysis

Serum FSH was measured using ELISA assays, AxSYM® FSH Reagent Pack (Abbott IMx; Abbott

Park, IL; sensitivity limit 0.37 mIU/mL). The inter- and intra-assay variations were <10%.

Statistics

For continuous variables, mean and SD were calculated and compared between the obstructive and nonobstructive groups by Mann-Whitney U-test. No statistical comparison was made for sperm recovery and pregnancy data because of the low number of patients in the fourth to sixth cycles (Table II, III, and IV).

RESULTS

The first TESA procedure was performed either as a diagnostic pretreatment trial or as part of a treatment ICSI cycle (Table IV). The repeated procedures were always part of treatment cycles. The mean testicular volume was significantly higher and the mean serum FSH concentration significantly lower in the obstructive compared to the nonobstructive group (Table I). In the obstructive group, 100% of the patients (22/22) were considered to have sufficient spermatozoa for ICSI (pretreatment trials) or a sufficient number of spermatozoa were found for ICSI for all recovered oocytes in the first TESA (Table II). In

Table III. Outcome of Testicular Sperm Aspiration (TESA) Regarding Sperm Retrieval, Sufficiency of Spermatozoa for ICSI, and Sufficiency for Cryopreservation in the Nonobstructive Group

TESA	<i>n</i>	Insufficient number of spermatozoa for ICSI (%)	Sufficient for ICSI but not for all recovered oocytes (%)	Sufficient for ICSI for all recovered oocytes (%)	Sufficient for ICSI for all recovered oocytes and for cryopreservation (%)
1st	34	1 (3)	13 (38)	18 (53) ^a	2 (6)
2nd	34	0	10 (29)	22 (65)	2 (6)
3rd	14	0	1 (7)	11 (79)	2 (14)
4th	5	0	4 (80)	1 (20)	0
5th	3	0	2 (67)	1 (33)	0
6th	1	0	0	1 (100)	0

^aincluding 10 pretreatment trials.

Table IV. Outcome of Testicular Sperm Aspiration (TESA) Regarding Fertilization, Pregnancy, and Ongoing/Delivery Rates in the Obstructive and the Nonobstructive Groups

TESA	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
Obstructive group						
No. of cycles	11 ^a	21 ^b	4 ^c			
No. of oocytes injected (mean $\pm$ SD)	9.5 $\pm$ 3.2	11.8 $\pm$ 6.3	7.8 $\pm$ 5.1			
No. of embryo transfers	11	19	4			
Interval between sperm retrievals (in days)						
Median		224	212			
Range		40–1125	146–330			
Fertilization rate per oocyte (%)	49.5	63.7	41.9			
No. of pregnancies per cycle	2/11	12/21	1/4			
No. of pregnancies per ET	2/11	12/19	1/4			
No. of ongoing/deliveries per cycle	2/11	11/21	1/4			
No. of ongoing/deliveries per ET	2/11	11/19	1/4			
Nonobstructive group						
No. of cycles	24 ^d	34	14	5	3	1
No. of oocytes injected (mean $\pm$ SD)	9.8 $\pm$ 5.7	8.1 $\pm$ 4.8	12.1 $\pm$ 4.6	14.8 $\pm$ 8.0	12.3 $\pm$ 6.5	11.0 $\pm$ 0
No. of embryo transfers	23	26	13	5	3	1
Interval between sperm retrievals (in days)						
Median		180	182.5	178	124	194
Range		68–991	120–871	117–720	117–139	194–194
Fertilization rate per oocyte (%)	41.5	50.9	40.2	65.4	59.5	45.5
No. of pregnancies per cycle	7/24	8/34	1/14	0/5	1/3	0/1
No. of pregnancies per ET	7/23	8/26	1/13	0/5	1/3	0/1
No. of ongoing/deliveries per cycle	2/24	6/34	1/14	0/5	1/3	0/1
No. of ongoing/deliveries per ET	2/23	6/26	1/13	0/5	1/3	0/1

^a Ten diagnostic pretreatment trials and one cycle where epididymal spermatozoa were used for ICSI are excluded.

^b One cycle where epididymal spermatozoa were used for ICSI is excluded.

^c Two cycles where epididymal spermatozoa were used for ICSI are excluded.

^d Ten diagnostic pretreatment trials are excluded.

27% of these patients (6/22) spermatozoa could be cryopreserved. In the second and third TESA, 96% (21/22) and 83% (5/6) had sufficient spermatozoa for ICSI for all recovered oocytes with or without cryopreservation of spermatozoa. The mean number of oocytes injected for the first, second, and third recoveries was 9.5, 11.8, and 7.8 respectively (Table IV). During the second and third TESA, there was one case in each group where the number of spermatozoa was decreased, resulting in inability to microinject all recovered oocytes. There were no total sperm recovery failures. The fertilization and pregnancy rates are presented in Table IV. The number of pregnancies per ET was 2/11, 12/19, and 1/4. In four cycles where both TESA and PESA were performed and the epididymal spermatozoa were used for ICSI, the outcome of ICSI was not included. In the obstructive group there were 2/36 cycles where ET was not performed. The reason was one case of no fertilization and another with no embryonic cleavage.

In the nonobstructive group, up to six repeated retrievals were performed (Table III). At the first TESA, 97% (33/34) had sufficient spermatozoa for ICSI. There were no sperm recovery failures during

the repeated TESA procedures. At the second to sixth retrievals, sufficient spermatozoa for ICSI for all recovered oocytes were found in 65, 79, 20, 33, and 100% of men. Only five men underwent more than three retrievals. The fertilization rate varied between 40.2% and 65.4%, with no tendency to decrease with the number of TESA (Table IV). In the nonobstructive group, ET did not occur in 10/81 cycles because of failed fertilization (8 cycles) and no embryonic cleavage (2 cycles).

The number of pregnancies per ET was 7/23, 8/26, and 1/13 after the first three retrievals. At the fourth to the sixth TESA procedure, the pregnancy rate per ET was 0/5, 1/3, and 0/1. There were no sperm recovery failures in any of the repeated TESA procedures, and the time interval between procedures seemed not to affect the outcome of sperm retrieval or the fertilization rate (Table V).

Except for minor pain and local swelling, there were no reports of clinically significant peroperative or postoperative complications leading to medical treatment or hospital care from any of the participants in the study. All the results were comparable between the three participating clinics.

Table V. Outcome of Repeated TESA Regarding Sperm Retrieval and Fertilization Rate in Relation to Time Interval Between TESA Procedures

Time interval	Obstructive group			Nonobstructive group		
	<3 months	3–6 months	>6 months	<3 months	3–6 months	>6 months
“TSEA Procedures (<i>n</i>)”	4	5	19	1	29	27
Insufficient number of spermatozoa for ICSI (%)	0	0	0	0	0	0
Sufficient for ICSI but not of all recovered oocytes (%)	0	0	1 (5)	0	7 (24)	4 (15)
Sufficient for ICSI of all recovered oocytes (%)	3 (75)	5 (100)	15 (79)	1 (100)	16 (55)	23 (85)
Sufficient for ICSI of all recovered oocytes and for cryopreservation (%)	1 (25)	0	3 (16)	0	6 (21)	0
Fertilization rate per oocyte (%)	74.2	37.3	62.5	57.1	47.0	49.8

DISCUSSION

In all azoospermic men with normal spermatogenesis, that is, patients with so-called obstructive azoospermia, spermatozoa may be recovered by open testicular biopsy (TESE) (17), by needle aspiration techniques such as TESA, and in most cases also by PESA (10). However, in men with nonobstructive azoospermia, variable sperm recovery success rates are reported using similar recovery techniques. Friedler *et al.* (11) reported a lower success rate of testicular sperm retrieval using the needle aspiration technique compared to an open biopsy technique. Another study by Lewin *et al.* (18) reported successful recovery of mature spermatozoa from needle aspiration in 59% of nonobstructive men, which is comparable with results with the biopsy technique. The authors claimed that several punctures from each testis (10–20 in their own patient material) are necessary to increase the chance of hitting a rare site of active spermatogenesis. Similar results were reported by Rosenlund *et al.* (19), who found the TESA procedure, provided that a 19-gauge needle was used, being comparable with TESE in terms of determining the presence of spermatozoa but inferior for histopathological evaluation. In contrast, in a prospective study by Ezech *et al.* (20), testicular sperm recovery was more effective using open biopsy compared to needle biopsy when both open biopsy and multiple punctures were performed on the same testis. Schlegel (21) reported that TESE combined with microdissection of testicular tubules improves sperm retrieval in men with nonobstructive azoospermia.

Attention has been drawn to the possible injuries to the testis because of devascularization

and inflammatory changes after testicular biopsy (13,14,22). In the publication from Schlegel and Su (13), impaired spermatogenesis for up to 6 months after TESE was reported. In a study published by Rosenlund *et al.* (15), PESA was repeated up to three times from the same unilateral epididymis in obstructive men with no negative effects on the outcome of sperm retrieval or fertilization rate.

In this study, we retrospectively studied repeated TESA from 22 obstructive and 34 nonobstructive men. The aspiration was at most repeated twice in the obstructive group and five times in the nonobstructive group. The sperm recovery outcome and the fertilization and pregnancy rate did not seem to change dramatically with repeated procedures, neither did the time interval between procedures appear to influence the outcome. A numerically lower rate of spermatozoa, sufficient for ICSI for all recovered oocytes, was found in the fourth and the fifth cycles. However, no statistical comparison was performed because of the low number of cycles at these trials.

Diagnostic sperm recovery procedures on all azoospermic men are routinely performed in one of the three participating clinics. Men with absence of viable spermatozoa will not be accepted for subsequent ICSI cycles. For the remaining patients the predictive value for successful sperm recovery in the treatment cycles is very high (10). In the obstructive cases, where the expectations of successful recovery is high, diagnostic procedure confirm the appearance of normal spermatogenesis. Cryopreservation of motile spermatozoa is often possible, and repeated procedures can be avoided.

TESA is a simple procedure and is well tolerated by patients. Local anesthesia is adequate in a large

number of patients (23). We had no reports of peroperative or postoperative complications in this study. If the recovery of testicular tissue is poor despite multiple needle aspirations, we recommend the use of a biopsy gun with a 14 or 16-gauge needle as a complement. Tuuri *et al.* (24) considered this method quite comparable to TESE and considered this the optimal method for sperm retrieval.

It is important to point out that testicular aspiration techniques seem to be performed in different ways in published studies. The simplest method is often named testicular fine needle aspiration (TEFNA), where a 21-gauge butterfly needle is usually inserted into the testicular mass with a single puncture. With the needle fixed in the testicular parenchyma, the syringe is used for multiple aspiration movements (25). With the TESA technique, a 19 gauge rather than a 21-gauge butterfly needle is inserted into the testis. Testicular tissue is sampled with a negative pressure by moving the needle backward and forward within the testis in rapid movements (9). On withdrawal, a core of testicular tissue extends between the tip of the needle and the testis. This technique was used in this study because at our centers it is considered the method of choice for testicular sperm recovery in nonobstructive men. In obstructive cases, TESA is used as a complement to PESA if the yield of epididymal sperm is not sufficient. TESA is considered less invasive than TESE, and tissue can be sampled from several areas deeper into the testis compared to the more superficial open biopsies. This could be an advantage if the spermatogenesis in nonobstructive men is focal, as suspected by Turek *et al.* (26). However, other reports have suggested the distribution of spermatogenesis to be homogeneous and multifocal (27). The location of spermatogenesis was studied by Hauser *et al.* (28). In this report the testis was considered heterogeneous, the spermatogenesis focal, and in most severe cases of testicular failure the low numbers of spermatozoa produced were found in the periphery and not in the deeper parts of the testis such as the rete testis.

In summary, this retrospective analysis suggests that the TESA needle aspiration technique can be repeated several times with no negative effect on recovery of mature spermatozoa or pregnancy outcome. The time interval between aspiration procedures seems to be of less importance. Even if no severe complications were detected, the present data does not rule out the possibilities of testicular damage after repeated aspirations. Further prospective studies are needed to determine whether TESA is

associated with a potential risk of temporary or permanent ischaemic testicular injury, which has been noted after open biopsy.

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REFERENCES

1. Temple-Smith PD, Southwick GJ, Yates CA, Trounson AO, de Kretser DM: Human pregnancy by in vitro fertilization (IVF) using sperm aspirated from the epididymis. *J In Vitro Fert Embryo Transf* 1985;2:119–122
2. Palermo G, Joris H, Devroey P, Van Steirteghem AC: Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. *Lancet* 1992;340:17–18
3. Silber SJ, Devroey P, Tournaye H, Van Steirteghem AC: Fertilizing capacity of epididymal and testicular sperm using intracytoplasmic sperm injection (ICSI). *Reprod Fert Dev* 1995;7:281–292
4. Mansour RT, Kamal A, Fahmy I, Tawab N, Serour GI, Aboulghar MA: Intracytoplasmic sperm injection in obstructive and non-obstructive azoospermia. *Hum Reprod* 1997;12:1974–1979
5. Kahraman S, Ozgur S, Alatas C, Aksoy S, Tasdemir M, Nuhoglu A, Tasdemir I, Balaban B, Biberoglu K, Schoysman R, Nijs M, Vanderzwalmen P: Fertility with testicular sperm extraction and intracytoplasmic sperm injection in non-obstructive azoospermic men. *Hum Reprod* 1996;11:756–760
6. Tournaye H, Devroey P, Liu J, Nagy Z, Lissens W, Van Steirteghem A: 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* 1994;61:1045–1051
7. Tucker MJ, Morton PC, Witt MA, Wright G: Intracytoplasmic injection of testicular and epididymal spermatozoa for treatment of obstructive azoospermia. *Hum Reprod* 1995;10:486–489
8. Craft I, Shrivastav P: Treatment of male infertility [letter; comment]. *Lancet* 1994;344:191–192
9. Craft I, Tsirigotis M: Simplified recovery, preparation and cryopreservation of testicular spermatozoa. *Hum Reprod* 1995;10:1623–1626
10. Westlander G, Hamberger L, Hanson C, Lundin K, Nilsson L, Soderlund B, Werner C, Bergh C: Diagnostic epididymal and testicular sperm recovery and genetic aspects in azoospermic men. *Hum Reprod* 1999;14:118–122
11. Friedler S, Raziel A, Strassburger D, Soffer Y, Komarovskiy D, Ron-El R: Testicular sperm retrieval by percutaneous fine needle sperm aspiration compared with testicular sperm extraction by open biopsy in men with non-obstructive azoospermia. *Hum Reprod* 1997;12:1488–1493
12. Tournaye H, Verheyen G, Nagy P, Ubaldi F, Goossens A, Silber S, Van Steirteghem AC, Devroey P: Are there any predictive

- factors for successful testicular sperm recovery in azoospermic patients? *Hum Reprod* 1997;12:80–86
13. Schlegel PN, Su LM: Physiological consequences of testicular sperm extraction. *Hum Reprod* 1997;12:1688–1692
 14. Ron-El R, Strauss S, Friedler S, Strassburger D, Komarovsky D, Raziel A: Serial sonography and colour flow Doppler imaging following testicular and epididymal sperm extraction. *Hum Reprod* 1998;13:3390–3393
 15. Rosenlund B, Westlander G, Wood M, Lundin K, Reismar E, Hillensjo T: Sperm retrieval and fertilization in repeated percutaneous epididymal sperm aspiration. *Hum Reprod* 1998;13:2805–2807
 16. Hamberger L, Sjogren A, Lundin K, Soderlund B, Nilsson L, Bergh C, Wennerholm UB, Wikland M, Svalander P, Jakobsson AH, Forsberg AS: Microfertilization techniques—the Swedish experience. *Reprod Fertil Dev* 1995;7:263–267
 17. Tournaye H, Liu J, Nagy PZ, Camus M, Goossens A, Silber S, Van Steirteghem AC, Devroey P: Correlation between testicular histology and outcome after intracytoplasmic sperm injection using testicular spermatozoa [see comments]. *Hum Reprod* 1996;11:127–132
 18. Lewin A, Reubinoff B, Porat-Katz A, Weiss D, Eisenberg V, Arbel R, Bar-el H, Safran A: Testicular fine needle aspiration: The alternative method for sperm retrieval in non-obstructive azoospermia. *Hum Reprod* 1999;14:1785–1790
 19. Rosenlund B, Kvist U, Ploen L, Rozell BL, Sjoblom P, Hillensjo T: A comparison between open and percutaneous needle biopsies in men with azoospermia. *Hum Reprod* 1998;13:1266–1271
 20. Ezech UI, Moore HD, Cooke ID: A prospective study of multiple needle biopsies versus a single open biopsy for testicular sperm extraction in men with non-obstructive azoospermia. *Hum Reprod* 1998;13:3075–3080
 21. Schlegel PN: Testicular sperm extraction: Microdissection improves sperm yield with minimal tissue excision. *Hum Reprod* 1999;14:131–135
 22. Harrington TG, Schauer D, Gilbert BR: Percutaneous testis biopsy: An alternative to open testicular biopsy in the evaluation of the subfertile man. *J Urol* 1996;156:1647–1651
 23. Gorgy A, Meniru GI, Naumann N, Beski S, Bates S, Craft IL: The efficacy of local anaesthesia for percutaneous epididymal sperm aspiration and testicular sperm aspiration. *Hum Reprod* 1998;13:646–650
 24. Tuuri T, Moilanen J, Kaukoranta S, Makinen S, Kotola S, Hovatta O: Testicular biopsy gun needle biopsy in collecting spermatozoa for intracytoplasmic injection, cryopreservation and histology. *Hum Reprod* 1999;14:1274–1278
 25. Tournaye H, Clasen K, Aytoz A, Nagy Z, Van Steirteghem A, Devroey P: Fine needle aspiration versus open biopsy for testicular sperm recovery: A controlled study in azoospermic patients with normal spermatogenesis. *Hum Reprod* 1998;13:901–904
 26. Turek PJ, Cha I, Ljung BM: Systematic fine-needle aspiration of the testis: Correlation to biopsy and results of organ “mapping” for mature sperm in azoospermic men. *Urology* 1997;49:743–748
 27. Silber SJ, Nagy Z, Devroey P, Tournaye H, Van Steirteghem AC: Distribution of spermatogenesis in the testicles of azoospermic men: The presence or absence of spermatids in the testes of men with germinal failure. *Hum Reprod* 1997;12:2422–2428
 28. Hauser R, Botchan A, Amit A, Ben Yosef D, Gamzu R, Paz G, Lessing JB, Yogev L, Yavetz H: Multiple testicular sampling in non-obstructive azoospermia—is it necessary? *Hum Reprod* 1998;13:3081–3085