MINERVA UROL NEFROL 2005;57:99-107

Sperm retrieval for intra-cytoplasmic sperm injection in non-obstructive azoospermia

G. M. COLPI [1], G. PIEDIFERRO [1], F. NERVA [1], D. GIACCHETTA [1], E. M. COLPI [2], E. PIATTI [1]

[1]*Andrology Unit, San Paolo Hospital Polo Universitario, Milan, Italy*
[2]*Obstetrics and Gynecology Unit University of Insubria, Varese, Italy*

Surgical testicular sperm retrieval for intra-cytoplasmic sperm injection (ICSI) purposes is the only possibility of biological fathering in case of non-obstructive azoospermia (NOA). Successful retrieval only correlates with histology, not with FSH values or testicular volume. Concurrent AZFa and AZFb microdeletions predict unsuccessful recovery. Testicular sperm extraction (TESE) (mean of successful retrievals in literature: 52.7%) is the technique of choice: we had successful retrievals in 100% of cases of hypospermatogenesis with > 5 spermatids/tubule (spd/tub), 81.8% of cases of hypospermatogenesis with <4 spd/tub, 50% of cases of maturation arrest, and 25% of cases of histologically pure Sertoli cell-only syndrome. Microsurgical TESE (mTESE) has been reported to increase successful retrievals: from 16.7-45% for standard TESE to 42.9-63.6% for mTESE, depending on the distribution of testicular histology in the various case studies; from 9 to 14 cases out of 22, respectively, in the only study in which TESE and mTESE were performed simultaneously on the same testis. Improvements in biological procedures for TESE retrievals can increase positive findings. TeFNA does not appear to be indicated in NOA, both because of its low success rates - which, in practice, are only positive in hypospermatogenesis - and because it is unable to detect any carcinomas *in situ*. Previous surgery of left varicocele in NOA could increase the chances of subsequent recovery. ICSI from TESE has lower birth rates in NOA than in obstructive azoospermia (OA) (19% *vs* 28%). Abortion rates are significantly higher following ICSI from NOA (11.5%) than from OA (2.5%) (P=0.001). Therefore, the prognosticated fertility of a couple with an NOA male is quite lower than for a couple with an OA male.

Key words: **Non obstructive azoospermia - TESE - Microsurgical TESE - TeFNA - ICSI - Testis.**

Surgical sperm retrieval from testes is a necessary pre-condition in order to give patients with non-obstructive azoospermia (NOA) a possibility of biological fathering, using their gametes for intra-cytoplasmic sperm injection (ICSI). As a matter of fact, still in the recent past, these patients were offered either adoption or artificial insemination from a donor (AID), which was ruled unlawful in Italy not long ago.

According to Jarow *et al.*,[1] NOA (previously referred to as secretory azoospermia) is thought to account for 50-60% of all cases of azoospermia. However, in more recent times, there have been no truly reliable estimates of the NOA/OA (OA = obstructive azoospermia) ratio in azoospermia - both because of the bias due to the pre-selection of azoospermic patients being referred to the various

Address reprint requests to: G. M. Colpi, Unità di Andrologia – Ospedale San Paolo, Via A. di Rudinì 8, 20142 Milano. E-mail: gmcolpi@yahoo.com

Centres based on the latter's specific activities of excellence, and because the division between OA and NOA is often, *de facto*, a presumptive one, since it is made solely according to clinical criteria (testicular volume, clinical history data, plasma FSH etc.), and not on the basis of the only unquestionable diagnostic criterion, *i.e.*, testicular histology. In addition, the documented existence of mixed forms with obstructive and non-obstructive components at the same time (either as normal spermatogenesis with seminal duct obstruction on one side and severe spermatogenic failure on the other side, or as seminal duct obstruction with concomitant moderate spermatogenic damage on both sides) [2] may complicate the correct attribution of some cases to OA or NOA groups. Based on testicular histology, pure OA cases only account for 15% of the azoospermic subjects we have observed in the past 2 years.

Aetiopathogenesis and histology of non-obstructive azoospermia

Apart from genetic causes (such as Klinefelter's syndrome and other chromosomal abnormalities, including Y microdeletions), whose prevalence is approximately 20%, NOA is due to *maldescensus testis*, complete and focal germ cell aplasia (either congenital or acquired), spermatogenic arrest, previous orchitis, systemic diseases, severe and long-standing varicocele, previous surgery damaging testicular vessels, testicular tumours, prolonged local exposure to heat, irradiation, drugs, toxics etc.

Testicular histology of NOA shows different degrees of spermatogenic alterations: complete tubular sclerosis; reduced-diameter tubules without any trace of germinal epithelium (pure Sertoli cell-only syndrome [SCOS]); patterns which are similar to the previously described one, but with residual focal spermatogenesis in rare, larger-calibre tubules (incomplete SCOS); complete or incomplete spermatocytic maturation arrests (*i.e.*, with no or very rare spermatids and spermatozoa); complete or incomplete spermatid maturation arrests; hypospermatogenesis (decreased number of cells in all spermatogenic stages); and mixed patterns.

Considering the extreme variability of the above-mentioned spermatogenic alterations, classification of histology findings by means of score systems, preferably by means of Johnsen's score, is strongly recommended.

In 1997, Silber *et al.*[3] argued that, in order for sperm to occur in ejaculate, an average of at least 4 mature spermatids per seminiferous tubule was needed (in case of normozoospermia or pure OA, there is an average of 17-35 per tubule). Therefore, in pure NOA, there are less than 4 mature spermatids per seminiferous tubule. This explains why, in cases of NOA (confirmed by repeated semen analyses), mature spermatids may nevertheless be frequently found in biological preparations from testicular tissue biopsy.

Contrary to Authors holding opposite views, but in much more dated studies,[4, 5] some believe that human spermatogenesis is randomly organised, and that any damages may therefore occur patchily, and not evenly. On this basis, in order to increase the chances of recovering sperm in NOA, many recommend that more than one testicular sampling should be performed.

Predictive markers of successful sperm retrieval

There is evidence that the chance of recovering testicular sperm in NOA correlates neither with FSH or testicular volume in isolation,[6-8] nor with both determinations:[9] cross-data on age, infertility time, hormones (FSH, LH, testosterone, prolactin), and testicular volume reach a predictive sensitivity of 80.8%.[10]

Recently, a formula was proposed [11] to calculate the chances of recovery (sensitivity: 71%; specificity: 71.4%) based on FSH, total testosterone (TT), and Inhibin B: C = [1 + exp (5.201 – 0.048 x FSH – 0.449 x TT – 0,021 x Inhibin B)](-1).

Conflicting views are reported on a possible predictive role of Inhibin B:[12] however, as yet, there are no conclusive data.

Microdeletions of the AZFa and AZFb re-

TABLE I.—*Successful retrievals with TESE.*

Authors	No. of TESE	Retrieval +	%
Kahraman *et al.* (1996) [26]	29	14	48.2
Mulhall *et al.* (1997) [27]	30	21	70
Schlegel *et al.* (1997) [28]	16	10	62
Friedler *et al.* (1997) [29]	37	16	43
Jesek *et al.* (1998) [30]	64	49	77
Ostad *et al.* (1998) [31]	81	23	28
Ezeh *et al.* (1998) [32]	35	22	63
Schulze *et al.* (1999) [33]	250	157	62.8
Amer *et al.* (1999) [34]	216	71	37.5
Ben-Yosef *et al.* (1999) [35]	55	33	60
Su *et al.* (1999) [36]	81	47	58
Ng *et al.* (2000) [37]	26	12	46.2
Kitamura *et al.* (2000) [38]	44	32	72.7
Friedler *et al.* (2002) [39]	83	32	39
Damani *et al.* (2002) [40]	23	15	65.2
Meseguer *et al.* (2003) [41]	12	5	41.6
Negri *et al.* (2003) [42]	107	53	49.5
Piediferro *et al.* (2004) [43]	92	59	64.1
Kubota *et al.* (2005) [44]	30	20	66.6
Total	1311	691	52.7

gions of the Y chromosome are a strongly unfavourable prognostic marker: AZFa deletion (in particular, USPY and DBY) would result in SCOS, and complete AZF deletion is always associated with no sperm upon testicular sperm extraction (TESE).[13, 14] Microdeletions in the AZFc region, which are frequently found in infertile males (13% of those with azoospermia and 7% of those with severe oligoasthenoteratozoospermia [OAT] syndrome [13, 15-18]) is thought to have a lower negative prognostic influence.[19-24] Non-expression of the protamin-2 gene (a spermatid marker) is also thought to be an unfavourable prognostic marker for sperm retrieval in NOA.[25]

The chance of recovering sperm upon TESE only correlates with histology, which shows a sensitivity of 96% and a specificity of 100%.[6]

Sperm retrieval techniques in non-obstructiveazoospermia

TESE enables positive sperm retrieval in 28-77% [26-45] of NOA cases documented by testicular histology (mean 52.7% by single TESE) (Table I [22-44]), substantially according to a previous review:[46] the variability of results is thought to be due to heterogeneity of histological patterns. With regard to multiple TESE, we found only 3 papers with testicular histological data:[31, 34, 45] the mean value of successful retrievals was 52.5% (Table II [31, 34, 45]). The only slight difference between this mean and the previously reported value with standard TESE can be explained by heterogeneity of histological data in considered case reports.

TABLE II.—*Successful retrievals with multiple TESE.*

Authors	No. of TESE	Retrieval +	%
Ostad *et al.* (1998) [31]	81	47	58
Amer *et al.* (1999) [34]	100	49	49
Hauser *et al.* (1998) [45]	55	28	50.9
Total	236	124	52.5

TESE shows excellent repeatability: in patients with successful sperm retrieval upon a first TESE, a second TESE repeated 3 months later (for ICSI with fresh sperm) yielded successful recovery in 75% of cases with single sampling, and in 94.7% with multiple samplings.[34]

With regard to the correlation between testicular histology and biological sperm recovery with TESE in NOA, Tournaye *et al.*[47] reported successful retrieval in 100% of cases of hypospermatogenesis, 62% of cases of incomplete maturation arrest, 48% of cases of complete maturation arrest, 86% of cases of incomplete SCOS, and 19% of cases of complete SCOS. More recently, Seo *et al.*[7] reported successful recoveries in 89.2% of cases of severe hypospermatogenesis, 62.5% of cases of maturation arrest, and 16.3% of cases of SCOS. Out of 92 TESEs - 59 of which (56.1%) with successful recovery -, Piediferro *et al.*[43] reported successful recoveries in 100% of 22 cases of hypospermatogenesis > 5 spermatids/tubule (spd/tub), 75% of 8 cases of hypospermatogenesis with 4<spd/tub<5, 81.8% of 22 cases of hypospermatogenesis with <4 spd/tub, 50% of 12 maturation arrests, and 25% of 28 cases of histologically pure SCOS, underlining the probable importance - for the purpose of successful retrieval - of the search time employed by the biologist before issuing a negative report: prolonging this maximum time from 1 h to 4 h resulted

TABLE III.—*Successful retrievals withTESE versus mTESE.*

Authors	TESE +	(%)	mTESE +	(%)	Increase
Schlegel (1999) [48]	10/22	45	17/27	63	+40%
Amer *et al.* (2000) [49]	30/100	30	47/100	47	+56%
Colpi *et al.* (2001) [50]	9/22	40.9	14/22	63.6	+55%
Okubo *et al.* (2002) [51]	4/17	24	8/1	48	+100%
Okada *et al.* (2002) [52]	4/24	16.7	33/74	44.6	+167%
Tsujimura *et al.* (2002) [53]	13/37	35.1	24/56	42.9	+22%

in an increase in successful recoveries, from 2 out of 5 to 16 out of 17, respectively, in the more severe cases of hypospermatogenesis.

These data suggest that there are islands of spermatogenesis in testicular pulp even in worst-prognosis histological conditions, such as SCOS.

In an effort to increase the chances of finding islands of spermatogenesis in sampled tissue, Schlegel [48] devised microsurgical TESE (mTESE), *i.e.* testicular pulp microdissection. By using an operating microscope, an attempt is made to identify larger-calibre and darker tubules, as well as tubules in the areas that are closer to vessels, and therefore better supplied with blood, *i.e.* more likely sites of residual spermatogenesis. This technique, involving book-like opening of the testis, allows the surgeon to perform multiple microsamplings of these tubules on the wide exposed pulp area - therefore in different areas and at different depths, thus making a sort of "testicular mapping". Various authors reported increased success rates with mTESE *versus* TESE (Table III [48-53]): while some [48, 52, 53] compared the 2 techniques between 2 different NOA patient groups, others used TESE on one testis and mTESE on the contralateral one with comparable histology.[49] Okubo *et al.*[51] only performed mTESE when TESE had been unsuccessful, doubling the number of total successful retrievals. Conversely, Colpi *et al.*[50] compared the 2 methods by performing both of them on the same testis. Schlegel [48] underlined that, with this method, less testicular pulp is sacrificed (on average 9.4 mg *vs* 720 mg for standard TESE), thus reducing the (nevertheless low [54]) risks of later endocrine deprivation in patients such as those with NOA, who usually have small testes. Moreover, the risk of vascular damage is reduced,[55, 56] the postoperative period is substantially unaltered, and immediate and later complications are very low - even lower than with TESE:[49, 53, 56, 57] Dardashti *et al.*[58] reported no scrotal haematoma (requiring surgical drainage) and no testicular atrophy in 107 mTESEs *vs* 3.4% (3/119 haematomas and 1/119 atrophies) in TESEs ($P<0.05$). Out of 223 mTESE procedures performed, Colpi *et al.* (2005) found no complications (haematomas, testicular atrophy, orchialgia), and detected no notable parenchymal or vascularisation abnormalities upon ultrasound follow-up 3-6 months later (unpublished data).

Percutaneous testicular sperm aspiration (TESA) with a fine needle (TeFNA) offers largely lower chances of successful retrieval in NOA than TESE: from a review, successful retrieval rates range from 7.1% to 64.7%; the mean value of these percentages is 29.9%, but the median is 21.1% only.[32, 39, 59-64]

In case of severe tubular damage, retrieval with TeFNA is very low. Data from patients whose testicular histology is available show that successful retrievals with TeFNA are achieved almost exclusively in hypospermatogenesis:[32, 62-65] this explains the higher rates reported with TeFNA in some case studies [62-64] in NOA subjects mainly suffering from hypospermatogenesis. In addition, some authors [32, 64-67] actually pre-select patients for possible subsequent TeFNA or TESE for ICSI purposes by subjecting them to prior diagnostic needle aspiration, aimed at establishing whether there is sperm or not, and based on which they give a diagnosis of the type of testicular damage. This, like all diagnoses based on cytologic preparations only, is not totally free from possible errors,[68] since classification of spermatogenetic cells on smears from needle aspiration is reliable in the hands only of highly trained cytologists.[69]

TABLE IV —*Sperm occurrence in ejaculate following varicocelectomy in NOA patients.*

Authors	No. of azoospermic patients	Sperm occurrence in ejaculate post-varicocelectomy	%
Negri *et al.* (1998) [74]	6	2	33.3
Matthews *et al.* (1998) [75]	22	12	55
Kim *et al.* (1999) [76]	28	12	43
Kadioğlu *et al.* (2001) [77]	24	5	21
Pasqualotto *et al.* (2003) [78]	15	7	47
Kruse *et al.* (2003) [79]	1	1	
Cakan *et al.* (2004) [80]	13	3	23
Schlegel *et al.* (2004) [81]	31	7	22
Aponte *et al.* (2004) [82]	39	21	54
Total	179	70	39.1

In 2 papers in which NOA was controlled by histology, and TeFNA and TESE were performed on the same testis, TeFNA recovered sperm in 14% *vs* 63% [32] and in 11% *vs* 43%,[29] respectively. Moreover, Khadra *et al.*[64] retrieved sperm by TeFNA from 45 (53.6%) out of 84 patients suffering from presumed NOA (not controlled by histology!); TESE, however, yielded successful retrievals in 28 out of the 39 TeFNA-negative subjects (+71%).

In an animal model (rat), TeFNA involves severe, progressive and irreversible damage of tubules along the needle's path, and - especially if performed at several sites [70] - causes extensive tubular atrophy; on the contrary, TESE causes localised cicatricial fibrosis, with decreased tubular volume and increased interstitial tissue in the adjacent parenchyma,[71] but leaves the rest of the testis unharmed.[70]

Carcinoma *in situ* of testis can be found in 1-5% of males with very severe semen anomalies,[33] and particularly in those with NOA: therefore, since it is more difficult to detect them in cytological than in histological preparations, this is an additional reason to refer NOA cases to TESE surgery with simultaneous biopsy for histology, instead of using needle aspiration techniques only.[33, 72]

How to increase sperm retrieval rates in non-obstructive azoospermia

Aydos *et al.* reported that, in patients with normal FSH, retrieval rates improve following administration of pFSH:[73] actually, results appear to be statistically better in subjects with hypospermatogenesis and focal spermatogenesis only, who show satisfactory results, already. These data have not been confirmed by any other studies yet.

There are increasingly frequent reports of sperm occurrence in ejaculate in NOA patients with varicocele following surgical treatment of the latter: this made it possible, sometimes, to perform ICSI with sperm from ejaculate, in some cases, even to use less invasive assisted reproductive procedures than ICSI, and, anecdotically, to obtain some spontaneous pregnancies (Table IV).[74-82] In these patients, sperm occurred in ejaculate only if spermatids were found upon testicular biopsy:[77, 80] sperm occurrence does not correlate with age, hormone assays, testicular volume, and degree of varicocele,[80] and is not durable, so that, under such circumstances, any semen samples with sperm should be cryopreserved.[78] Sperm reoccurrence in ejaculate proves a varicocelectomy-induced improvement in spermatogenesis: therefore, in case of varicocele in NOA patients, this should be repaired, at least in order to increase the chances of subsequent successful surgical retrieval (testicular telomerase activity - a spermatid marker - is thought to have predictive value).[83]

Some changes in biological work were reported to be able to increase successful retrieval rates: removed tissue digestion with type IV collagenase in order to better release gametes from the epithelial lattice of seminiferous tubules;[84] use of a stereomicroscope

during biological handling of tubules;[85] or increase in the time employed for biological search of any sperms in the suspension obtained from testicular tissue fragmentation.[43]

Intracytoplasmatic sperm injection and non-obstructive azoospermia

Contrary to the early data reported in literature, ICSI performed with sperms retrieved from testes is currently acknowledged to yield poorer results in NOA patients than in OA patients.[86-88]

ICSI from TESE has lower birth rates in NOA than in OA (19% *vs* 28%).[89] Abortion rates are significantly higher following ICSI from NOA (11.5%) than from OA (2.5%) [90] (P=0.001).

The risk of genetic alterations is proportional to spermatogenic damage, and is higher in NOA than in severe OAT-syndrome.[88]

Therefore, at the beginning of their path towards ICSI - female factors being equal -, the prognosticated fertility of a couple with an NOA male is quite lower than for a couple with an OA male; as a matter of fact, the far lower chance of recovering sperm in NOA than in OA must preliminarily be added to the lower success rate of ICSI in NOA.

Conclusions

In order to - at least partially – reduce the handicap of an NOA couple *versus* an OA couple with respect to ICSI, it is necessary to:

1) Make a prior attempt to retrieve and, in case, cryopreserve sperm from testes in an NOA male, before subjecting his woman to ovarian stimulation and oocyte pick-up, especially in order to reduce the risk of having to use semen from a donor (which, by the way, is now forbidden by Italian law) because no homologous sperms were retrieved. For the time being, the results of ICSI with fresh sperm from TESE do not seem to show any significant differences from those obtained with cryopreserved sperm from TESE.[35, 89, 91, 92]

2) Have recourse to retrieval techniques offering the highest success rates (TESE or mTESE, instead of TeFNA).

3) Optimise biological work relating to search and cryopreservation of the sperm retrieved from testes, as well as thawing and checking their viability immediately before ICSI.

4) If an NOA patient also suffers from clinically relevant varicocele, he should undergo varicocele surgery some months before the attempt to retrieve testicular sperm - providing that his partner is not sufficiently old as to advise against any additional waiting period before ICSI, in order not to reduce the latter's success rates.

Riassunto

Il recupero di spermatozoi per iniezione intracitoplasmatica di spermatozoi nelle azoospermie non-ostruttive

Il recupero chirurgico di spermatozoi testicolari finalizzato all'iniezione intracitoplasmatica di sperma (*intra-cytoplasmic sperm injection*, ICSI) rappresenta l'unica possibilità di paternità biologica in caso di azoospermia non ostruttiva (*non-obstructive azoospermia*, NOA). La positività di un recupero correla solo con l'esame istologico, non con i valori di FSH o con il volume testicolare. Le microdelezioni contemporanee dell'AZFa e dell'AZFb predicono recupero negativo. La TESE (media dei recuperi positivi in letteratura: 52,7%) è la tecnica d'elezione: si sono avuti recuperi positivi nel 100% delle ipospermatogenesi con > 5 spermatidi/tubulo (spd/tub), nell'81,8% delle ipospermatogenesi con <4Spd/tub, nel 50% degli arresti maturativi e nel 25% delle sindromi a sole cellule di Sertoli pure all'istologia. Dalla letteratura si evince che la TESE microchirurgica (mTESE) sembra aumentare i recuperi positivi: dal 16,7-45% della TESE standard al 42,9-63,6% della mTESE, a seconda della distribuzione dell'istologia testicolare nelle varie casistiche studiate; rispettivamente da 9 a 14 casi su 22 nell'unico studio in cui TESE e mTESE sono state effettuate in contemporanea sullo stesso testicolo. Perfezionamenti delle procedure biologiche sui prelievi da TESE possono aumentare i recuperi positivi. La TeFNA non appare indicata nelle NOA, e per i suoi bassi tassi di successo, in pratica positivi solo nelle ipospermatogenesi, e perché incapace di detectare eventuali carcinoma *in situ*. La chirurgia preliminare di un coesistente varicocele sinistro in soggetti NOA potrebbe aumentare le probabilità di un successivo recupero. La ICSI da TESE ha nelle NOA tassi di gravidanza a termine più

bassi che nelle azoospermie ostruttive (*obstructive azoospermia*, OA) (19% *vs* 28%). Il tasso di aborto è significativamente più elevato a seguito di ICSI con spermatozoi da NOA (11,5%) che da OA (2,5%) (P=0,001). Pertanto la prognosi di fertilità di una coppia con maschio NOA risulta alquanto inferiore rispetto a una coppia con maschio OA.

Parole chiave: Azoospermia - Spermatozoi - Iniezione intracitoplasmatica di spermatozoi.

References

1. Jarow JP, Espleland MA, Lipshultz LI. Evaluation of the azoospermic patient. J Urol 1989;142:62-6.
2. Colpi GM, Negri L, Benaglia R, Nerva F, Piediferro G, Bulfamante G. Only a full diagnostic surgery allows a correct classification of azoospermic men entering a TESE-ICSI programme. Hum Reprod 2000;15 Suppl 1:245.
3. 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;12:2422-8.
4. Steinberger E, Tjioe DY. A method for quantitative analysis of human seminiferous epithelium. Fertil Steril 1968;19:959-61.
5. Skakkebaek NE, Heller CG. Quantification of human seminiferous epithelium. I. Histological studies in twenty-one fertile men with normal chromosome complements. J Reprod Fertil 1973;32:379-89.
6. Zheng J, Huang X, Li C. Predictive factors for successful sperm recovery in azoospermia patients. Zhonghua Wai Ke Za Zhi 2000;38:366-8.
7. Seo JT, Ko WJ. Predictive factors of successful testicular sperm recovery in non-obstructive azoospermia patients. Int J Androl 2001;24:306-10.
8. Hauser R, Botchan A, Yogev L, Gamzu R, Ben Yosef D, Lessing JB *et al*. Probability of sperm detection in nonobstructive azoospermic men undergoing testicular sperm extraction procedures unrelated to clinical parameters. Arch Androl 2002;48:301-5.
9. Piediferro G, Nerva F, Piacentini I, Piticchio G, Scroppo FI, Castiglioni F *et al*. Il ruolo del volume testicolare e del FSH plasmatico nel fenotipo delle azoospermie non-ostruttive. Giorn It Androl 2004;11:145.
10. Samli MM, Dogan I. An artificial neural network for predicting the presence of spermatozoa in the testes of men with nonobstructive azoospermia. J Urol 2004;171:2354-7.
11. Tsujimura A, Matsumiya K, Miyagawa Y, Takao T, Fujita K, Koga M *et al*. Prediction of successful outcome of microdissection testicular sperm extraction in men with idiopathic nonobstructive azoospermia. J Urol 2004;172:1944-7.
12. Vernaeve V, Brugnon F, Tournaye H. Inhibin B, predictive factor for testicular sperm recovery? Gynecol Obstet Fertil 2004;32:767-70.
13. Foresta C, Ferlin A, Rossi A, Salata E, Tessari A. Alteration of spermatogenesis and Y chromosome microdeletions. Analysis of the DAZ gene family. Minerva Endocrinol 2002;27:193-207.
14. Foresta C, Moro E, Ferlin A. Prognostic value of Y deletion analysis. The role of current methods. Hum Reprod 2001;16:1543-7.
15. Silber SJ, Alagappan R, Brown LG, Page DC. Y chromosome deletions in azoospermic and severely oligozoospermic men undergoing intracytoplasmic sperm injection after testicular sperm extraction. Hum Reprod 1998;13:3332-7.
16. Foresta C, Ferlin A, Moro E, Marin P, Rossi A, Scandellari C. Microdeletion of chromosome Y in male infertility: role of the DAZ gene. Ann Ital Med Int 2001;16:82-92.
17. Repping S, van Daalen SK, Korver CM, Brown LG, Marszalek JD, Gianotten J *et al*. A family of human Y chromosomes has dispersed throughout northern Eurasia despite a 1.8-Mb deletion in the azoospermia factor c region. Genomics 2004;83:1046-52.
18. Yen PH. Putative biological functions of the DAZ family. Int J Androl 2004;27:125-9.
19. Brandell RA, Mielnik A, Liotta D, Ye Z, Veeck LL, Palermo GD *et al*. AZFb deletions predict the absence of spermatozoa with testicular sperm extraction: preliminary report of a prognostic genetic test. Hum Reprod 1998;13:2812-5.
20. Foresta C, Ferlin A, Moro E. Deletion and expression analysis of AZFa genes on the human Y chromosome revealed a major role for DBY in male infertility. Hum Mol Genet 2000;9:1161-9.
21. Foresta C, Moro E, Rossi A, Rossato M, Garolla A, Ferlin A. Role of the AZFa candidate genes in male infertility. J Endocrinol Invest 2000;23:646-51.
22. Schlegel PN. The Y chromosome. Reprod Biomed Online 2002;5:22-5.
23. Ferlin A, Moro E, Rossi A, Dallapiccola B, Foresta C. The human Y chromosome's azoospermia factor b (AZFb) region: sequence, structure, and deletion analysis in infertile men. J Med Genet 2003;40:18-24.
24. Hopps CV, Mielnik A, Goldstein M, Palermo GD, Rosenwaks Z, Schlegel PN. Detection of sperm in men with Y cromosome microdeletions of the AZFa, AZFb and AZFc regions. Hum Reprod 2003;18:1660-5.
25. Song GJ, Lee H, Park Y, Lee HJ, Lee YS, Seo JT, Kang IS. Expression pattern of germ cell-specific genes in the testis of patients with nonobstructive azoospermia: usefulness a molecular marker to predict the presence of testicular sperm. Fertil Steril 2000;73:1104-8.
26. Kahraman S, Ozgur S, Alatas C, Aksoy S, Tasdemir M, Nuhoglu A *et al*. Fertility with testicular sperm extraction and intracytoplasmic sperm injection in non-obstructine azoospermic men. Hum Reprod 1996;11:765-60.
27. Mulhall JP, Burgess CM, Cunningham D, Carson R, Harris D, Oates RD. Presence of mature sperm in testicular parenchyma of men with nonobstructive azoospermia: prevalence and predictive factors. Urology 1997;49:91-5.
28. Schlegel PN, Palermo GD, Goldstein M, Menendez s, Zaninovic N, Veeck LL *et al*. Testicular sperm extraction with intracytoplasmic sperm injection for nonobstructive azoospermia. Urology 1997;49:435-40.
29. Friedler S, Raziel A, Strassburger D, Soffer Y, Komaro*v*sky 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;12:1488-93.
30. Jesek D, Knuth UA, Schulze W. Successful testicular sperm extraction (TESE) in spite of high serum follicle stimulating hormone and azoospermia: correlation between testicular morphology, TESE results, semen analysis and serum hormone values in 103 infertile men. Hum Reprod 1998;13:1230-4.
31. Ostad M, Liotta D, Ye Z, Schlegel PN. Testicular sperm extraction for nonobstructive azoospermia: results of a multibiopsy approach with optimized tissue dispersion. Urology 1998;52:692-6.
32. Ezeh UIO, Moore HDM, 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;13:3075-80.
33. Schulze W, Thoms F, Knuth UA. Testicular sperm extraction: comprehensive analysis with simultaneously performed histology in 1418 biopsies from 766 subfertile men. Hum Reprod 1999;14:82-96.
34. Amer M, Haggar SE, Moustafa T, Abd El-Naser, Zohdy W. Testicular sperm extraction: impact to testicular histology on outcome, number of biopsies to be performed and optional time for repetition. Hum Reprod 1999;14:3030-4.
35. Ben-Yosef D, Yogev L, Hauser R, Yavetz H, Azem F, Yovel I *et al.* Testicular sperm retrieval and cryopreservation prior to initiating ovarian stimulation as the first line approach in patients with non-obstructive azoospermia. Hum Reprod 1999;14:1794-801.
36. Su LM, Palermo GD, Goldstein M, Veeck LL, Rosenwaks Z, Schlegel PN. Testicular sperm extraction with intracytoplasmic sperm injection for non obstructive azoospermia: testicular histology can predict success of sperm retrieval. J Urol 1999;161:112-6.
37. Ng HY, Lau YL, Yeung SB, So WK, Tam PC, Ho PC. Testicular sperm extraction and intracytoplasmic sperm injection in non-obstructive azoospermia. Chin Med J (Engl) 2000;113:246-50.
38. Kitamura M, Nishimura K, Miura H, Komori K, Koga M, Fujioka H *et al.* Predictor factor for TESE-ICSI for non-obstructive azoospermia. Nippon Hinyokika Gakkai Zasshi 2000;91:589-94.
39. Friedler S, Raziel A, Schachter M, Strassburger D, Bern O, Ron-El R. Outcome of first and repeated testicular sperm extraction and ICSI in patients with non-obstructive azoospermia. Hum Reprod 2002;17:2356-61.
40. Damani MN, Master V, Meng MV, Burgess C, Turek P, Oates RD *et al.* Postchemoterapy ejaculatory azoospermia: fatherood with sperm from testis tissue with intracytoplasmic sperm injection. J Clin Oncol 2002;15:930-6.
41. Meseguer M, Garrido N, Remohi J, Pellicer A, Simon C, Martinez-Jabaloyas JM *et al.* Testicular sperm extraction (TESE) and ICSI in patients with permanent azoospermia after chemotherapy. Hum Reprod 2003;18:1281-5.
42. Negri L, Albani E, Di Rocco M, Morreale G, Novara P, Levi-Setti PE. Testicular sperm extraction in azoospermic men submitted to bilateral orchipexy. Hum Reprod 2003;18:2534-9.
43. Piediferro G, Giacchetta D, Nerva F, Mancini M, Sagone P, Contalbi GF *et al.* Accorgimenti biologici per incrementare i recuperi positivi di spermatozoi mediante TESE. Giorn It Androl 2004;1:157.
44. Kubota Y, Tamaki M, Maeda S, Katsumata Y, Moriwaky T, Tashiro K *et al.* Clinical result of testicular sperm extraction (TESE) to male-factor inferility in Toyota Memorial Hospital. Hinyokika Kiyo 2005;51:9-11.
45. Hauser R, Botchan A, Amit A, Ben Yosef D, Gamzu R, Paz G *et al.* Multiple testicular sampling in non-obstructive azoospermia: is it necessary? Hum Reprod 1998;13:3081-5.
46. Vernaeve V. Infertility treatment in non-obstructive azoospermia. Proefschrift voor het bekomen van de graad van Doctor in de Medische, Promotor H. Tournaye, Co-promotors P. Devroey & A. Van Steirteghem. Vrije Universiteit Brussel, Academiejaar 2003-2004; Brussel, Belgium, 2004.
47. Tournaye H, Liu J, Nagy PZ, Camus M, Goosens A, Silber S *et al.* Correlation between testicular histology and outcome after intracytoplasmic sperm injection using testicular spermatozoa. Hum Reprod 1996;11:127-32.
48. Schlegel PN. Testicular sperm extraction: microdissection improves sperm yield with minimal tissue excision. Hum Reprod 1999;14:131-5.
49. Amer M, Ateyah A, Hany R, Zohdy W. Prospective comparative study between microsurgical and conventional testicular sperm extraction in non-obstructive azoospermia: follow-up by serial ultrasound examinations. Hum Reprod 2000;15:653-6.
50. Colpi GM, Piediferro G, Nerva F, Scroppo FI. TESE microchirurgica nelle azoospermie non ostruttive. Giorn It Andr 2001;8:133.
51. Okubo K, Ogura K, Ichioka K, Terada N, Matsuta Y, Yoshimura K *et al.* Testicular sperm extraction for non-obstructive azoospermia: results with conventional and microsurgical techniques. Hinyokika Kiyo 2002;48:275-80.
52. Okada H, Dobashi M, Yamazaki T, Hara I, Fujisawa M, Arakawa S *et al.* Conventional *versus* microdissection testicular sperm extraction for non obstructive azoospermia. J Urol 2002;168:1063-7.
53. Tsujimura A, Matsumiya K, Miyagawa Y, Tohda A, Miura H, Nishimura K *et al.* Conventional multiple or microdissection testicular sperm extraction: a comparative study. Hum Reprod 2002;17:2924-9.
54. Shill T, Bals-Pratsch M, Kupker W, Sandmann J, Johannisson R, Diedrich K. Clinical and endocrine follow-up of patients after testicular sperm extraction. Fertil Steril 2003;79:281-6.
55. Schlegel PN, Su LM. Physiology consequences of testicular sperm extraction. Hum Reprod 1997;12:1688-92.
56. Silber SJ. Microsurgical TESE and distribution of spermatogenesis in non-obstructive azoospermia. Hum Reprod 2000;15:2278-84.
57. Wood S, Thomas K, Sephton V, Troup S, Kingsland CR, Lewis-Jones DI. Postoperative pain, complications and satisfaction rates in patients who undergo surgical sperm retrieval. Fertil Steril 2003;79:56-62.
58. Dardashti K, Williams RH, Goldstein M. Microsurgical testis biopsy: a novel technique for retrieval of testicular tissue. J Urol 2000;163:1206-7.
59. Tournaye H. Surgical sperm recovery for intracytoplasmic sperm injection: which method is to be preferred? Hum Reprod 1999;14 Suppl 1:71-81.
60. Westlander G, Hamberger L, Hanson C, Lundin K, Nilsson L, Soderlund B *et al.* Diagnostic epididymal and testicular sperm recovery and genetic aspects in azoospermic men. Hum Reprod 1999;14:118-22.
61. Lewin A, Reubinoff B, Porat-Katz A, Weiss D, Eisenberg V, Arbel R *et al.* Testicular fine needle aspiration: the alternative method for sperm retrieval in non-obstructive azoospermia. Hum Reprod 1999;14:1785-90.
62. Fasouliotis SJ, Safran A, Porat-Katz A, Simon A, Laufer N, Lewin A. A high predictive value of the first testicular fine needle aspiration in patients with non-obstructive azoospermia for sperm recovery at the subsequent attempt. Hum Reprod 2002;17:139-42.
63. Makhseed M, Al Salem MH, Ahmed MA. Percutaneous testicular sperm aspiration and intracytoplasmic sperm injection in obstructive and non-obstructive azoospermia: an easy alternative to TESE and MESA. Urol Int 2002;68:86-90.
64. Khadra AA, Abdulhadi I, Ghunain S, Kilani Z. Efficiency of percutaneous testicular sperm aspiration as a mode of sperm collection for intracytoplasmic sperm injection in nonobstructive azoospermia. J Urol 2003;169:603-5.
65. Aridogan IA, Bayazit Y, Yaman M, Ersoz C, Doran S. Comparison of fine-needle aspiration and open biopsy of testis in sperm retrieval and histopathologic diagnosis. Andrologia 2003;35:121-5.
66. Craft I, Tsirigotis M, Courtauld E, Farrer-Brown G. Testicular needle aspiration as an alternative to biopsy for the assessment of spermatogenesis. Hum Reprod 1997;12:1483-7.

67. Zukerman Z, Orvieto R, Avrech OM, Weiss DB, Gottschalk-Sabag S, Bar-On E *et al*. Is diagnostic testicular fine needle aspiration necessary in azoospermic men before sperm aspiration/extraction for intracytoplasmic sperm injection cycles? Assist Reprod Genet 2000;17:92-6.
68. Salihu HM, Aliyu MH. Sperm retrieval in infertile males: comparison between testicular sperm extraction and testicular sperm aspiration techniques. Wien Klin Wochenschr 2003;115:370-9.
69. Meng MV, Cha I, Ljung BM, Turek PJ. Relationship between classic histological pattern and sperm findings on fine needle aspiration map in infertile men. Hum Reprod 2000;15:1973-7.
70. Shufaro Y, Prus D, Laufer N, Simon A. Impact of repeated fine needle aspiration (TEFNA) and testicular sperm extraction (TESE) on the microscopic morphology of the testis: an animal model. Hum Reprod 2002;17:1795-9.
71. Tash JA, Schlegel PN. Histologic effects of testicular sperm extraction on the testicle in men with nonobstructive azoospermia. Urology 2001;57:334-7.
72. Piediferro G, Contalbi GF, Nerva F, Colpi GM. Carcinoma *in situ* in azoospermia non-ostruttiva sfuggito alla TeFNA e diagnosticato con TESE: case report. Giorn It Androl 2004;11:123.
73. Aydos K, Unlu C, Demirel LC, Evirgen O, Tolunay O. The effect of pure FSH administration in non-obstructive azoospermic men on testicular sperm retrieval. Eur J Obstet Gynecol Reprod Biol 2003;108:54-8.
74. Negri L, Colpi GM, Scroppo FI, Grugnetti C. Changes of therapeutic strategies for infertile males after varicocele surgery. Int J Androl 1998;21:25.
75. Matthews GJ, Matthews ED, Goldstein M. Induction of spermatogenesis and achievement of pregnancy after microsurgical varicocelectomy in men with azoospermia and severe oligoasthenospermia. Fertil Steril 1998;70:71-5.
76. Kim ED, Leibman BB, Grinblat DM, Lipshultz LI. Varicocele repair improves semen parameters in azoospermic men with spermatogenic failure. J Urol 1999;162:737-40.
77. Kadioglu A, Tefekli A, Cayan S, Kandirali E, Erdemir F, Tellaloglu S. Microsurgical inguinal varicocele repair in azoospermic men. Urology 2001;57:328-33.
78. Pasqualotto FF, Lucon AM, Hallak J, Goes PM, Saldanha LB, Arap S. Induction of spermatogenesis in azoospermic men after varicocele repair. Hum Reprod 2003;18:108-12
79. Kruse R, Schuppe HC, Malms J, Hanneken S, Ruzicka T, Neumann NJ. Anti-inflammatory and varicocele treatment in nonobstructive azoospermia. Andrologia 2003;35:217-9.
80. Cakan M, Altug U. Induction of spermatogenesis by inguinal varicocele repair in azoospermic men. Arch Androl 2004;50:145-50.
81. Schlegel PN, Kaufmann J. Role of varicocelectomy in men with nonobstructive azoospermia. Fertil Steril 2004;81:1585-8.
82. Aponte R, Carrino M, Fontana B, Masala D. Varicocele e dispermia grave: una giusta indicazione alla terapia chirurgica? In: E. Belgrano editor. Andrologia 2004. Roma: CIC edizioni internazionali; 2004. p. 143-6.
83. Giannakis D, Baltogiannis D, Tsoukanelis K, Loutradis D, Miyagawa I, Makrydimas G *et al*. Role of testicular tissue telomerase assay for the prediction of the presence of testicular spermatozoa in azoospermic men with varicoceles, pre- and post-varicocelectomy. Andrologia 2004;36:111-22.
84. Crabbe E, Verheyen G, Silber S, Tournaye H, Van de Velde H, Goossens A *et al*. Enzymatic digestion of testicular tissue may rescue the intracytoplasmic sperm injection cycle in some patients with non-obstructive azoospermia. Hum Reprod 1998;13:2791-6.
85. Kamal A, Fahmy I, Mansour RT, Abou-Setta AM, Serour GI, Aboulghar MA. Selection of individual testicular tubules from biopsied testicular tissue with a stereomicroscope improves sperm retrieval rate. J Androl 2004;25:123-7.
86. Monzo A, Kondylis F, Lynch D, Mayer J, Jones E, Nehchiri F *et al*. Outcome of intracytoplasmic sperm injection in azoospermic patients: stressing the liaison between the urologist and reproductive medicine specialist. Urology 2001;58:69-75.
87. Vernaeve V, Tournaye H, Osmanagaoglu K, Verheyen G, Van Steirteghem A, Devroey P. Intracytoplasmic sperm injection with testicular spermatozoa is less successful in men with nonobstructive azoospermia than in men with obstructive azoospermia. Fertil Steril 2003;79:529-33.
88. Silber S, Munné S. Chromosonal abnormalities in embryos derived from testicular sperm extraction (tese) in men with non-obstructive azoospermia. Proceedings EAA International Symposium "Genetics of Male Infertility: from Research to Clinic", 2003, October 2-4, Florence, Italy.
89. Schwarzer JU, Fiedler K, Hertwig I, Krusmann G, Wurfel W, Schleyer M *et al*. Sperm retrieval procedures and intracytoplasmatic spermatozoa injection with epididymal and testicular sperms. Urol Int 2003;70:119-23.
90. Borges E Jr, Rossi-Ferragut LM, Pasqualotto FF, dos Santos DR, Rocha CC, Iaconelli A Jr. Testicular sperm results in elevated miscarriage rates compared to epididymal sperm in azoospermic patients. Sao Paulo Med J 2002;l20:122-6.
91. Gil-Salom M, Romero J, Rubio C, Ruiz A, Remohi J, Pellicer A. Intracytoplasmic sperm injection with cryopreserved testicular spermatozoa. Mol Cell Endocrinol 2000;169:15-9.
92. Sousa M, Cremades N, Silva J, Oliveira C, Ferraz L, Teixeira da Silva J *et al*. Predictive value of testicular histology in secretory azoospermic subgroups and clinical outcome after microinjection of fresh and frozen-thawed sperm and spermatids. Hum Reprod 2002;17:1800-10.