Correlation between testicular histology and outcome after intracytoplasmic sperm injection using testicular spermatozoa

H.Tournaye[1,4] J.Liu[1], P.Z.Nagy[1], M.Camus[1], A.Goossens[2] S.Silber[3], A.C.Van Steirteghem[1] and P.Devroey[1]

[1]Centre for Reproductive Medicine, [2]Department of Human Pathology, University Hospital, Dutch-speaking Brussels Free University (Vrije Universiteit Brussel), Laarbeeklaan 101, B-1090 Brussels, Belgium, [3]St. Luke's Hospital, 224 South Woods Mill Road, St Louis, MO 63017, USA

[4]To whom correspondence should be addressed

A comprehensive study is presented of a series of 124 infertile men undergoing testicular sperm retrieval for intracytoplasmic sperm injection (ICSI). In this study we correlated the histological changes observed in the testicular tissue with the results of the wet preparation and the outcome after ICSI using testicular spermatozoa. In all patients with normal spermatogenesis and hypospermatogenesis spermatozoa were recovered from the wet preparation. The sperm recovery rate was 84% in patients with incomplete germ-cell aplasia and maturation arrest, while in patients with complete germ-cell aplasia or maturation arrest this figure was 76%. In these patients more specimens were sampled and fewer spermatozoa were recovered. Since no spermatozoa were recovered in only 10 patients, ICSI with testicular sperm was performed in the remaining 114 couples (91.9%). The normal fertilization rate was 57.8%. The fertilization rate was significantly lower in couples among whom the husband showed germ-cell aplasia and maturation arrest. Overall, 55.2% of normally fertilized oocytes developed into embryos showing ≤50% of anucleate fragments. There were no major differences between the different histological categories in terms of embryonic development in vitro. The overall pregnancy rates per testicular sperm extraction (TESE) procedure, per ICSI procedure and per transfer were respectively 36.3, 39.5 and 43.7%. The overall implantation rate per embryo (sacs/embryos replaced) was 20.3%. A lower implantation rate was observed in couples among whom the husband had maturation arrest (not statistically significant). The above data show that testicular biopsies may have an important therapeutic role in the management of infertility in azoospermic patients.

Key words: azoospermia/intracytoplasmic sperm injection/maturation arrest/Sertoli-cell-only syndrome/testicular spermatozoa

Introduction

Since the introduction of intracytoplasmic sperm injection (ICSI) (Palermo et al., 1992; Van Steirteghem et al., 1993a),

the number of patients with male-factor infertility requesting artificial insemination with donor sperm (AID) has decreased substantially. However, as assisted reproductive technologies for treating male-factor infertility shifted from conventional in-vitro insemination to micro-assisted insemination, the origin of the spermatozoa used has shifted too. Initially ejaculated spermatozoa were used, followed by epididymal spermatozoa (Temple-Smith et al., 1985) and finally testicular spermatozoa (Schoysman et al., 1993a). The introduction of ICSI using epididymal spermatozoa (Tournaye et al., 1994) and testicular spermatozoa (Craft et al., 1993) has led to a marked improvement in the fertility prognosis for many azoospermic patients. One category of patients, however, remains in a rather hopeless situation. These patients suffer from azoospermia because of primary testicular failure, a condition assumed to have an extremely poor prognosis. From a histological viewpoint, testicular failure can be classified according to different categories: germ-cell aplasia (or Sertoli-cell-only syndrome), maturation arrest, germ-cell hypoplasia (or hypospermatogenesis) and tubular sclerosis (Levin, 1979). In the case of germ cell aplasia and maturation arrest, focal spermatogenesis may still be present histologically (Levin, 1979). Retrieval of testicular spermatozoa, i.e. 'testicular sperm extraction' (TESE), from individuals with primary testicular failure followed by ICSI, is a recent advance in the treatment of male-factor infertility (Devroey et al., 1995). In the present study we investigated whether the histological changes observed in the testicular tissue correlated with the outcome after ICSI using testicular spermatozoa.

Materials and methods

From March 1994 till March 1995, 124 patients had a testicular biopsy to recover spermatozoa for ICSI. Thirty-eight of these patients had a history involving presence of a few spermatozoa once in their ejaculate at a previous semen analysis. However, at the time of oocyte retrieval no spermatozoa could be observed in at least two semen samples and testicular biopsy was performed for sperm recovery. This condition is referred to as virtual azoospermia. In 86 patients no spermatozoa were observed at all in the semen; these patients are referred to as absolute azoospermic patients. All patients had a karyotype assessment and in patients with congenital bilateral absence of the vas deferens (CBAVD) screening of the most frequent CFTR-gene mutations was performed in both partners as described previously (Tournaye et al., 1994; Silber et al., 1995b)

Testicular sperm extraction was performed under general or local anaesthesia. General anaesthesia was invariably used when the patient presented with clinical features of primary testicular failure, i.e. bilateral small testicular volumes (< 15 ml) or increased serum follicle stimulating hormone (FSH) (>9 IU/l).

After unilateral hemiscrototomy a small testicular incision was made and a specimen was sampled using a pair of curved scissors. This specimen was then transferred into a Petri dish (Falcon Plastics, Becton-Dickinson, Aalst, Belgium), filled with ~1 ml modified HEPES-buffered Earle's medium supplemented with 0.4% human serum albumin (HSA) (Belgian Red Cross, Brussels, Belgium). Peroperative microscopical examination of a wet preparation of the shredded specimens was performed at ×400 according to Jow *et al.* (1993). If spermatozoa were observed, no further testicular incision was made. If microscopical assessment did not show any sperm cells, a testicular incision at another site was made. In some instances repeated sampling was needed in order to observe spermatozoa and biopsies were taken from the contralateral testis whenever necessary. One single testicular specimen per testis was sent for histology. This specimen was routinely fixed in Bouin's solution and stained with haematoxylin and eosin for visualization of histological details. Classification of the histological findings was according to Levin (1979).

Sperm analysis was performed according to the World Health Organization (WHO) guidelines taking dilution by Earle's medium into account (WHO, 1993). For ICSI, the spermatozoa were recovered directly from the pellet after centrifuging the supernatant of the shredded tissue from the Petri dish at 300 *g* for 5 min (Nagy *et al.*, 1995a). Occasionally the sperm suspension was layered on a two-layer Percoll (Pharmacia, Upsalla, Sweden) gradient (95 and 47.5%) when the suspension contained too many other cells.

All female partners were superovulated using a gonadotrophin-releasing hormone analogue (GnRHa) suppression protocol using Buserelin (Suprefact nasal spray: Hoechst, Frankfurt, Germany) with human menopausal gonadotrophins (HMG) (Humegon: Organon, Oss, The Netherlands; Pergonal: Serono, Brussels, Belgium). Oocyte–cumulus complexes (OCC) were recovered 36 h after administration of 10 000 IU of human chorionic gonadotrophin (HCG). After the surrounding cumulus and corona cells had been removed, the nuclear maturation of the oocytes was assessed under an inverted microscope. Metaphase II oocytes were injected with one single spermatozoon into the ooplasm. These procedures have been described elsewhere (Van Steirteghem *et al.*, 1993; Liu *et al.*, 1994). Further culture of injected oocytes was performed in 25 µl micro drops of B2 medium (bioMérieux, Montalieu Vercieu, France) under lightweight paraffin oil. Fertilization was confirmed after 16–18 h if two distinct pronuclei were observed under an inverted microscope. Cleavage was assessed 24 h later and embryos were classified according to their morphological appearance: excellent embryos had no anucleate fragments, good embryos had <20% of fragments, fair embryos showed between 20 and 50% of fragments and poor embryos had >50% of fragments. All embryos with less than 50% of their volume filled with anucleate fragments were considered for transfer. Cleaving embryos were replaced into the uterine cavity ~48 h after the ICSI procedure. In one couple in which the husband presented with a 47,XXY karyotype, a preimplantation diagnosis by fluorescent in-situ hybridization (FISH) was performed in order to assess the karyotype of the embryo before transfer (Staessen *et al.*, 1995).

A rise in serum HCG on two consecutive occasions from 11 days after transfer indicated pregnancy. Clinical pregnancy was defined by the presence of a gestational sac at ultrasonography at approximately 7 weeks of pregnancy. Patients were advised to participate in a follow-up study of the children born after micro-injection. Supernumerary embryos were cryopreserved whenever possible according to freezing protocols reported earlier (Van Steirteghem *et al.*, 1994). Statistical analysis was performed on the percentage values of the variables within each cycle in order to take the independence of observations within each cycle into account. Global significance was tested by the Kruskal–Wallis test and paired comparisons were performed by means of the Mann–Whitney *U*-test if significant differences occurred. Pregnancy rates were compared by means of the χ^2 test. All tests were performed at the 5% level of significance.

Results

Patients undergoing testicular biopsies for sperm retrieval for ICSI ranged from 21 to 69 in age (39.1 ± 7.6, average ± SD). Their partners ranged from 24 to 45 (33.9 ± 4.9) in age. The duration of infertility was 7.2 ± 5.0 on average (± SD), ranging from 1 to 23 years. Table I gives an overview of the histological findings of the 124 biopsies and the findings after wet preparation. Two patients showed extensive tubular and peritubular sclerosis. One of these patients showed focal normal spermatogenesis, the other one showed focal maturation arrest. The latter patient was the only one which presented with a karyotype anomaly, i.e. a 47,XXY karyotype. In two patients, one presenting with incomplete germ-cell aplasia and one with normal spermatogenesis, carcinoma-in-situ (CIS) cells were found at histology. Both patients had a history of crypt-orchidism. In 10 patients no spermatozoa could be recovered for ICSI: in eight, no spermatozoa were observed either after wet preparation peroperatively or after centrifugation and/or Percoll preparation, while in two patients no spermatozoa were found for ICSI although one and two spermatozoa respectively were observed after peroperative wet preparation. Table II shows the mean numbers of biopsies taken per patient and the mean numbers of spermatozoa retrieved from the biopsies according to the major histological categories. Motile spermatozoa were found in 73 testicular preparations (58.8%) and this mainly in biopsies with normal histology or with germ-cell hypoplasia. In these two categories less samples were taken (*P* < 0.0001 by the Kruskal–Wallis test) and more spermatozoa were recovered (*P* < 0.004 by the Kruskal–Wallis test).

In 114 couples ICSI was performed using testicular sperma-tozoa (91.9%). A total of 1397 metaphase-II oocytes were microinjected of which 158 were damaged (11.3%). There were no significant differences between the different histological categories in terms of numbers of oocytes injected or damaged. Of the 1239 successfully injected oocytes, 716 showed two pronuclei after 18 h (57.8%). Forty-nine oocytes showed three pronuclei (3.9%) and 45 showed only one pronucleus (3.6%). Table III shows these data for the different major histological categories. The normal fertilization rate was lower in patients with germ-cell aplasia and maturation arrest (*P* < 0.002). The normal cleavage rate was 55.2%: 395 cleaving embryos showed ≤50% of anucleate fragments. Table IV shows the data on embryonic development, embryo transfers and implantation. There were no major differences between the different histo-logical categories in terms of embryonic development *in vitro*. After replacing a total of 290 embryos in 103 embryo transfers 45 pregnancies were obtained. For 34 patients 177 super-numerary embryos were cryopreserved.

The overall pregnancy rates per TESE procedure, per ICSI procedure and per transfer were respectively 36.3, 39.5 and 43.7%. There were no significant differences between the groups studied in terms of pregnancy rates. The overall

Table 1. Histological findings and findings after wet preparation in 124 patients undergoing testicular biopsy

	Germ-cell aplasia		Maturation arrest		Germ-cell hypoplasia	Normal
	Complete	Incomplete	Complete	Incomplete		
Presence of spermatozoa at histology	–	+	–	+	+	+
	6	19	11	7	11	70
History of spermatozoa in previous ejaculate(s)						
+	1	11[a]	6	4	3	13[a]
–	5	8	5[b]	3	8	57[b]
Presence of spermatozoa in wet preparation						
+	4[c,d]	18[c]	9[d]	4	11	70[b]
–	2	1	2[b]	3	0	0

[a]Including one patient with carcinoma-in-situ cells.
[b]Including one patient with extensive tubular sclerosis.
[c]Including one patient with sperm found after peroperative wet preparation not found at the time of intracytoplasmic sperm injection (ICSI).
[d]Including one patient with no sperm found after peroperative wet preparation but with sperm found at the time of ICSI.

Table II. Mean number of testicular biopsies taken per patient and mean numbers of spermatozoa retrieved from the biopsy according to the major histological categories

	Germ-cell aplasia	Maturation arrest	Germ-cell hypoplasia	Normal
No. of TESE procedures	25	18	11	70
No. of biopsies taken				
Average ± SD[a]	2.8 ± 2.5[b]	4.2 ± 4.5[c]	1.5 ± 0.8	1.2 ± 0.5[d]
Range (min–max)	1–12	1–20	1–3	1–4
No. of spermatozoa recovered ($\times 10^6$)				
Average ± SD[e]	0.02 ± 0.04	0.08 ± 0.1	0.84 ± 1.52	0.49 ± 0.85
Range (min–max)	0–0.14	0–0.40	0–4.68	0–5.23
No. of biopsies with motile sperm[f]	8 (32.0)	6 (33.3)	6 (54.5)	53 (75.7)

[a]$P < 0.0001$ by Kruskal–Wallis test.
[b]Significantly different from [d]($P < 0.0001$ by the Mann–Whitney U-test).
[c]Significantly different from [d]($P < 0.0001$ by the Mann–Whitney U-test).
[e]$P < 0.004$ by Kruskal–Wallis test.
[f]Percentages within parentheses.

Table III. Results of intracytoplasmic sperm injection (ICSI) according to histological findings at testicular biopsy[a]

	Germ-cell aplasia	Maturation arrest	Germ-cell hypoplasia	Normal
No. of TESE procedures	25	18	11	70
No. of ICSI procedures	20 (80%)	13 (72%)	11 (100%)	70 (100%)
No. of mature oocytes injected (A)	266	163	156	812
Average ± SD	13.3 ± 6.9	12.5 ± 7.0	14.2 ± 7.5	11.6 ± 7.2
No. of oocytes damaged	48 (18%)	10 (6.2%)	10 (6.4%)	90 (11.1%)
No. of oocytes successfully injected (B)[b]	218 (82%)	153 (93.8%)	146 (93.6%)	722 (88.9%)
No. of oocytes showing 2-PN[c,d]	96 (44.0%)[e]	70 (45.7%)[f]	99 (67.8%)[g]	451 (62.5%)[h]
Average ± SD	4.8 ± 3.3	5.4± 4.9	9.0 ± 5.2	10.3 ± 4.8
No. of oocytes showing 3-PN[c]	15 (6.9%)	1 (0.7%)	4 (2.7%)	29 (4.0%)
No. of oocytes showing 1-PN[c]	11 (5.0%)	6 (3.9%)	4 (2.7%)	24 (3.3%)

[a]ICSI cycles: $n = 114$.
[b]Percentage of A within parentheses.
[c]Percentage of B within parentheses.
[d]$P < 0.002$ by Kruskal–Wallis test.
[e]Significantly different from [g]($P < 0.03$ by the Mann–Whitney U-test).
[e]Significantly different from [h]($P < 0.002$ by the Mann–Whitney U-test).
[f]Significantly different from [g]($P < 0.02$ by the Mann–Whitney U-test).
[f]Significantly different from [h]($P < 0.002$ by the Mann–Whitney U-test).
TESE = testicular sperm extraction; PN = pronuclear.

implantation rate per embryo (sacs/embryos replaced) was 20.3%. However, a lower implantation rate was observed in couples with maturation arrest (significance not reached). In the group with germ-cell aplasia, one patient delivered a healthy set of triplets and five pregnancies, including one twin pregnancy, are still ongoing. Two biochemical pregnancies

Table IV. Embryonic development and embryo transfers after intracytoplasmic sperm injection (ICSI) with testicular spermatozoa according to histological findings at testicular biopsy[a]

	Germ-cell aplasia	Maturation arrest	Germ-cell hypoplasia	Normal
No. of ICSI procedures	20	13	11	70
No. of oocytes showing 2-PN (A)	96	70	99	451
No. of cleaving embryos (B)[b]	76 (79.2%)	43 (61.4%)	82 (82.8%)	363 (80.5%)[a]
No. of excellent embryos[c]	1 (1.3%)	9 (20.9%)	11 (13.4%)	32 (8.8%)
No. of good embryos[c]	42 (55.2%)	28 (65.1%)	47 (57.3%)	208 (57.3%)
No. of fair embryos[c]	15 (19.7%)	5 (11.6%)	15 (18.3%)	78 (21.5%)
No. of transfers (C)	19	12	10	62
No. of embryos (D)	56	32	31	171
Av ± SD	2.5 ± 1.5	2.7± 1.4	3.1 ± 1.1	2.8 ± 1.0
No. of pregnancies[d]	6 (31.6%)	6 (50.0%)	6 (60.0%)	27 (43.5%)
No. of implantations[e]	11 (19.6%)	4 (12.5%)	7 (22.5%)	37 (21.6%)

[a]Differences not significant.
[b]Percentages of A within parentheses.
[c]Percentages of B within parentheses.
[d]Percentages of C within parentheses.
[e]Percentages of D within parentheses.
PN = pronuclear.

were observed in the group with maturation arrest. In one of these couples the husband was a 47,XXY Klinefelter. Four singleton pregnancies are still ongoing. In the couples with germ-cell hypoplasia all six pregnancies are still ongoing, including one twin pregnancy. In the group showing normal testicular histology, one biochemical pregnancy and four miscarriages were observed. One patient had a vanishing twin with an ongoing singleton pregnancy. Seven patients have delivered: four singletons and three twins. The remaining 14 pregnancies, including three twin and two triplet pregnancies, are still ongoing.

Discussion

Since the introduction of ICSI, testicular spermatozoa can be successfully used to alleviate infertility because of obstructive (Schoysman et al., 1993a,b; Devroey et al., 1994; Silber et al., 1995 a,b) and non-obstructive azoospermia (Devroey et al., 1995; Tournaye et al., 1995). In this study we have reviewed a series of patients in whom testicular sperm retrieval for ICSI was performed and have examined the outcome in these patients according to the histological findings of the testicular biopsy.

No spermatozoa were observed in only eight out of 124 cases. Patients in whom spermatozoa were once observed in one of their previous ejaculates did not always show spermatozoa in the wet preparation of their testicular biopsy.

When patients showed normal spermatogenesis or germ-cell hypoplasia in their biopsy, spermatozoa were invariably recovered from the wet preparation. In contrast, in the study by Jow et al. (1993), spermatozoa were not found after wet preparation of testicular specimens of four out of 11 (36%) patients with germ-cell hypoplasia, of seven out of nine patients with maturation arrest (77%) and in all nine patients with germ-cell aplasia (100%). However, in the present series in about three out of four patients showing maturation arrest or germ-cell aplasia, spermatozoa were recovered. Nevertheless, the mean number of biopsies taken shows that in cases with aplasia or maturation arrest more biopsies had to be taken in an attempt to recover spermatozoa. Some patients from these

histological categories needed only one biopsy in order to retrieve spermatozoa while in others even after 20 biopsies no spermatozoa were found. In 13 patients spermatozoa were observed in the wet preparation but not at the histology. This lack of correlation between the histological findings and the findings in the wet preparation can be explained by the fact that only one sample per testis was sent for histology while for wet preparation samples were taken until spermatozoa were found or until the whole testicular surface had been randomly sampled. This finding stresses the shortcomings of a unique testicular specimen for making a correct histological diagnosis. The above findings also indicate that active spermatogenesis is focal or limited to a small number of seminiferous tubules. It therefore seems preferable that the testicular biopsy should be taken by an open excisional technique under general anaesthesia unless a preliminary biopsy has revealed a normal spermatogenesis or germ-cell hypoplasia. In these patients spermatozoa might also be recovered through fine-needle aspiration technique (FNA) (Foresta et al., 1992; Mallidis and Baker, 1994). Whether FNA will allow testicular sperm recovery in patients with incomplete germ-cell aplasia or incomplete maturation arrest also remains to be proven, in view of the difficulties reported of getting a conclusive cytological diagnosis (Gottschalk-Sabag et al., 1995). The open, excisional technique as used in this series allows larger samples to be taken from different sites while maintaining full control for bleeding from the testicular tissue, thus maximizing the chance of spotting an active region but minimizing the risk of testicular haematoma.

In two patients, both with a history of cryptorchidism, CIS cells were observed in the testicular histology. For the infertile male population the presence of CIS cells is reported in 1.1% of cases with most such patients having a history of cryptorchidism (Skakkebaek, 1978). Patients with this finding should have an assessment for tumour markers, i.e. alphafetoprotein and beta-HCG and a scrotal ultrasound. They should also have a testicular biopsy of the contralateral testis if this has not yet been done. Since ~50% of these CIS lesions will develop into testicular carcinoma (Skakkebaek et al.,

1978), unilateral orchidectomy has to be performed rather than radiotherapy when the presence of an invasive tumour is diagnosed or suspected.

In all patients the numbers of spermatozoa recovered were limited. Patients with germ-cell aplasia and maturation arrest showed a lower total number of spermatozoa retrieved, while in these patients generally a higher number of biopsies had to be taken. In two patients spermatozoa were observed in the wet preparation but no longer after further preparation for ICSI. In two other patients the opposite was true: no spermatozoa were found in the wet preparation but spermatozoa were found after preparation for ICSI.

As reported previously (Nagy et al., 1995a), in this series too the overall fertilization rate after ICSI with testicular spermatozoa was lower than after ICSI with ejaculated spermatozoa. The fertilization rates using spermatozoa from patients with normal histological findings or germ-cell hypoplasia were significantly higher than in patients with maturation arrest or germ-cell aplasia. This may be due to the fact that in the latter categories fewer motile spermatozoa were available for injection. Injecting a motile spermatozoon is one of the important prerequisites for achieving normal fertilization with ICSI (Nagy et al., 1995b). Since both germ-cell aplasia and maturation arrest are assumed to have a genetic origin (Vogt et al., 1992; Martin-du Pan and Campana, 1993; Reijo et al., 1995), genetic factors might also explain the decrease in fertilization rates in these cases.

Once fertilization was achieved, embryonic development was comparable across the different categories. The overall implantation rate of 20.3% is comparable to the implantation rates as observed after ICSI using ejaculated and epididymal spermatozoa (Nagy et al., 1995a). The lowest implantation rate was observed in couples in which the husband showed maturation arrest at his testicular histology. One might again speculate, as mentioned above, that the genetic origin of this condition causes a reduction in implantation rate.

From the above results, it may be concluded that in azoospermic patients presenting with normal testicular histology or germ-cell hypoplasia, spermatozoa may be invariably recovered. However, in patients with germ-cell aplasia and maturation arrest, spermatozoa may not always be found. In only one out of four of such cases in this retrospective series could spermatozoa not be recovered. Patients should be counselled about this risk and may consider the use of donor spermatozoa in case no spermatozoa can be retrieved. The above data also mean that testicular biopsies have an important diagnostic and therapeutic role in all azoospermic patients, including those clinically referred to as suffering from primary testicular failure. The case of the 47,XXY Klinefelter patient is unique in this respect. Although the husband had extreme hypogonadism (testicular volume <5 ml) and an elevated serum concentration of FSH (24.8 IU/l), we were able to recover a few spermatozoa from his testicular biopsy in order to inject four mature oocytes. After replacing one cleaving embryo with a 46,XX karyotype according to FISH, a biochemical pregnancy did occur (highest HCG level 210 IU/l). To our knowledge, this is the first report of a pregnancy through artificial reproductive techniques with spermatozoa from a 47,XXY (non-mosaic) Klinefelter patient.

Most current textbooks recommend testicular biopsy only in patients with azoospermia or severe oligozoospermia showing normal levels of FSH and normal testicular size. However, elevated FSH levels do not preclude a normal spermatogenesis and, therefore a correct diagnosis of non-obstructive azoospermia can only be made by testicular biopsy (Hauser et al., 1995; Tournaye et al., 1995). At the present time, however, the reasons for performing testicular biopsy are not only diagnostic. By means of ICSI, testicular spermatozoa can be successfully used to alleviate azoospermic infertility irrespective of the changes in testicular histology associated with the azoospermia. Nevertheless, the results from this study show that in some patients, especially those with maturation arrest, results may be less good. These patients need more biopsies to be taken and have the lowest sperm recovery rate.

Larger series are needed to confirm the results from this retrospective study. Of the 54 patients with abnormal histological findings, 25 had virtual azoospermia, i.e they had a history of the presence of at least one sperm cell in their ejaculate. This obviously means they had focally active spermatogenesis in at least one of their testes. If this series were limited only to patients without such a history, i.e. absolute azoospermic patients, the sperm recovery failure rate would probably be much higher. Currently we are conducting a prospective study in order to identify factors predicting sperm recovery failure in such patients. The role of a preliminary biopsy before a combined ICSI–TESE cycle is probably limited in this respect. A preliminary testicular biopsy may be predictive only if normal spermatogenesis or germ-cell hypoplasia is observed. However, if complete or incomplete maturation arrest or germ-cell aplasia is encountered, no definitive conclusion can be drawn. It is even possible that no spermatozoa may be found in the wet preparation for ICSI when the preliminary histology has showed incomplete maturation arrest or incomplete germ-cell aplasia. In cases where maturation arrest or germ-cell aplasia were observed without focal spermatogenesis, there may still be a chance of recovering testicular spermatozoa by sampling more testicular tissue during the ICSI–TESE cycle. At present, we therefore suggest that TESE should be offered to every azoospermic infertile patient in order to retrieve spermatozoa for ICSI only after extensive counselling with a view to the possible use of donor sperm in case sperm recovery should fail. Since extreme oligozoospermia or azoospermia may have a genetic origin (Vogt et al., 1992; Jarvi et al., 1995; Reijo et al., 1995) the use of testicular spermatozoa for ICSI may raise some concern (Seamark and Robinson, 1995). Counselling about the risk of transmitting a genetic trait responsible for infertility is therefore imperative in those couples willing to undergo ICSI with testicular spermatozoa.

References

Craft, I., Bennett, V. and Nicholson, N. (1993) Fertilising ability of testicular spermatozoa. (letter) *Lancet*, **342**, 864.

Devroey, P., Liu, J., Nagy, Z., Tournaye, H., Silber, S.J. and Van Steirteghem, A.C. (1994) Normal fertilization of human oocytes after testicular sperm extraction and intracytoplasmic sperm injection. *Fertil. Steril.*, **62**, 639–641.

Devroey, P., Liu, J., Nagy, P., Goossens, A., Tournaye, H., Camus, M., Van Steirteghem, A. and Silber, S. (1995) Pregnancies after testicular sperm extraction (TESE) and intracytoplasmic sperm injection (ICSI) in non-obstructive azoospermia. *Hum. Reprod.*, **10**, 1457–1460.

Foresta, C., Varotto, A. and Scandellari, C. (1992) Assessment of testicular cytology by fine needle aspiration as a diagnostic parameter in the evaluation of the azoospermic subject. *Fertil. Steril.*, **57**, 858–865.

Gottschalk-Sabag, S., Weiss, D.B., Folb-Zacharow, N. and Zukerman, Z. (1995) Is one testicular specimen sufficient for quantitative evaluation of spermatogenesis? *Fertil. Steril.*, **64**, 399–402.

Hauser, R., Temple-Smith, P. D., Southwick, G. J. and de Kretser, D. (1995) Fertility in cases of hypergonadotropic azoospermia. *Fertil. Steril.*, **63**, 631–636.

Jarvi, K., Zielenski, J., Wilschanski, M., Durie, P., Buckspan, M., Tullis, E. *et al.* (1995) Cystic fibrosis transmembrane conductance regulator and obstructive azoospermia. *Lancet*, **354**, 1578.

Jow, W.W., Steckel, J., Schlegel, P., Magid, M.S. and Goldstein, M. (1993) Motile sperm in human testis biopsy specimens. *J. Androl.*, **14**, 194–198.

Levin, H.S. (1979) Testicular biopsy in the study of male infertility. Its current usefulness, histologic techniques, and prospects for the future. *Hum. Pathol.*, **10**, 569–584.

Liu, J., Nagy, Z., Joris, H., Tournaye, H., Devroey, P. and Van Steirteghem, A.C. (1994) Intracytoplasmic sperm injection does not require special treatment of the spermatozoa. *Hum. Reprod.*, **9**, 1127–1130.

Mallidis, C. and Baker, G.H.W. (1994) Fine needle tissue aspiration of the testis. *Fertil. Steril.*, **61**, 367–375.

Martin-du-Pan, R.C. and Campana, A. (1993) Physiopathology of spermatogenic arrest. *Fertil. Steril.*, **60**, 937–946.

Nagy, Z., Liu, J., Janssenswillen, C., Silber, S., Devroey, P. and Van Steirteghem, A. (1995a) Comparison of fertilization, embryo development and pregnancy rates after intracytoplasmic sperm injection using ejaculated, fresh and frozen-thawed epididymal and testicular spermatozoa. *Fertil. Steril.*, **63**, 808–815.

Nagy, Z., Liu, J., Joris, H., Bocken, G., Tournaye, H., Devroey, P. and Van Steirteghem, A. (1995b) The result of intracytoplasmic sperm injection is not related to any of the three basic sperm parameters. *Hum. Reprod.*, **10**, 1123–1129.

Reijo, R., Lee,T., Salo, P., Alagappan, R., Brown, L.G., Rosenberg, M. *et al.* (1995) Diverse spermatogenic defects in humans caused by Y chromosome deletions encompassing a novel RNA-binding protein gene. *Nature Genet.*, **10**, 383–393.

Schoysman R., Van der Zwalmen, P., Nijs, M., Segal-Bertin, C., Geerts, L. and Van de Casseye, M. (1993a) Successful fertilization by testicular spermatozoa in an in-vitro fertilization programme (letter). *Hum. Reprod.*, **8**, 1339–1340.

Schoysman R., Van der Zwalmen, P., Nijs, M., Segal, L., Segal-Bertin, C., Geerts, L., Van Roosendaal, E. and Schoysman, D. (1993b) Pregnancy after fertilization with human testicular sperm. (letter) *Lancet*, **342**, 1237.

Seamark, R.F. and Robinson, J.S. (1995) Potential health hazards of assisted human reproduction. *Hum. Reprod.*, **10**, 1321.

Silber, S.J., Van Steirteghem, A.C., Liu, J., Nagy, Z., Tournaye, H. and Devroey, P. (1995a) High fertilization and pregnancy rate after intracytoplasmic sperm injection with spermatozoa obtained from testicle biopsy. *Hum. Reprod.*, **10**, 148–153.

Silber, S.J., Nagy, Z., Liu, J., Tournaye, H., Lissens, W., Ferec, C., Liebaers, I., Devroey, P. and Van Steirteghem, A.C. (1995b) The use of epididymal and testicular spermatozoa for intracytoplasmic sperm injection: the genetic implications for male infertility. *Hum. Reprod.*, **10**, 2031–2043.

Skakkebaek, N.E. (1978) Carcinoma *in situ* of the testis: frequency and relationship to invasive germ cell tumors in infertile men. *Histopathology*, **2**, 157–170.

Staessen, C., Coonen, E., Van Assche, E., Liu, J., Bonduelle, M., Tournaye, H., Devroey, P., Van Steirteghem A. and Liebaers, I. (1995) Preimplantation diagnosis of an embryo from a 47,XXY Klinefelter patient: case report.

Eleventh Annual meeting of the European Society for Human Reproduction and Embryology, Hamburg. *Hum. Reprod.*, **10** (Suppl. 2), Abstract 133.

Temple-Smith, P.D., Southwick, G.J., Yates, C.A., Trounson, A.D. and de Kretser, D.M. (1985) Human pregnancy by *in vitro* fertilization (IVF) using sperm aspirated from the epididymis. *J. In Vitro Fert. Embryo Transf.*, **2**, 119–122.

Tournaye, H., Devroey, P., Liu, J., Nagy, Z., Lissens, W. and Van Steirteghem, A. (1994) Microsurgical epididymal sperm aspiration and intracytoplasmic sperm injection: a new effective approach to infertility as a result of congenital bilateral absence of the vas deference. *Fertil. Steril.*, **61**, 1045–1051.

Tournaye, H., Camus, M., Goossens, A., Nagy, Z., Silber, S., Van Steirteghem, A.C. and Devroey, P. (1995) Recent concepts in the management of infertility because of non-obstructive azoospermia. *Hum. Reprod.*, **10**, (Suppl. 1) 115–119.

Van Steirteghem, A.C., Liu, J., Joris, H., Nagy, Z. Janssenswillen, C., Tournaye, H., *et al.* (1993) Higher success rate by intracytoplasmic sperm injection than subzonal insemination. Report of a second series of 300 consecutive treatment cycles. *Hum. Reprod.*, **8**, 1055–1060.

Van Steirteghem, A.C., Van der Elst, J., Van den Abbeel, E., Joris, H., Camus, M. and Devroey, P. (1994) Cryopreservation of supernumerary multicellular human embryos obtained after intracytoplasmic sperm injection. *Fertil. Steril.*, **62**, 775–780.

Vogt, P., Chandley, A.C., Hargreave, T.B., Keil, R., Ma, K. and Sharkey, A. (1992) Microdeletions in interval 6 of the Y chromosome of males with idiopathic sterility point to disruption of AZF, a human spermatogenesis gene. *Hum. Genet.*, **89**, 491–496.

World Health Organization (1992) *Laboratory Manual for the Examination of Human Semen and Semen–Cervical Mucus Interaction*, 3rd edn. Cambridge University Press, Cambridge.