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Sperm retrieval and live birth rates in presumed Sertoli-cell-only syndrome in testis biopsy: a single centre experience

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SUMMARY

We aimed to investigate sperm retrieval rates (SRR) by testicular sperm extraction (TESE), factors affecting SRR, and fertilization rate (FR), implantation rate (IR), clinical pregnancy rate (CPR) and live birth rate (LBR) in patients with presumed Sertoli-cell-only syndrome in testis biopsy (SCOS). We retrospectively evaluated files of 134 patients with SCOS who underwent TESE. Group I were patients in whom spermatozoa were retrieved and Group II were patients in whom no spermatozoa could be retrieved. SRR, Follicle stimulating hormone (FSH), Luteinizing hormone (LH), and testosterone levels, and the volume of testicles were compared between groups. In addition, FR, IR, CPR and LBR were determined. Sperm retrieval was achieved in 37 (27.6%) patients (Group I), and the remaining 97 (72.4%) patients made Group II. There were no significant differences in age, infertility time, testicular volume, serum FSH, LH and testosterone levels between Groups I and II ($p > 0.05$). Intracytoplasmic sperm injection (ICSI) was performed in 36 patients. FR, IR, and CPR were 60.86 ± 23.03, 36.53 ± 41.78 and 51.3% respectively. Cycle and patient based LBRs were 37.8 and 45.1% respectively. SRR in SCOS is lower than patients with non-obstructive azoospermia (NOA) in general. No parameters to predict spermatozoa retrieval were determined. In patients with SCOS, ICSI achieves similar live birth rate to other patients with NOA.

INTRODUCTION

Non-obstructive azoospermia (NOA) refers to absence of spermatozoa in semen analysis caused by minimal or no production of fully developed spermatozoa in the testicles. Roughly 1% of all men and 10% of infertile men are influenced by testicular failure as a consequence of NOA (Su *et al.*, 1999). Testicular sperm extraction (TESE) along with intracytoplasmic sperm injection (ICSI) is a first-line treatment for infertility caused by NOA (Devroey *et al.*, 1995). The pregnancy and implantation rates are lower than those reported by using spermatozoa from patients with obstructive azoospermia (Kahraman *et al.*, 1996a; Aboulghar *et al.*, 1997; Ghazzawi *et al.*, 1998). Hypospermatogenesis, maturation arrest and Sertoli-cell-only syndrome (SCOS), with or without focal spermatogenesis are the commonest histological patterns of patients with NOA.

The SCOS is characterized by azoospermia and complete lack of germ cells in testicular biopsies which exhibit only Sertoli cells (Del Castillo *et al.*, 1947). SCOS is reported in 10.8–44% of patients with NOA (Sasagawa *et al.*, 2001; Mitchell *et al.*, 2011). The sperm retrieval rates (SRR) in patients with SCOS are generally reported to be lower than NOA caused by other factors (Donoso *et al.*, 2007). To our knowledge no study has specifically examined the results of ICSI in SCOS patients.

We aimed to investigate the results of ICSI in SCOS patients. We retrospectively screened testicular biopsy results of patients who underwent TESE because of NOA and identified the patients with SCOS. We evaluated factors that may affect sperm retrieval rate in SCOS. Fertilization rate (FR), implantation rate (IR), clinical pregnancy rate (CPR) and live birth rate (LBR) were also assessed in those couples whom spermatozoa were obtained.

MATERIALS AND METHODS

We retrospectively reviewed the files of 567 patients with NOA who underwent TESE between September 2003 and July 2011, and identified patients in whom SCOS was reported at pathological examination. We excluded 24 patients with karyotype abnormalities and Y chromosome microdeletion. The results of remaining 543 patients were evaluated. The presence of azoospermia was confirmed by at least two semen analyses. Serum

FSH, LH and testosterone levels were measured in all patients. Testicular volume was measured in all patients by using a Prader orchidometer. Informed consent was obtained from all patients before the operation.

TESE technique

In patients with testicular volume larger than 5 cc conventional TESE was performed. In patients with testicular volume smaller than 5 cc microdissection TESE was performed. In patients who underwent conventional TESE, microdissection TESE was also performed if the conventional technique failed to retrieve spermatozoa. Conventional and microdissection TESE procedures were performed under spinal anaesthesia. A longitudinal incision was made to obtain a larger operation field, especially in those with testicular volume smaller than 5 cc. For conventional TESE, the tunica albuginea was incised for approximately 5 mm at the upper pole near the head of the epididymis. If no spermatozoa were seen in the initial sample, subsequent samples were taken from the middle part and the lower pole of the testis opposite the rete testis. When there was not a sufficient number of spermatozoa in the tubules obtained from these fields, the procedure was continued (maximum seven biopsies) until sufficient spermatozoa were collected. The procedure was terminated when sufficient spermatozoa were retrieved. When no spermatozoa could be detected in three poles through the conventional technique, microdissection TESE was performed by enlarging the middle incision vertically (combined technique). For microdissection TESE, the subtunical vessels were identified under the surgical microscope and avoided. Direct examination of the testicular parenchyma was performed at $\times 20$ to $\times 40$ magnification with an operating microscope. Small samples were excised from large, opaque seminiferous tubules. The procedure was terminated when a sufficient volume of spermatozoa had been retrieved for intracytoplasmic sperm injection. When there were no spermatozoa in one testis, these procedures were performed on the contralateral testis. At the same session, a surgically obtained small tissue specimen was placed in Bouin's solution and sent to the histopathology laboratory.

To investigate factors that may affect sperm retrieval in patients with SCOS, patients were divided into two groups based on the presence of spermatozoa in TESE. Group I were patients in whom spermatozoa were retrieved and Group II were patients in whom no spermatozoa could be retrieved. Age, infertility period, serum FSH, LH and testosterone levels, and testicular volume were compared between groups. To evaluate TESE and ICSI results in SCOS patients, SRR, and in those whom ICSI was performed FR, IR, CPR and LBR were determined.

In group I, spermatozoa were either used for ICSI and/or cryopreservation. In 33 patients spermatozoa were cryopreserved and in four patients (one owing to positive hepatitis B antigen, three owing to patient preference) same day ICSI was performed using fresh spermatozoa.

Statistical analysis

Statistical analysis was performed using the statistical package SPSS v 17.0. For each continuous variable, normality was checked by Kolmogorov Smirnov and Shapiro–Wilk tests and by histograms. Comparison between groups was made with Mann Whitney-U test for data not normally distributed. Values of $p < 0.05$ were considered statistically significant.

RESULTS

Spermatozoa were retrieved in 252 of 543 (46.4%) patients with NOA in whom TESE was performed. In 32 patients no pathology reports were available. In 511 patients with histopathology reports, 134 (26.2%) patients had presumed SCOS. These 134 patients formed the study cohort. Sperm retrieval was achieved in 37 (27.6%) patients. These 37 patients formed Group I, and the remaining 97 (72.4%) patients in whom sperm retrieval failed made Group II.

There were no significant differences in patient age, mean infertility period, mean values of FSH, LH, testosterone levels and testis volume between Group I and Group II (Table 1).

In the whole group, 50 patients who had testicular volume smaller than 5 mL underwent microdissection TESE and spermatozoa were retrieved in 10 patients (20%). Conventional TESE was performed on 84 patients. Spermatozoa were retrieved in 14 patients (16.7%). In 70 of the 84 patients in whom no spermatozoa could be detected through the conventional technique, further microdissection TESE (combined technique) was performed. Sperm retrieval was achieved in 13 of these 70 patients (18.6%) with combined technique.

In Group I, 10 patients (27%) underwent upfront microdissection because of small testicular volume. Of the remaining 27, TESE was started by conventional technique and spermatozoa were retrieved in 14 patients (37.8%). In the other 13 (35.1%), no spermatozoa were retrieved by conventional technique, so microdissection was performed to retrieve spermatozoa (Table 2). Nineteen patients had unilateral and 18 had bilateral TESE. In this group, 2 patients had second TESE after unsuccessful ICSI and no spermatozoa could be retrieved at this time.

In Group II, 40 had microdissection and 57 had combined TESE. Six had unilateral (solitary testis) and 91 had bilateral TESE.

At the time of this report ICSI was still not performed in 3 of 37 patients in Group I, therefore they were excluded from the FR and LBR analyses. In 34 patients ICSI was performed at 40 cycles. The mean age of the spouses at the time of ICSI was 31 ± 5.3 years. In 3 (8.1%) patients no fertilization occurred and second cycle was not performed. In 6 of remaining 31 patients, ICSI had to be performed for second time (37 cycles) using frozen spermatozoa. A total of 37 cycles were executed in 31 patients. Nineteen clinical (51.3%) and three biochemical (8.1%) pregnancies were achieved (Fig. 1). FR, IR and CPR were calculated as 60.86 ± 23.03, 36.53 ± 41.78 and 51.3% respectively. Cycle and patient based LBRs were 37.8 and 45.1% respectively. Number of oocytes and number of transferred embryos are given in Table 3.

Table 1 Comparison of clinical and laboratory data for patients in groups I and II

	Group I	Group II	*p*
Age (years)	34.5 ± 5.6	33.8 ± 6.3	0.501
Infertility time (years)	8.3 ± 5.6	7.3 ± 5.6	0.285
FSH (mIU/mL)	18.9 ± 9.0	19.8 ± 8.0	0.643
LH (mIU/mL)	8.0 ± 4.6	8.8 ± 4.8	0.359
Testosterone (ng/mL)	4.4 ± 1.6	4.2 ± 1.6	0.846
Testis volume (mL)	11.2 ± 6.2	10.4 ± 6.2	0.459

FSH, follicle-stimulating hormone; LH, luteinizing hormone. Values are given as mean ± SD.

Table 2 TESE technique in groups I and II

	Conventional (n)	Combined (n)	Microdissection (n)	Total
Group I	14	13	10	37
Group II	0	57	40	97
Total	14	70	50	134

Figure 1 Flow chart of ICSI for 37 patients in group I.

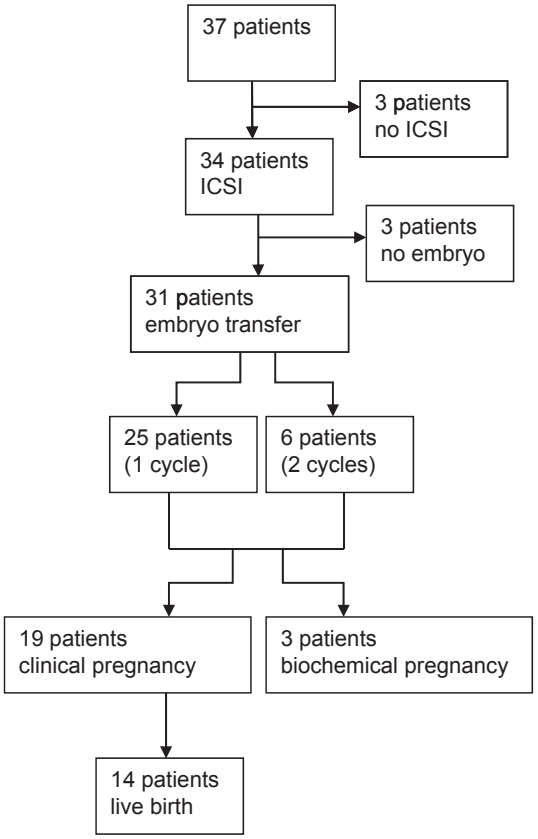

Table 3 Fertilization, implantation, pregnancy and birth rates in patients with SCOS

Fertilization rate	60.86 ± 23.03
Implantation rate	36.53 ± 41.78
Cancelled	8.1 (3/37)
Number of oocyte in OPU	16.26 ± 7.93
MII oocyte number	13.81 ± 6.65
Number of transferred embryos	2.6 ± 0.83
Clinical pregnancy rate	51.3 (19/37)
Biochemical pregnancy rate	8.1 (3/37)
Live birth rate (cycles)	37.8 (14/37)
Live birth rate (patients)	45.1 (14/31)

OPU, oocyte pick-up. Numbers are given as percentages (%).

In six patients two cycles were performed (Fig. 1). In the first cycle, pregnancy did not occur after embryo transfer in four patients and the other two patients had spontaneous abortion. In the second cycle, fertilization and embryo transfer was successful in all six patients. Three of these patients had no pregnancy, two patients had biochemical and one patient had clinical pregnancies. Only one live birth was achieved.

None of the patients showed any acute or chronic complications after TESE. We were not able to perform post-operative serum testosterone level measurements in most of the patients because of low patient compliance therefore post-operative testicular failure could not be evaluated.

DISCUSSION

There are many studies investigating SRR and results of ICSI in patients with NOA (Kahraman *et al.*, 1996b; Meseguer *et al.*, 2003). Parameters affecting spermatozoa retrieval and success of surgical technique have also been examined (Turunc *et al.*, 2010; Ghalayini *et al.*, 2011). However, no studies specifically investigate these factors in SCOS patients. We tried to establish SRR, predictive factors for sperm retrieval, FR, IR, PR and LBR for patients with presumed SCOS in testis biopsy. To our knowledge, this is the first such study in patients with SCOS.

SRR in TESE operations ranges from 41.6 to 89.6% (Devroey *et al.*, 1995; Vernaeve *et al.*, 2006). Our overall SRR in NOA patients was 45.5%. In various reports SCOS rates range from 10.8 to 44% in patients with NOA. We found that SCOS was the pathologic diagnosis in 29.5% of the patients who underwent TESE for NOA in our centre. SRR in patients with SCOS ranges from 16.3 to 38.7% in various reports (Seo & Ko, 2001; Vernaeve *et al.*, 2006). Our SRR rate in SCOS was 27.6%.

Clinically, testicular volume is correlated with spermatogenesis and SRR (Colpi *et al.*, 2009; Turunc *et al.*, 2010; Ghalayini *et al.*, 2011). On the other hand, some investigators report that differences in testicular volumes in patients with NOA were not associated with SRR (Hibi *et al.*, 2005). Turunc *et al.* determined positive correlation between testicular volume and SRR in patients with NOA (Turunc *et al.*, 2010). In their study, SRR was significantly low in patients with testicular volume less than 5 mL. They pointed out that SRR with TESE would be low in patients with NOA and low testicular volume. We did not determine any relation between testicular volume and SRR in patients with SCOS.

TESE can be performed by conventional, microdissection, and combined techniques. Success rates vary, but microdissection technique has the highest SRR (Okada *et al.*, 2002; Mulhall *et al.*, 2005; Ramasamy *et al.*, 2005; Turunc *et al.*, 2010). In a retrospective study, SRR were 16.7 and 44.6% in conventional and microdissection groups respectively. Subgroup analysis revealed that only SCOS had a significantly higher SRR with microdissection procedure, but not men with maturation arrest (Okada *et al.*, 2002).

In a study by Ramasamy *et al.*, SRR were 32 and 57% with conventional and microdissection TESE respectively (Ramasamy *et al.*, 2005). In SCOS patients, SRR were 6.2 and 26.9% with conventional and microdissection TESE (Ghalayini *et al.*, 2011). Mulhall *et al.* found no difference between two procedures. However, microdissection TESE was more successful than conventional procedure in testicular atrophy (testicular volume less than 10 mL) (Mulhall *et al.*, 2005). Direct microdissection was performed in 50 patients with testicular atrophy (volume less than 5 mL). We observed that microdissection TESE complementary to conventional method in patients with testicular volume larger than 5 mL raises the success rate of SRR. Our results indicate that combining microdissection when conventional TESE fails causes successful sperm retrieval in 18.6% of the patients (13 of 70 patients).

No lower limit of testicular volume for the absence of spermatozoa has been identified. Spermatozoa are often retrieved from testes with volumes less than 5 mL by microdissection TESE. Thus, small testicular volume itself does not preclude successful microdissection TESE (Tsujimura, 2007). Considering the potential complications (haematoma, fibrosis and androgen decline) of conventional TESE in testicular atrophy, we only performed microdissection in patients with testes < 5 mL.

Although some studies found a negative relation (Ghalayini *et al.*, 2011) between elevated serum FSH level and SRR, the others showed no significant association (Turunc *et al.*, 2010). In a study SRR was lower in patients with serum FSH level less than 15 IU/mL compared with patients with higher values (Ramasamy *et al.*, 2009). We found no relation between FSH and SRR in patients with SCOS.

Fertilization and pregnancy live birth rates were studied in patients with NOA. In NOA, FR, IR,CPR, LBR has been reported as 38.6–68% (Kahraman *et al.*, 1996b; Meseguer *et al.*, 2003; Raman & Schlegel, 2003; Vernaeve *et al.*, 2004; Haimov-Kochman *et al.*, 2010; Fadini *et al.*, 2011), 11.3% (Fadini *et al.*, 2011), 21–46% (Raman & Schlegel, 2003; Bromage *et al.*, 2007) and 20–43% (Raman & Schlegel, 2003; Bromage *et al.*, 2007) respectively. There has been no report in patients with SCOS. Our fertilization and pregnancy rates in patients with SCOS are similar with the previous reports of patients with NOA. This implies that in patients with SCOS, fertilization, pregnancy and birth rates are similar to other NOA patients, once successful sperm retrieval is achieved.

CONCLUSIONS

Sperm retrieval rate in SCOS is lower than in patients with NOA in general. In patients with presumed SCOS in testis biopsy, age, infertility time, serum FSH, LH, testosterone levels and testicular volume were not useful to predict spermatozoa retrieval. On the other hand, ICSI achieved similar live birth rate in patients with SCOS, as other patients with NOA. In patients with small testes and those who already have the diagnosis of SCOS, microdissection TESE increases the sperm retrieval rates.

REFERENCES

Aboulghar MA, Mansour RT, Serour GI, Fahmy I, Kamal A, Tawab NA & Amin YM. (1997) Fertilization and pregnancy rates after intracytoplasmic sperm injection using ejaculate semen and surgically retrieved sperm. *Fertil Steril* 68, 108–111.

Bromage SJ, Falconer DA, Lieberman BA, Sangar V & Payne SR. (2007) Sperm retrieval rates in subgroups of primary azoospermic males. *Eur Urol* 51, 534–539.

Colpi GM, Colpi EM, Piediferro G, Giacchetta D, Gazzano G, Castiglioni FM, Magli MC & Gianaroli L. (2009) Microsurgical TESE versus conventional TESE for ICSI in nonobstructive azoospermia: a randomized controlled study. *Reprod Biomed Online* 18, 315–319.

Del Castillo EB, Trabucco A & De la Balze FA. (1947) Syndrome produced by absence of the germinal epithelium without impairment of the sertoli or leydig cells. *J Clin Endocrinol Metab* 7, 493–502.

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

Donoso P, Tournaye H & Devroey P. (2007) Which is the best sperm retrieval technique for non-obstructive azoospermia? A systematic review. *Hum Reprod Update* 13, 539–549.

Fadini R, Colpi E, Mignini Renzini M, Coticchio G, Comi R, Mastrolilli M, Guarnieri T, Villa A & Dal Canto M. (2011) Outcome of cycles of oocyte in vitro maturation requiring testicular sperm extraction for nonobstructive azoospermia. *Fertil Steril* 96, 321–323.

Ghalayini IF, Al-Ghazo MA, Hani OB, Al-Azab R, Bani-Hani I, Zayed F & Haddad Y. (2011) Clinical comparison of conventional testicular sperm extraction and microdissection techniques for non-obstructive azoospermia. *J Clin Med Res* 3, 124–131.

Ghazzawi IM, Sarraf MG, Taher MR & Khalifa FA. (1998) Comparison of the fertilizing capability of spermatozoa from ejaculates, epididymal aspirates and testicular biopsies using intracytoplasmic sperm injection. *Hum Reprod* 13, 348–352.

Haimov-Kochman R, Prus D, Farchat M, Bdolah Y & Hurwitz A. (2010) Reproductive outcome of men with azoospermia due to cryptorchidism using assisted techniques. *Int J Androl* 33, e139–e143.

Hibi H, Ohori T, Yamada Y, Honda N & Asada Y. (2005) Probability of sperm recovery in non-obstructive azoospermic patients presenting with testes volume less than 10 ml/FSH level exceeding 20 mIU/ml. *Arch Androl* 51, 225–231.

Kahraman S, Ozgür S, Alatas C, Aksoy S, Balaban B, Evrenkaya T *et al.* (1996a) High implantation and pregnancy rates with testicular sperm extraction and intracytoplasmic sperm injection in obstructive and non-obstructive azoospermia. *Hum Reprod* 11, 673–676.

Kahraman S, Ozgür S, Alataş C, Aksoy S, Taşdemir M, Nuhoğlu A *et al.* (1996b) Fertility with testicular sperm extraction and intracytoplasmic sperm injection in non-obstructive azoospermic men. *Hum Reprod* 11, 756–760.

Meseguer M, Garrido N, Remohí J, Pellicer A, Simón C, Martínez-Jabaloyas JM & Gil-Salom M. (2003) Testicular sperm extraction (TESE) and ICSI in patients with permanent azoospermia after chemotherapy. *Hum Reprod* 18, 1281–1285.

Mitchell V, Robin G, Boitrelle F, Massart P, Marchetti C, Marcelli F & Rigot JM. (2011) Correlation between testicular sperm extraction outcomes and clinical, endocrine and testicular histology parameters in 120 azoospermic men with normal serum FSH levels. *Int J Androl* 34, 299–305.

Mulhall JP, Ghaly SW, Aviv N & Ahmed A. (2005) The utility of optical loupe magnification for testis sperm extraction in men with nonobstructive azoospermia. *J Androl* 26, 178–181.

Okada H, Dobashi M, Yamazaki T, Hara I, Fujisawa M, Arakawa S & Kamidono S. (2002) Conventional versus microdissection testicular sperm extraction for nonobstructive azoospermia. *J Urol* 168, 1063–1067.

Raman JD & Schlegel PN. (2003) Testicular sperm extraction with intracytoplasmic sperm injection is successful for the treatment of nonobstructive azoospermia associated with cryptorchidism. *J Urol* 170, 1287–1290.

Ramasamy R, Yagan N & Schlegel PN. (2005) Structural and functional changes to the testis after conventional versus microdissection testicular sperm extraction. *Urology* 65, 1190–1194.

Ramasamy R, Lin K, Veeck Gosden L, Rosenwaks Z, Palermo GD & Schlegel PN. (2009) High serum FSH levels in men with nonobstructive azoospermia does not affect success of microdissection testicular sperm extraction. *Fertil Steril* 92, 590–593.

Sasagawa I, Yazawa H, Suzuki Y, Tateno T, Ichiyanagi O, Kobayashi T, Matsuki S & Nakada T. (2001) Reevaluation of testicular biopsies of males with nonobstructive azoospermia in assisted reproductive technology. *Arch Androl* 46, 79–83.

Seo JT & Ko WJ. (2001) Predictive factors of successful testicular sperm recovery in non-obstructive azoospermia patients. *Int J Androl* 24, 306–310.

Su LM, Palermo GD, Goldstein M, Veeck LL, Rosenwaks Z & Schlegel PN. (1999) Testicular sperm extraction with intracytoplasmic sperm injection for non-obstructive azoospermia: testicular hystology can predict success of sperm retrieval. *J Urol* 161, 112–116.

Tsujimura A. (2007) Microdissection testicular sperm extraction: prediction, outcome, and complications. *Int J Urol* 14, 883–889.

Turunc T, Gul U, Haydardedeoglu B, Bal N, Kuzgunbay B, Peskircioglu L & Ozkardes H. (2010) Conventional testicular sperm extraction combined with the microdissection technique in nonobstructive azoospermic patients: a prospective comparative study. *Fertil Steril* 94, 2157–2160.

Vernaeve V, Krikilion A, Verheyen G, Van Steirteghem A, Devroey P & Tournaye H. (2004) Outcome of testicular sperm recovery and ICSI in patients with non-obstructive azoospermia with a history of orchidopexy. *Hum Reprod* 19, 2307–2312.

Vernaeve V, Verheyen G, Goossens A, Van Steirteghem A, Devroey P & Tournaye H. (2006) How successful is repeat testicular sperm extraction in patients with azoospermia? *Hum Reprod* 21, 1551–1554.