Introduction

Klinefelter syndrome is the most common numerical chromosomal abnormality found in infertile men. It is encountered in 11% of azoospermic men and one in 500 male newborn infants (Forest et al., 1999; Schiff et al., 2005). The genetic abnormality results from meiotic non-disjunction resulting in a 47,XXY genotype in the vast majority of the cases (Harari et al., 1995). However, 3–10% of affected cases are mosaic with a 46,XY/47,XXY genotype (Griffin and Wilson, 1987; Harari et al., 1995).

In cases with a 46,XY/47,XXY mosaic karyotype, spermatozoa can usually be recovered from the ejaculate, and fertilization has ensued after intracytoplasmic sperm injection (ICSI) (Harari et al., 1995; Bielanska et al., 2000). Patients with non-mosaic Klinefelter syndrome are generally azoospermic because of primary testicular failure, although in some cases focal spermatogenesis is present and testicular spermatozoa have been recovered and successfully used for ICSI (Palermo et al., 1998; Reubinoff et al., 1998).

There are limited data in the literature on testicular sperm extraction (TESE) and ICSI in patients with Klinefelter syndrome. The aim of this study was to evaluate TESE and ICSI performance in 33 men with Klinefelter syndrome and compare it with a control group of 113 patients with non-obstructive azoospermia and normal karyotype.
Materials and methods

A single practitioner’s (MG) experience with attempted treatment of men with non-obstructive azoospermia associated with Klinefelter syndrome over a 3-year period was retrospectively analysed. At least 20 cells were cytogenetically analysed for each peripheral karyotype analysis. The study and control groups were based on consecutive patients with azoospermia seeking fertility treatment at the study centre. Thirty-three patients had non-mosaic 47,XXY genotype and underwent 39 TESE cycles. A total of 113 consecutive patients with non-obstructive azoospermia and normal peripheral karyotype served as the control group and underwent 130 TESE cycles. No patients in either the Klinefelter or control groups had undergone a previous TESE attempt elsewhere.

Pre-operative evaluation included a complete history and physical examination. In both groups, pre-operative hormonal treatment was not planned, since it has not been shown to improve successful surgical retrieval of spermatozoa (Schiff et al., 2005). Similarly, a diagnostic testicle biopsy was not performed, since it does not accurately predict successful surgical retrieval of spermatozoa at TESE (Schiff et al., 2005).

TESE was performed under local anaesthesia by widely opening the testis in an equatorial plane. Microdissection was carried out with examination of the seminiferous tubules using an operating microscope (Carl Zeiss, OPMI Pico Surgical Microscope) at ×20 magnification. Enlarged seminiferous tubules were selected, removed and evaluated by an embryologist. Each sample was mechanically cut and dispersed in 1 ml of G-IVF (VitroLife, Kungsbacka, Sweden) supplemented with 10% human serum albumin (VitroLife, Kungsbacka, Sweden) in a Petri dish (Falcon Plastics, Becton-Dickinson). Each specimen was evaluated under phase-contrast microscope at ×200 magnification. If intact spermatozoa were noted, the procedure was terminated. If no spermatozoa were seen, microdissection of additional areas of testicular parenchyma was carried out and additional samples were taken. Great care was exercised to avoid injury to the testicular vasculature and removal of testicular interstitial tissue that contains Leydig cells. After dissection, the tunica albuginea was closed with 5–0 polypropylene. As a routine policy, all TESE procedures were performed 1 day prior to oocyte retrieval.

The biopsy specimen was collected in a sterile conic tube (Falcon Plastics, Becton-Dickinson). Following washing with the gradient method (Isolate sperm separation medium; Irvine Scientific, Santa Ana, California, USA), the prepared sample was incubated at 37°C with 7% CO₂ in air. In the morning of the scheduled oocyte retrieval day, the sample was transferred into a Petri dish (Falcon Plastics, Becton-Dickinson) and covered with oil (VitroLife, Kungsbacka, Sweden) for identification and collection of spermatozoa.

Pituitary desensitization with gonadotrophin-releasing hormone agonists and ovarian stimulation with gonadotrophins was performed for the female partner as previously described (Bukulmez et al., 2000). The criterion for human chorionic gonadotrophin (HCG; Profasi, Serono, Istanbul, Turkey) administration was the presence of three or more follicles exceeding 17 mm in diameter. Oocyte retrieval was carried out under general anaesthesia using vaginal ultrasound-guided puncture of follicles 36 h after HCG administration.

The most morphologically normal motile spermatozoa were selected for ICSI. Where all spermatozoa had morphological defects, spermatozoa with fully developed tails and grossly normal heads were injected. Vitality testing of immotile spermatozoa was not performed. The presence of fertilization was evaluated by examining oocytes 12–17 h after injection for the presence of distinct two pronuclei and two polar bodies.

As a routine policy, no further TESE attempt was recommended for those couples with unsuccessful surgical retrieval of spermatozoa with TESE. However, a repeat TESE attempt was highly recommended for those couples with successful retrieval of spermatozoa, but who had failed to conceive. Routinely, when motile spermatozoa were identified at the first examination in the laboratory, pieces of testicular tissue were cryopreserved; however, in this study only cycles using fresh tissue were included.

Embryos were graded on day 3 according to a 1–4 scoring system (with 1 being the best), which was based on fragmentation, cell symmetry and blastomere number (Hardarson et al., 2001). The embryos with even blastomeres and no fragmentation were graded as grade 1, the embryos with even blastomeres and <20% fragmentation as grade 2a, the embryos with uneven blastomeres and no fragmentation as grade 2b, the embryos with uneven blastomeres and <20% fragmentation as grade 2ab. The embryos with 20–50% fragmentation and >50% fragmentation were graded as grade 3 and 4 embryos respectively (Hardarson et al., 2001). Grades 1–3 were considered as transferable embryos.

Embryo biopsy and preimplantation genetic diagnosis (PGD) was performed for all available grade 1–3 embryos with six cells or more on the morning of day 3. Using mechanical zona dissection, one blastomere was removed and fluorescence in-situ hybridization (FISH) was performed using the probes for chromosomes X, Y, 13, 18, and 21.

Because PGD was carried out for the Klinefelter syndrome group, embryo transfer was performed on day 5. Day 3 embryo transfer was performed in the control group (no PGD required). All the procedures of embryo transfer were performed with a soft catheter under transabdominal ultrasonography. Luteal phase was supported by daily vaginal progesterone suppositories (Crinone; Serono, Istanbul, Turkey) starting 1 day after oocyte retrieval.

Clinical pregnancy was defined as the presence of an intrauterine gestational sac with fetal heart beat at transvaginal ultrasonography.

The statistical analyses were performed using Statistics Package for Social Sciences version 13.0 (SPSS Inc., Chicago, IL, USA). The normal distribution of the variables was tested with the Kolmogorov–Smirnov test. Parametric and numeric variables were compared with independent samples t-test. Non-normally distributed metric variables were analysed using Mann–Whitney U-test. The chi-squared test and Fisher’s exact test were used to analyse nominal variables in the form of frequency tables.
Results

In the Klinefelter syndrome group, the mean male age at the time of TESE was 32.0 ± 6.4 (range: 22–46 years) (Table 1). The mean female age was 28.5 ± 6.1 (range: 20–44 years). The respective figures in the control group were 34.3 ± 5.8 (range: 22–49 years) and 29.9 ± 5.4 (19–42 years). The mean age of the patients with Klinefelter syndrome was significantly lower than the control group ($P < 0.05$).

Spermatozoa were successfully retrieved from 22 attempts (56%) at 39 TESE procedures in the Klinefelter syndrome group (Table 1). This figure was 44% (57/130) in the control group. In the control group, the mean male age was comparable among those with successful and unsuccessful surgical retrieval of spermatozoa. However, within the Klinefelter group, the mean male age of those with successful retrieval of spermatozoa was significantly younger when compared with those with unsuccessful retrieval of spermatozoa (29.6 ± 5.3 versus 35.1 ± 6.6, $P = 0.008$).

The successful retrieval rates of spermatozoa at the first TESE attempt in the Klinefelter and control groups were 52 and 40% respectively. These figures were 83 and 71% respectively in the second or further TESE attempts.

There was no significant difference in the fertilization rates of three groups of spermatozoa used for ICSI in patients with Klinefelter syndrome, namely: (i) all motile spermatozoa used for ICSI (66%); (ii) all immotile spermatozoa used for ICSI (54%); and (iii) number of oocytes exceeding the number of available motile spermatozoa and therefore motile and immotile spermatozoa used for ICSI (54%). However, there appears to be a trend for a higher fertilization rate in the all-motile sperm group. The respective figures were 67, 42 and 56% in the control group ($P < 0.001$ for all motile and all immotile subgroups).

Following biopsy and FISH procedure, a FISH result was available in 71 of 75 (95%) of the embryos. A normal result was obtained for the chromosomes analysed in 42 of the 71 embryos (59%). This rate of euploidy is concordant with 54% quoted by Staessen et al. (2003). The types of aneuploidy were as follows: 14 monosomies (chromosomes 13, 18 and 21), 10 trisomies (chromosomes 13, 18 and X), two triploidy and three chaotic.

Of the 22 successful TESE and ICSI cycles within the Klinefelter syndrome group, four (18%) did not reach embryo transfer; two due to total fertilization failure and two due to poor embryo quality. Within the control group, of the 57 successful TESE and ICSI cycles, 12 (21%) did not reach embryo transfer; four due to total fertilization failure and eight due to poor embryo quality.

The embryological data and pregnancy outcome is given in Table 2. The clinical pregnancy rates per embryo transfer of

### Table 1. The baseline characteristics of the Klinefelter syndrome and control (non-obstructive azoospermia with normal karyotype) groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Klinefelter syndrome</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>33</td>
<td>113</td>
</tr>
<tr>
<td>No. of TESE attempts</td>
<td>39</td>
<td>130</td>
</tr>
<tr>
<td>Male age years (mean ± SD)</td>
<td>32.0 ± 6.4</td>
<td>34.3 ± 5.8</td>
</tr>
<tr>
<td>Female age years (mean ± SD)</td>
<td>28.5 ± 6.1</td>
<td>29.9 ± 5.4</td>
</tr>
<tr>
<td>Successful retrieval of spermatozoa/total TESE attempts (%)</td>
<td>22/39 (56)</td>
<td>57/130 (44)</td>
</tr>
<tr>
<td>Successful retrieval of spermatozoa/first TESE attempt (%)</td>
<td>17/33 (52)</td>
<td>45/113 (40)</td>
</tr>
<tr>
<td>Successful retrieval of spermatozoa/2nd or further TESE attempt (%)</td>
<td>5/6 (83)</td>
<td>12/17 (71)</td>
</tr>
</tbody>
</table>

*P < 0.05; there were no other statistically significant differences between the two groups. TESE = testicular sperm extraction.

### Table 2. The embryological data and pregnancy outcome of the Klinefelter syndrome and control (non-obstructive azoospermia with normal karyotype) groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Klinefelter syndrome</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cumulus–oocyte complexes (mean ± SD)</td>
<td>13.1 ± 7.7</td>
<td>11.2 ± 6.4</td>
</tr>
<tr>
<td>Fertilization rate (%)</td>
<td>48</td>
<td>54</td>
</tr>
<tr>
<td>No. of embryos transferred (mean ± SD)</td>
<td>2.4 ± 1.2</td>
<td>2.7 ± 1.0</td>
</tr>
<tr>
<td>Biochemical pregnancy/embryo transfer (%)</td>
<td>11/18 (61)</td>
<td>19/46 (41)</td>
</tr>
<tr>
<td>Clinical pregnancy/embryo transfer (%)</td>
<td>7/18 (39)</td>
<td>15/46 (33)</td>
</tr>
<tr>
<td>Implantation rate (%)</td>
<td>23</td>
<td>26</td>
</tr>
<tr>
<td>Live birth rate/embryo transfer (%)</td>
<td>28</td>
<td>26</td>
</tr>
</tbody>
</table>

There were no statistically significant differences between the two groups.
both Klinefelter syndrome (39%) and control groups (33%) were comparable. The respective figures for the implantation rates of the both groups were 23 and 26%. The live birth rates per embryo transfer of the Klinefelter and control groups were 28 and 26% respectively.

Discussion

The first healthy live births achieved with successful surgical retrieval of testicular spermatozoa and ICSI in patients with Klinefelter syndrome were reported in 1998 (Palermo et al., 1998; Reubinoff et al., 1998). Since then, several case reports (Denschlag et al., 2004; Yarali and Bozdag, 2006; Greco et al., 2008) and case-series have been published (Levron et al., 2000; Friedler et al., 2001; Ulug et al., 2003; Vernaeve et al., 2003; Schiff et al., 2005; Kyono et al., 2007). There is, however, still a paucity of data on the successful surgical retrieval of spermatozoa as well as pregnancy outcome of ICSI cycles in such patients.

The available published series of the TESE procedure performed in patients with Klinefelter syndrome is given in Table 3. Successful retrieval of testicular spermatozoa has been reported in 35–72% of the TESE attempts (Table 3). Of these seven case series, microscopic TESE was performed in two (Ulug et al., 2003; Schiff et al., 2005), whereas macroscopic TESE was performed in the remaining five (Tournaye et al., 1996; Levron et al., 2000; Friedler et al., 2001; Vernaeve et al., 2003; Kyono et al., 2007). In the current study in which microscopic TESE was performed, spermatozoa could be retrieved at TESE in 56 and 44% of males with Klinefelter syndrome and non-obstructive azoospermia with normal karyotype, respectively (Table 1). Madgar et al. (2002) reported that testicular volume, serum testosterone concentrations and results of the HCG testing are important predictive factors for successful retrieval of spermatozoa. However, in other studies, as neither clinical/biological factors (Westlander et al., 2001; Vernaeve et al., 2004) nor a diagnostic testicular biopsy (Schiff et al., 2005) predict successful sperm recovery in patients with non-obstructive azoospermia irrespective of peripheral karyotype, a diagnostic testicular biopsy is not routinely carried out before an attempted TESE and ICSI cycle in such patients.

As a routine policy, TESE is performed 1 day prior to the scheduled oocyte retrieval. The main reason for this policy is to avoid oocyte retrieval when no spermatozoa are noted following extensive laboratory search which not infrequently takes hours of examination. There appears to be a trend for a higher fertilization rate in the all-motile spermatozoa used for ICSI sub-group in both the Klinefelter and control groups, but reaching statistical significance only in the control group. The data reflect that an acceptable fertilization and pregnancy outcome may still be achieved even when all surgically retrieved spermatozoa are immotile in males with Klinefelter syndrome or non-obstructive azoospermia with normal karyotype.

Due to possible increased risk of increased gonosome number in the spermatozoa of patients with Klinefelter syndrome, PGD may be offered to such couples (Staessen et al., 2003). A significantly increased risk of abnormalities was observed in embryos of patients with Klinefelter syndrome (46%) compared with those of control couples with X-linked diseases (23%) (Staessen et al., 2003). A significantly increased risk of abnormalities was noted for sex chromosomes and autosomes (chromosomes 18 and 21) (Kahraman et al., 2003; Staessen et al., 2003). In contrast, Kruse et al. (1998) reported hyperhaploid spermatozoa only in 7.5% of retrieved spermatozoa from men with Klinefelter syndrome (Kruse et al., 1998). Similarly, Levron et al. (2000) noted that the majority (94%) of the testicular spermatozoa from patients with Klinefelter syndrome had a normal pattern of sex chromosome segregation. In the present series, the rate of normal embryos following PGD was 59% in patients with Klinefelter syndrome, which was concordant with the data (54%) of Staessen et al. (2003).

So far as is known, the current study is the third largest published series on the performance of TESE and ICSI treatment in

Table 3. Comparison of the available published case series of testicular sperm extraction (TESE) procedures performed in patients with Klinefelter syndrome.

<table>
<thead>
<tr>
<th>Study</th>
<th>No. of TESE procedures</th>
<th>TESE with successful sperm retrieval (%)</th>
<th>Clinical pregnancy/embryo transfer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tournaye et al. 1996</td>
<td>10</td>
<td>4 (40)</td>
<td>0/4</td>
</tr>
<tr>
<td>Levron et al. 2000</td>
<td>20</td>
<td>8 (40)</td>
<td>4/8</td>
</tr>
<tr>
<td>Friedler et al. 2001</td>
<td>12</td>
<td>5 (42)</td>
<td>5/10</td>
</tr>
<tr>
<td>Vernaeve et al. 2003</td>
<td>50</td>
<td>24 (48)</td>
<td>Not available</td>
</tr>
<tr>
<td>Ulug et al. 2003</td>
<td>11</td>
<td>6 (55)</td>
<td>2/6</td>
</tr>
<tr>
<td>Schiff et al. 2005</td>
<td>54</td>
<td>39 (72)</td>
<td>22/39</td>
</tr>
<tr>
<td>Kyono et al. 2007</td>
<td>17</td>
<td>6 (35)</td>
<td>7/9a</td>
</tr>
<tr>
<td>Present study</td>
<td>39</td>
<td>22 (56)</td>
<td>7/18</td>
</tr>
</tbody>
</table>

*a Five cycles using fresh TESE and the remaining five cycles were performed with cryopreserved–thawed spermatozoa.

*b Six cycles using fresh TESE and the remaining three cycles were performed with cryopreserved–thawed spermatozoa.
patients with Klinefelter syndrome. It is concluded that TESE–ICSI performance in patients with Klinefelter syndrome is comparable with that of patients with non-obstructive azoospermia and normal karyotype. The drawback of the present study is that PGD and day 5 transfer was performed in patients with Klinefelter syndrome, whereas day 3 transfer without PGD was employed in the control group. This is due to the policy that routine day 3 transfer is done at the study institute except for PGD cases; PGD is performed in couples with structural and numerical chromosomal disorders when day 5 transfer is carried out. Since preimplantation genetic screening has not been reported to improve implantation rates in couples with normal karyotype (American Society for Reproductive Medicine, 2007) it was not employed in the control group.

References


Bukulmez O, Yarali H, Yucel A et al. 2000 Intracytoplasmic sperm injection versus in vitro fertilization for patients with a tubal factor as their sole cause of infertility: a prospective, randomized trial. Fertility and Sterility 73, 38–42.


Madgar I, Dor J, Weissenberg R et al. 2002 Prognostic value of the clinical and laboratory evaluation in patients with nonmosaic Klinefelter syndrome who are receiving assisted reproductive therapy. Fertility and Sterility 77, 1167–1169.


Ulug U, Bener F, Akman MA et al. 2003 Partners of men with Klinefelter syndrome can benefit from assisted reproductive technologies. Fertility and Sterility 80, 903–906.


Declaration: The authors report no financial or commercial conflicts of interest.

Received 23 August 2008; refereed 12 September 2008; accepted 9 February 2009.