Asian Journal of Andrology (2021) 23, 59–63
www.asiaandro.com; www.ajandrology.com

Open Access

ORIGINAL ARTICLE

Male Fertility

Sperm retrieval rates and clinical outcomes for patients with different causes of azoospermia who undergo microdissection testicular sperm extraction-intracytoplasmic sperm injection

Hong-Liang Zhang[1,2,3,4,*], Lian-Ming Zhao[1,2,3,*], Jia-Ming Mao[1,2,3], De-Feng Liu[1,2,3], Wen-Hao Tang[1,2,3,4], Hao-Cheng Lin[1,2,3], Li Zhang[2], Ying Lian[2], Kai Hong[1,2,3], Hui Jiang[1,2,3,4]

The aim of our study was to compare the sperm retrieval rates (SRRs) and clinical outcomes of patients with different causes of azoospermia who underwent microdissection testicular sperm extraction-intracytoplasmic sperm injection (micro-TESE-ICSI). We conducted a retrospective study at the Reproductive Medicine Center of Peking University Third Hospital in Beijing, China, from January 2014 to December 2017. This study examined 769 patients with nonobstructive azoospermia who underwent 347 cycles of micro-TESE-ICSI. Patients with azoospermia were classified into Group A (Klinefelter syndrome, *n* = 284, 125 cycles), Group B (azoospermia Y chromosome factor c [*AZFc*] microdeletion, *n* = 91, 64 cycles), Group C (cryptorchidism, *n* = 52, 39 cycles), Group D (previous mumps and bilateral orchitis, *n* = 23, 23 cycles), and Group E (idiopathic azoospermia, *n* = 319, 96 cycles). Clinical characteristics, SRR, embryonic development, and pregnancy outcomes of the patients were compared between all groups. Patients in Group D had the highest and most successful SRR. The average SRR for all patients was 46.0%. The rates of clinical pregnancy, implantation, and live birth in Group D were 78.3%, 65.0%, and 74.0%, respectively, which were higher than those in all other groups ($P < 0.05$). Group B patients had the lowest clinical pregnancy, implantation, and live birth rates of all groups ($P < 0.05$). No differences were found in the miscarriage rate or birth defects among the groups ($P > 0.05$). Patients with orchitis had the highest SRR and best clinical outcomes. Although *AZFc* microdeletion patients had a higher SRR, their clinical outcomes were worse.

Asian Journal of Andrology (2021) **23**, 59–63; doi: 10.4103/aja.aja_12_20 published online: 21 April 2020

Keywords: azoospermia; intracytoplasmic sperm injection; microdissection testicular sperm extraction; pregnancy outcomes; sperm retrieval rate

INTRODUCTION

Azoospermia, defined as the absence of spermatozoa in the ejaculate after assessment of centrifuged semen on at least two occasions, occurs in approximately 10% of male infertility cases.[1] Clinically, azoospermia is divided into obstructive azoospermia (OA) and nonobstructive azoospermia (NOA), which accounts for 60% of cases of defective spermatogenesis.[2] The etiology of NOA can be classified as acquired, congenital, or idiopathic.[3] Causes of congenital NOA mainly include genetic abnormalities, such as Klinefelter syndrome (KS), azoospermia Y chromosome factor (*AZFa*, *AZFb*, and *AZFc*) microdeletions, and cryptorchidism. Acquired causes of NOA mainly include trauma, testicular torsion, varicocele, inflammation, drugs, radiation, and testicular spermatogenetic dysfunction caused by other factors (cryptorchidism, pituitary tumor, and idiopathic hypogonadotropic sexual hypofunction). Idiopathic NOA is diagnosed when known etiologies are excluded.[4] Recently, intracytoplasmic sperm injection (ICSI) using microdissection sperm retrieval (micro-TESE) has been commonly applied to NOA patients, and is generally considered to be a standard method, with high sperm retrieval rate (SRR) and minimal tissue loss.[5,6] Most studies of micro-TESE-ICSI for NOA patients have mainly focused on comparing sperm retrieval and clinical pregnancy outcomes with those of OA patients.[4,7,8] However, there is limited data on clinical pregnancy outcomes, which are important in clinical practice for couples with OA, for distinct NOA with different etiologies. Therefore, the aim of our study was to compare the SRRs and clinical outcomes of patients who had different causes of NOA and who were undergoing micro-TESE-ICSI treatment.

PATIENTS AND METHODS

Patients

We collected medical data retrospectively from the Reproductive Medicine Center of Peking University Third Hospital in Beijing (China)

[1]Department of Urology, Peking University Third Hospital, Beijing 100191, China; [2]Department of Reproductive Medicine Center, Peking University Third Hospital, Beijing 100191, China; [3]Department of Andrology, Peking University Third Hospital, Beijing 100191, China; [4]Department of Human Sperm Bank, Peking University Third Hospital, Beijing 100191, China.
*These authors contributed equally to this work.
Correspondence: Dr. K Hong (kenhong99@hotmail.com) or Dr. H Jiang (jianghui55@163.com)
Received: 13 September 2019; Accepted: 14 January 2020

from January 2014 to December 2017. Azoospermia was confirmed by testing at least two semen samples. Data regarding medical history; physical examination; assessments of sex hormones, including follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone (T) levels; and genetic abnormalities were recorded for each patient. All enrolled participants in our study fulfilled the following criteria: (1) complete clinical data; (2) absence of preexisting chronic diseases, including hypertension, diabetes mellitus, and heart, kidney, hematological, and autoimmune diseases; (3) absence of female infertility factors, including anovulation, hormonal infertility, tubal factors, and endometriosis; (4) ages of couples from 23 years to 35 years; and (5) body mass index (BMI) of all couples from 18 kg m^{-2} to 30 kg m^{-2}.

In total, 769 cases of azoospermia who underwent 347 cycles of micro-TESE-ICSI were identified and studied. Patients were classified into Group A (KS, $n = 284$, 125 cycles), Group B (*AZFc* microdeletion, $n = 91$, 64 cycles), Group C (cryptorchidism, $n = 52$, 39 cycles), Group D (previous mumps and bilateral orchitis, $n = 23$, 23 cycles), and Group E (idiopathic azoospermia, $n = 319$, 96 cycles) according to different causes of NOA. The clinical characteristics, in particular, SRR, embryonic development, and pregnancy outcomes, of the patients were compared between all groups. The study protocol was approved by the Ethics Committee of Peking University Third Hospital, and all patients provided written informed consent before the study.

Micro-TESE

Micro-TESE was performed under general anesthesia, and a mid-line scrotal incision was made on the median raphe of the scrotum. Testicular parenchyma was exposed and directly examined for dilated tubule areas under an operating microscope (×12 to ×24 magnification). The seminiferous tubules were teased with 21G needles and placed into a G-MOPS-plus buffer (Vitrolife, Goteborg, Sweden). The collected tubules were finely minced into a homogeneous suspension and examined for the presence of spermatozoa using an inverted microscope (TS100, Nikon, Tokyo, Japan). Samples were positive when at least one sperm was identified. If fresh oocytes could be obtained on the same or the next day of sperm retrieval, fresh sperm were used for the ICSI procedure. If not, sperm were cryopreserved with sperm-freezing solution (Vitrolife). The testicular suspension was diluted 1:1 with the sperm-freezing solution in a 2-ml straw. The straw was incubated at room temperature for 10 min, then placed horizontally on a styrofoam board in a liquid nitrogen bath for 30 min, and then the straw was stored in liquid nitrogen vapor. When thawing, the straws were put in a 37°C incubator for 15 min, and then the sperm were collected from the cryoprotectant by two washes in culture medium, each with centrifugation at 300*g* for 10 min (BY-160A, BY-CENTRIFUGE, Beijing, China). The resulting samples were incubated in culture medium (Vitrolife) for later use.

Ovarian stimulation and oocyte retrieval

Ovarian stimulation was a long protocol performed with a combination of gonadotrophin-releasing hormone (GnRH) analogs (Cetrotide, Merck Serono, Amsterdam, the Netherlands), FSH (Gonal-F alfa, Merck Serono), and human chorionic gonadotrophin (HCG; Choriogonadotropin alfa, Merck Serono). Oocyte retrieval involved vaginal ultrasound-guided puncture of follicles 36 h and 38 h after HCG administration. The oocytes were maintained in G-MOPS-plus (Vitrolife) and placed in a 37°C incubator with 5% CO_2, 5% O_2, and 90% N_2. Cumulus cells were removed by pipetting and exposure to hyaluronidase 2 h after retrieval (Type VIII; Sigma Chemical Company, St. Louis, MO, USA).

ICSI and embryo transfer

ICSI was performed as described in detail elsewhere.[9] Fertilization was assessed by the presence of two pronuclei (2pn) and two polar bodies 17–19 h after insemination. Embryo transfer was performed on day 3 after oocyte retrieval. Embryos were scored according to the Society for Assisted Reproductive Technology (SART) scoring system,[10] and only the best embryos were selected for transfer. The number of transferred embryos was usually limited to two to reduce the risk of a multiple pregnancy.

Definitions

The fertilization rate was the number of 2pn zygotes in all mature metaphase II (MII) stage oocytes. Clinical pregnancy was defined as a rising serum HCG level at least 12 days after embryo transfer. Clinical pregnancy was confirmed by the presence of a gestational sac during ultrasound examination in the 5th week after transfer. The miscarriage rate was the number of miscarriage cycles divided by the number of clinical pregnancy cycles. Indicators evaluating embryonic development included the number of oocytes and MII oocytes that were retrieved, the fertilization rate, the number of transferred embryos, and the good-quality embryo rate. Clinical outcomes included the cumulative pregnancy, implantation, cumulative live birth, and cumulative miscarriage rates, as well as the cumulative number of birth defects.

Statistical analysis

Statistical analyses were performed using Statistical Package for Social Sciences version 22.0 (IBM Corp., Armonk, NY, USA). Data were expressed as the mean ± standard deviation, and categorical data were expressed as percentages. $P < 0.05$ was considered statistically significant.

RESULTS

The characteristics of all patients are described in **Table 1**. The mean male age was 28.6 ± 4.0 years, and the mean T level was 6.08 ± 9.97 ng ml^{-1} in Group A; both of these parameters were the lowest in all groups. Significance was reached compared with Groups B, C, and E ($P < 0.05$). Female age and testicular volume in Group A were 26.7 ± 3.5 years and 1.92 ± 0.8 ml, respectively, which were the lowest in all groups. The FSH and LH levels were higher in Group A than for any other groups ($P < 0.05$). There were no significant differences in other patient characteristics among the different groups.

Table 2 presents the SRRs of all groups. Patients in Group D had the highest and most successful SRR, which was 100%. The average SRR for all patients in our study was 46.0%.

Embryonic development for all groups is summarized in **Table 3**. The fertilization rate was 27.9% ± 19.1% in Group B, which was the lowest among the groups, and 65.1% ± 19.3% in Group D, which was the highest among the groups (both $P < 0.05$). No difference was found for other aspects of embryonic development, including the average number of oocytes retrieved, average number of MII oocytes, oocyte maturation rate, and average number of transferred embryos for all the groups.

The pregnancy outcomes for all patients are shown in **Table 4**. The rates of clinical pregnancy, implantation, and live birth in Group D were 78.3%, 65.0%, and 74.0%, respectively, which were higher than those in any other groups (all $P < 0.05$). Patients in Group B had the lowest clinical pregnancy, implantation, and live birth rates for all the groups (all $P < 0.05$). No differences were found in the miscarriage rate or in birth defects among all the groups ($P > 0.05$).

Table 1: Characteristics of all patients

Characteristics	*Group A (n=284)*	*Group B (n=91)*	*Group C (n=52)*	*Group D (n=23)*	*Group E (n=319)*
Age (year)					
Male[a]	28.6±4.0	31.3±4.8	31.3±3.4	29.8±4.6	30.9±5.1
Female[b]	26.7±3.5	30.3±3.8	30.3±3.1	29.1±3.9	29.7±4.5
BMI (kg m^{-2})					
Male	24.89±4.54	24.86±3.17	25.79±2.98	25.93±2.91	24.97±3.78
Female	22.20±3.54	21.59±2.86	21.78±2.45	21.08±2.89	22.39±3.18
FSH (mIU ml^{-1})[c]	35.15±15.96	18.77±9.72	24.59±12.73	28.29±11.47	20.55±10.86
LH (mIU ml^{-1})[c]	18.38±8.33	8.38±4.52	11.03±5.37	13.11±4.94	8.96±4.66
T (ng ml^{-1})[a]	6.08±9.97	10.31±4.17	10.43±5.91	7.54±3.24	9.30±4.84
Testicular volume (ml)[b]	1.92±0.82	8.81±3.27	8.49±3.43	6.24±3.24	8.13±3.97

Data are expressed as mean±s.d.. [a]Values in Group A was the lowest among all the groups, and significance was reached compared with Groups B, C, or E (P<0.05); [b]values in Group A was the lowest of all the groups, and significance was reached compared with Groups B, C, D, or E (P<0.05); [c]values in Group A was the highest of all the groups (P<0.05). s.d.: standard deviation; FSH: follicle-stimulating hormone; LH: luteinizing hormone; T: testosterone; BMI: body mass index

Table 2: The sperm retrieval rate using micro-testicular sperm extraction

	Group A (n=284)	*Group B (n=91)*	*Group C (n=52)*	*Group D (n=23)*	*Group E (n=319)*
Number of micro-TESEs	300	102	59	25	337
Number of micro-TESEs that resulted in sperm	127	67	39	23	98
SRR (%)	44.7	73.6	75.0	100.0	30.7
Average SRR (%)			46.0		

TESE: testicular sperm extraction; SRR: sperm retrieval rate

Table 3: Embryonic development for all groups

	Group A (n=284)	*Group B (n=91)*	*Group C (n=52)*	*Group D (n=23)*	*Group E (n=319)*
Number of cycles	125	64	39	23	96
Number of oocytes	15.63±8.82	12.81±6.31	13.64±7.12	16.02±9.75	14.22±7.99
Number of MII oocytes	12.66±7.29	10.92±5.68	11.23±6.28	13.09±8.51	10.95±5.98
Oocyte maturation rate (%)	82.1±14.8	85.9±14.4	82.7±14.0	81.0±14.8	82.0±16.9
Fertilization rate (%)[a,b]	54.4±20.9	27.9±19.1	45.4±28.4	65.1±19.3	45.7±22.8
Number of transferred embryos	1.72±0.57	1.34±0.76	1.69±0.57	1.74±0.45	1.61±0.70

Data are expressed as mean±s.d.. [a]Values in Group B was the lowest of all the groups (P<0.05); [b]values in Group D was the highest of all the groups (P<0.05). MII oocytes: metaphase II oocytes; s.d.: standard deviation

Table 4: Pregnant outcomes for all groups

	Group A (n=284)	*Group B (n=91)*	*Group C (n=52)*	*Group D (n=23)*	*Group E (n=319)*
Number of cycles	125	64	39	23	96
Clinical pregnancy rate[a,b], % (*n*/total)	54.4 (68/125)	20.3 (13/64)	53.9 (21/39)	78.3 (18/23)	46.9 (45/96)
Implantation rate[a,b], % (*n*/total)	36.4 (80/220)	19.8 (17/86)	37.9 (25/66)	65.0 (26/40)	38.7 (60/155)
Live birth rate[a,b], % (*n*/total)	50.4 (63/125)	18.8 (12/64)	46.2 (18/39)	74.0 (17/23)	40.6 (39/96)
Miscarriage rate, % (*n*/total)	5.9 (4/68)	7.7 (1/13)	9.5 (2/21)	5.6 (1/18)	13.3 (6/45)
Birth defects (*n*)	3	0	0	0	0

[a]Values in Group B was the lowest of all the groups (P<0.05); [b]values in Group D was the highest of all the groups (P<0.05)

DISCUSSION

In recent years, as micro-TESE technology has increasingly improved, more NOA patients have been able to conceive their offspring through this technology. However, no systematic study has reported the SRRs or related clinical outcomes for patients with different causes of NOA, key parameters that also concern NOA patients in clinical practice. Therefore, our center analyzed relevant recent data to study the SRRs and clinical outcomes for patients with different causes of NOA and who underwent micro-TESE-ICSI, in order to provide relevant guidance for clinical work.

Klinefelter syndrome is the most frequent chromosomal abnormality associated with NOA, with an estimated prevalence ranging from 1:500 to 1:700 in newborn males, and is characterized by infertility, elevated FSH and LH levels, normal or reduced T level, small testicular volume, and testicular hypofunction.[11] As these characteristics are quite apparent, patients with KS can be diagnosed and treated early.[12] Our data showed that the FSH and LH levels were the highest and that female age and testicular volume were the lowest in KS patients compared with all other groups (both $P < 0.05$). Both male age (28.6 ± 4.0 years) and T levels in KS were the lowest compared with all the groups, and significance was reached compared with Groups B, C, or E ($P < 0.05$). Our data were in accordance with the characteristics of KS patients.

We also investigated the SRRs of patients with different causes of NOA. We found that the average SRR was 46.0%, which is in agreement

with previous studies showing SRRs from 40%–60%.[13,14] In our study, the highest SRR (100%) was for patients with orchitis. So far, we have not found a report describing the SRR for patients with orchitis who undergo micro-TESE treatment. We speculate that the reason for the high SRR may be that NOA in most of these patients was caused by mumps, and therefore the infertility caused by this acquired infection was less likely to affect the fertility of their offspring.[15] Moreover, the SRR for patients with cryptorchidism in our study was also very high (75.0%). Previous studies have shown that men with cryptorchidism had a good chance of sperm retrieval when undergoing micro-TESE.[16,17] Bernie *et al*.[14] found that men with cryptorchidism tended to have a slightly higher SRR (74%) than that of other NOA patients, which is consistent with our results. Interestingly, the SRR was 73.3% for patients with the *AZFc* microdeletion in our study. Y chromosome microdeletions are the most important genetic etiology of male infertility, and *AZFc* is the most frequently microdeleted region in these NOA patients, accounting for more than 60% of all *AZF* microdeletions.[18] Many studies recommend TESE for patients with *AZFc* microdeletion, as sperm is typically present in the testes of these men.[18] For instance, Oates *et al*.[18] discovered sperm in 67% of testes in these patients undergoing TESE. However, the SRR of *AZFc* microdeletion patients who underwent micro-TESE treatment was limited. Recently, Sabbaghian *et al*.[19] showed that patients with the *AZFc* microdeletion had a more successful SRR of 43.7%, which was lower than that in our study. Consistent with our results, An *et al*.[8] found that *AZFc* microdeletion patients had an SRR of 70%. The SRR for KS patients was slightly lower than that of other patients (44.7%), which is in line with a previous study (40%).[20] Testicular hypofunction and the high FSH levels of KS patients may be a possible explanation for the low SRR, as reports have shown that men with azoospermia with high FSH levels have lower SRRs, and FSH was a suggested predictive measure for sperm retrieval.[20] The SRR for KS patients in our study was consistent with results from a previous meta-analysis, which showed that the SRR for KS patients was close to 50%.[21] In our study, patients with idiopathic azoospermia had the lowest SRR (30.7%), which was consistent with that of a previous report.[22] As related data were limited, the possible reasons for this need to be explored in further research.

In terms of clinical outcomes, our results demonstrated that patients with orchitis had the highest rates of fertilization, clinical pregnancy, implantation, and live birth, and that the patients with *AZFc* microdeletion had the lowest rates of fertilization and clinical pregnancy among all patients ($P < 0.05$). As mentioned above, the better clinical outcomes of patients with orchitis may be attributed to their infertility being caused by an acquired infection.[15] We found only one report concerning *AZFc* microdeletions. In 2018, Sabbaghian *et al*.[19] reported a fertilization rate of approximately 36% for patients with *AZFc* microdeletions who underwent micro-TESE-ICSI treatment, but no pregnancy was observed in the authors' center. The low fertilization and pregnancy rates for these patients were in agreement with our results. Notably, Liu *et al*.[23] reported that patients with oligozoospermia and an *AZFc* microdeletion and who were undergoing conventional TESE-ICSI treatment also had lower fertilization rates, which may indicate that the clinical reproductive outcomes for *AZFc* microdeletion patients remain adverse despite the use of sperm retrieval methods. In addition, although KS patients had a relatively low SRR in our study, their clinical outcomes were relatively good. A recent meta-analysis[21] showed that the current micro-TESE method for patients with KS resulted in clinical pregnancy and live birth rates close to 50%, which is consistent with our data. Our results also showed that patients with cryptorchidism and idiopathic azoospermia had relatively good clinical outcomes. One study using TESE-ICSI for patients with cryptorchidism reported fertilization, pregnancy, and live birth rates of 43.3%, 30%, and 20%, respectively.[24] To date, no report has addressed the outcomes from micro-TESE-ICSI for patients with cryptorchidism or idiopathic azoospermia.

Presently, new problems are arising as preimplantation genetic diagnosis (PGD) provides patients with the possibility to select female embryos for transfer, especially for KS and *AZFc* microdeletion patients who have a genetic etiology of male infertility. In our clinical practice, we are concerned with helping and adequately counseling patients with infertility as they make their own choices to become parents, especially for couples who decide to pursue PGD. Because of the high economic burden on patients and the debatable risks to offspring from PGD, our center does not recommend PGD for KS patients. None of the patients who enrolled in our study underwent the PGD procedure.

To the best of our knowledge, the current study is the first to systematically explore the SRRs and clinical outcomes for patients with NOA due to different etiologies who undergo micro-TESE-ICSI treatment. Our study provided the largest sample size to date, although the retrospective and single-center nature of our data present key limitations for further consideration. We also lacked a long-term follow-up plan after delivery.

CONCLUSION

Our study found that patients with orchitis have the highest SRR and best clinical outcomes. Although *AZFc* microdeletion patients had a higher SRR, their clinical outcomes were worse. A higher SRR does not indicate good outcomes nor does a lower SRR indicate poor outcomes. These findings will help formulate our suggestions to patients and provide relevant guidance for clinical work. As related data are very limited, more evidence is needed through studies with larger samples and different communities to investigate this aspect in future.

AUTHOR CONTRIBUTIONS

HJ conceived and designed the study. KH supported substantial contributions to the study conception and design. LMZ analyzed and interpreted the data. HLZ drafted the article. DFL, JMM, WHT, HCL, LZ, and YL collected the data. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declare no competing interests.

ACKNOWLEDGMENTS

This research was sponsored by the National Key Research and Development Project (SQ2018YFC100243), National Key Research and Development Project (2016YFC1000302), National Key Research and Developmental Program of China (2018YFC1003600), Young Scientists Fund of the National Natural Science Foundation of China (Grant No. 81601272), Clinical Medicine PlusX-Young Scholars Project, Peking University (Grant No. 2102018237), Beijing Municipal Natural Science Foundation (7182177), and National Key Research and Development Program of China (Grant No. 2017YFC1002001).

REFERENCES

1 Irvine DS. Epidemiology and aetiology of male infertility. *Hum Reprod* 1998; 13 Suppl 1: 33–44.
2 McLachlan RI, Rajpert-De Meyts E, Hoei-Hansen CE, de Kretser DM, Skakkebaek NE. Histological evaluation of the human testis – approaches to optimizing the clinical value of the assessment: mini review. *Hum Reprod* 2007; 22: 2–16.
3 Anniballo R, Ubaldi F, Cobellis L, Sorrentino M, Rienzi L, *et al*. Criteria predicting the absence of spermatozoa in the Sertoli cell-only syndrome can be used to improve success rates of sperm retrieval. *Hum Reprod* 2000; 15: 2269–77.

4 Tavukcuoglu S, Al-Azawi T, Al-Hasani S, Khaki AA, Khaki A, *et al.* Using fresh and frozen testicular sperm samples in couples undergoing ICSI-MicroTESE treatment. *J Reprod Infertil* 2013; 14: 79–84.
5 Ramasamy R, Lin K, Gosden LV, Rosenwaks Z, Palermo GD, *et al.* High serum FSH levels in men with nonobstructive azoospermia does not affect success of microdissection testicular sperm extraction. *Fertil Steril* 2009; 92: 590–3.
6 Kalsi J, Thum MY, Muneer A, Abdullah H, Minhas S. In the era of micro-dissection sperm retrieval (m-TESE) is an isolated testicular biopsy necessary in the management of men with non-obstructive azoospermia? *BJU Int* 2012; 109: 418–24.
7 Bocca S, Moussavi V, Brugh V, Morshedi M, Stadtmauer L, *et al.* ICSI outcomes in men undergoing TESE for azoospermia and impact of maternal age. *Andrologia* 2017; 49: e12617.
8 An G, Zou Z, Flannigan R, Liu J, Du H, *et al.* Outcome of oocyte vitrification combined with microdissection testicular sperm extraction and aspiration for assisted reproduction in men. *Med Sci Monit* 2018; 24: 1379–86.
9 Al Hasani S, Kupker W, Baschat AA, Sturm R, Bauer O, *et al.* Mini-swim-up: a new technique of sperm preparation for intracytoplasmic sperm injection. *J Assist Reprod Genet* 1995; 12: 428–33.
10 Racowsky C, Stern JE, Gibbons WE, Behr B, Pomeroy KO, *et al.* National collection of embryo morphology data into Society for Assisted Reproductive Technology Clinic Outcomes Reporting System: associations among day 3 cell number, fragmentation and blastomere asymmetry, and live birth rate. *Fertil Steril* 2011; 95: 1985–9.
11 Morris JK, Alberman E, Scott C, Jacobs P. Is the prevalence of Klinefelter syndrome increasing? *Eur J Hum Genet* 2008; 16: 163–70.
12 Gravholt CH, Chang S, Wallentin M, Fedder J, Moore P, *et al.* Klinefelter syndrome: integrating genetics, neuropsychology, and endocrinology. *Endocr Rev* 2018; 39: 389–423.
13 Ghalayini IF, Al-Ghazo MA, Hani OB, Al-Azab R, Bani-Hani I, *et al.* Clinical comparison of conventional testicular sperm extraction and microdissection techniques for non-obstructive azoospermia. *J Clin Med Res* 2011; 3: 124–31.
14 Bernie AM, Ramasamy R, Schlegel PN. Predictive factors of successful microdissection testicular sperm extraction. *Basic Clin Androl* 2013; 23: 5.
15 Zhang S, Li J, Zhou G, Qin S, Xin N, *et al.* Infertility treatment for azoospermic patients with a history of infectious parotitis (mumps) using combined techniques. *Hum Fertil (Camb)* 2013; 16: 266–8.
16 Ramasamy R, Padilla WO, Osterberg EC, Srivastava A, Reifsnyder JE, *et al.* A comparison of models for predicting sperm retrieval before microdissection testicular sperm extraction in men with nonobstructive azoospermia. *J Urol* 2013; 189: 638–42.
17 Raman JD, Schlegel PN. Testicular sperm extraction with intracytoplasmic sperm injection is successful for the treatment of nonobstructive azoospermia associated with cryptorchidism. *J Urol* 2003; 170: 1287–90.
18 Oates RD, Silber S, Brown LG, Page DC. Clinical characterization of 42 oligospermic or azoospermic men with microdeletion of the *AZFc* region of the Y chromosome, and of 18 children conceived via ICSI. *Hum Reprod* 2002; 17: 2813–24.
19 Sabbaghian M, Mohseni Meybodi A, Rafaee A, Saba S, Zamanian M, *et al.* Sperm retrieval rate and reproductive outcome of infertile men with azoospermia factor c deletion. *Andrologia* 2018; 50: e13052.
20 Zitzmann M, Nordhoff V, von Schonfeld V, Nordsiek-Mengede A, Kliesch S, *et al.* Elevated follicle-stimulating hormone levels and the chances for azoospermic men to become fathers after retrieval of elongated spermatids from cryopreserved testicular tissue. *Fertil Steril* 2006; 86: 339–47.
21 Corona G, Pizzocaro A, Lanfranco F, Garolla A, Pelliccione F, *et al.* Sperm recovery and ICSI outcomes in Klinefelter syndrome: a systematic review and meta-analysis. *Hum Reprod Update* 2017; 23: 265–75.
22 Cissen M, Meijerink AM, D'Hauwers KW, Meissner A, van der Weide N, *et al.* Prediction model for obtaining spermatozoa with testicular sperm extraction in men with non-obstructive azoospermia. *Hum Reprod* 2016; 31: 1934–41.
23 Liu XY, Wang RX, Fu Y, Luo LL, Guo W, *et al.* Outcomes of intracytoplasmic sperm injection in oligozoospermic men with Y chromosome AZFb or AZFc microdeletions. *Andrologia* 2017; 49: 1–6.
24 Haimov-Kochman R, Prus D, Farchat M, Bdolah Y, Hurwitz A. Reproductive outcome of men with azoospermia due to cryptorchidism using assisted techniques. *Int J Androl* 2010; 33: e139–43.