MEDICAL SCIENCE MONITOR

CLINICAL RESEARCH

e-ISSN 1643-3750
© Med Sci Monit, 2018; 24: 6968-6974
DOI: 10.12659/MSM.912613

Received: 2018.08.08
Accepted: 2018.09.11
Published: 2018.10.01

Pregnancy and Neonatal Outcomes in Azoospermic Men After Intracytoplasmic Sperm Injection Using Testicular Sperm and Donor Sperm

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABE Yang Yu
EF Qi Xi
CD Yuan Pan
BD Yuting Jiang
C Hongguo Zhang
F Linlin Li
AG Ruizhi Liu

Center for Reproductive Medicine, Center for Prenatal Diagnosis, The First Hospital, Jilin University, Changchun, Jilin, P.R. China

Corresponding Author: Ruizhi Liu, e-mail: lrz410@126.com
Source of support: This work was supported by the National Nature Science Fund under grant No. 81471515 from China

Background: The safety of intracytoplasmic sperm injection (ICSI) with testicular sperm in azoospermic men has been a concern. We evaluated ICSI outcomes, including neonatal outcomes, in children born using testicular sperm or donor sperm.

Material/Methods: Ninety-nine males with nonobstructive azoospermia (NOA) who underwent microdissection testicular sperm extraction (micro-TESE) and 126 males with obstructive azoospermia (OA) were included in this study. Sixty-one patients with NOA used donor sperm for ICSI on the day of oocyte retrieval when no spermatozoa were identified by micro-TESE on the day before oocyte retrieval. ICSI outcomes were compared among OA, donor, and NOA groups.

Results: There was no statistical difference in terms of female partner characteristics among OA, donor, and NOA groups. The normal fertilization rate (P=0.005), high quality embryo rate (P=0.014), implantation rate (P<0.001), clinical pregnancy rate (P=0.015), live birth rate (P=0.043) were significant lower in the NOA group, compared with the donor sperm group. The normal fertilization rate was significant lower in the NOA group than the OA group (P<0.001), but the live birth rate was not significantly lower (P=0.058). The high-quality embryo rate (P=0.014) and implantation rate (P=0.009) were lower in the OA group than the donor group. No differences between groups were observed in our study regarding neonatal parameters of the infants born.

Conclusions: The fertilization and pregnancy outcomes were negatively affected by using testicular sperm from males with NOA. Once a live birth was achieved, there was no difference in neonatal outcomes.

MeSH Keywords: Azoospermia • Infertility, Male • Sperm Injections, Intracytoplasmic • Tissue Donors

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/912613

2476 4 — 32

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Background

Azoospermia affects approximately 1% of all males and 10% to 15% of all infertile males, and it can be divided into obstructive azoospermia (OA) and nonobstructive azoospermia (NOA) [1]. NOA, caused by testicular failure, accounts for 60% of all azoospermia cases [2]. Intracytoplasmic sperm injection (ICSI) as a first-line treatment offers a chance for patients to become biological parenthood [3]. Testicular sperm extraction (TESE) combined with ICSI for treatment of OA was first introduced in 1993 [4]. Soon after, testicular spermatozoa obtained from patients with NOA were also successfully used in ICSI and resulted in successful pregnancies [5,6]. Additionally, microdissection testicular sperm extraction (micro-TESE) as the most effective sperm retrieval technology can increase the sperm retrieval rate (SRR) and minimal postoperative complications [7,8].

Although many studies have examined the outcomes of ICSI using testicular sperm in cases of NOA, data in the literature are still controversial. Some studies have suggested that the fertilization rate in males with OA was higher than males with NOA, but the pregnancy rate of partners was similar [9–11]. Some reports showed that both the fertilization rate and pregnancy rate in female partners of males with OA were higher, compared with males with NOA [12–14]. Others agreed that there were no differences in the ICSI outcomes between males with OA and NOA [15–17]. However, only a few studies compared the neonatal profile of babies born after ICSI from males with OA and NOA [9,13,18,19]. Due to the increased chromosomal aberrations in testicular spermatozoa from patients with NOA [20,21], concerns of the risk of congenital anomaly in children born after ICSI with testicular spermatozoa are pertinent. To date, the neonatal health of children born after ICSI using testicular spermatozoa from patients with NOA is not well documented.

Therefore, we performed a retrospective study to evaluate ICSI outcomes, neonatal profile, and obstetrical outcomes from in testicular spermatozoa from NOA males compared to OA males. Moreover, we compared these results with patients who received donor sperm for ICSI, due to failed spermatozoa retrieved by micro-TESE.

Material and Methods

This is a comparative study that received institutional review board approval from Medical Ethics Committee of First Hospital of Jilin University (2015-259) and written informed consent was obtained from patients.

Patients

We retrospectively studied 225 consecutive male patients with azoospermia who had ICSI procedures from 2012 to 2017 in the Centre for Reproductive Medicine and Prenatal Diagnosis of the First Hospital of Jilin University. Ninety-nine male patients with NOA underwent micro-TESE by the same surgeon. One-hundred and twenty-six consecutive male patients with OA had successful testicular spermatozoa retrieval by testicular sperm aspiration (TESA). All patients were confirmed to have azoospermia by analyzing of at least 2 centrifuged semen samples according to World Health Organization criteria. Patients had a complete history and physical examination, including endocrine profile, age, urologic evaluations, and genetic analysis. Karyotype and Y chromosomal microdeletion analyses were performed. Patients with complete AZFa and AZFb microdeletions were excluded. Seventeen patients had a 47, XXY karyotype and 3 patients had AZFc microdeletions. The average volume of both testicles was used for the analysis. All the procedures were performed by the same surgeon. Exclusion criteria for female patients was karyotype abnormality.

Testicular sperm recovery

TESA procedure was performed for male patients with OA. Under local anesthesia with 2% lidocaine, a 21-gauge needle attached to a 20-mL syringe was inserted through the scrotal skin into the testicle. Negative pressure was created by pulling the syringe plunger while the tip of the needle was moved gently in and out of the testicle, and the needle was then pulled up slowly. The testis tissue was placed in culture dishes for immediate microscopic examination.

The procedure of micro-TESE has been described previously in detail [7]. Briefly, under general anesthetic, a transverse, midscrotal incision was made over the left or right testis. The tunica vaginalis was opened to visualize the tunica albuginea. An equatorial incision was widely opened over the tunica albuginea under an operative microscope, taking care to avoid vasculature injury. Microdissection was then performed to identify larger and more opaque seminiferous tubules at between 12× and 18× magnification under an operating microscope. Successful retrievals were defined as the presence of sperm. If no spermatozoa were seen during micro-TESE, the testicular tissue was thoroughly examined for the presence of spermatozoa in the embryology laboratory 12 to 24 hours later. Tissue specimen was placed in Bouin solution and sent for histopathological analysis.

The micro-TESE procedure was performed on the day before oocyte retrieval. When no spermatozoa were identified by micro-TESE, donor sperm were offered to patients on the day of oocyte retrieval.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Ovarian stimulation

Long luteal down-regulation protocol was used in the patient's female partner. Gonadotrophin releasing hormone agonist (Tryptorelin, Ferring, Germany) administration was given in the mid luteal phase of the previous cycle. Ovarian stimulation started by administration recombinant follicle-stimulating hormone (FSH) (Gonal-F; Merck Serono, Switzerland) when serum FSH was follicular diameter was <5 mm. Ovulation was triggered with a single dose of recombinant human chorionic gonadotropin (Ovidrel, EMD Serono, Switzerland) when 2 or more ovarian follicles reached 18 mm or more in diameter. Oocytes were retrieved 36 to 38 hours after the administration of rhCG (human chorionic gonadotropin, a recombinant formulation)

ICSI procedure

Retrieved oocytes were incubated in Quinn-1020 (SAGE, Inc., USA) medium supplemented with 5% human serum albumin (SAGE, Inc., USA). Selected spermatozoa were washed into the PVP droplet to remove surrounding debris particles, which could be deleterious for oocyte and resulted embryo. Metaphase II oocytes were injected with normal morphology, and whenever possible, motile spermatozoa for ICSI. ICSI was performed under warmed-stage microscope (Olympus, Tokyo, Japan) at 200× magnification using a Hafman optic system, equipped with hydraulic micromanipulation (Eppendorf, Hamburg, Germany). Oocytes were assessed to determine whether fertilization had occurred at 17 to 19 hours after ICSI. Fertilization was considered to be normal if 2 pronuclei (PN) and 2 polar bodies were identified. The fertilization rate was calculated as the percentage of metaphase II oocytes forming 2 PN. After 72 hours fertilization, according to modified PETER cleavage stage embryos scoring system based on the blastomeric number and symmetry and cytoplasm fragmentation to assess the day 3 embryo quality [22]. Two embryos were transferred into the female partner's uterine cavity on day 3 after oocyte retrieval.

Serum hCG concentrations were measured 14 days after embryo transfer. Clinical pregnancy was defined as the presence of a gestational sac confirmed by ultrasound examination at the fourth week after embryo transfer. Pregnancy rate was defined as babies/embryo transfer.

Statistical analysis

All statistical data were analyzed with SPSS, version 17.0 (SPSS Inc.). For quantitative data such as testis volume, age, and hormone levels, independent-sample *t*-tests were performed for comparisons. The qualitative variables, such as fertilization rate and live birth rate, were evaluated by the chi-square or Fisher's exact test. $P < 0.05$ was considered statistically significant.

Result

There was no statistical difference in terms of female characteristics among the OA, donor, and NOA groups. Of the 99 male patients with NOA, 38 patients (38.4%) had successful sperm retrieval by micro-TESE. All 126 male patients (100%) with OA had successful sperm retrieval. Male patients with NOA had significant smaller testicular size (6.2 *vs.* 13.9 mL, $P<0.001$), higher FSH and luteinizing hormone (LH) levels (24.6 *vs.* 4.9 mIU/ml, $P<0.001$ and 13.0 *vs.* 4.4 mIU/mL, $P<0.001$, respectively), lower testosterone and inhibin B levels (11.3 *vs.* 13.6 pg/mL, $P=0.026$ and 44.2 *vs.* 182.5 pg/mL, $P<0.001$, respectively) (Table 1). The etiologies of the OA group and the NOA group are showed in Table 1. The SRR was higher in male patients with hypospermatogenesis (100%) than male patients with tubular hyalinization (51.9%), Sertoli cell-only (28.6%), and maturation arrest (16.7%). No differences were found between positive SRR and negative SRR in terms of male age, testicular size, FSH, LH, testosterone, and inhibin B levels (Table 2). Six male patients (35.3%) with 47, XXY karyotype and 2 male patients (66.7%) with AZFc microdeletions had successful sperm retrieval. Three male patients with 47, XXY karyotype were reported to have ongoing pregnancies. One child was delivered where sperm was from a male participant with AZFc microdeletions.

The ICSI outcomes are shown in Table 3. Thirty-eight NOA patients with positive SRR had 44 ICSI cycles. Sixty-one patients used donor sperm for ICSI. In the groups of NOA, donor sperm, and OA, there were no differences in terms of maternal age, estradiol, and progesterone levels on the day of hCG administration, and normal 2-pronuclear zygote (2PN). The number of maturity oocytes retrieved in the NOA group was more than in the OA group (12.6±5.0 *vs.* 11.2±5.9 respectively, $P=0.02$). The normal fertilization rate was significant lower in the NOA group than in the donor sperm group (63.0% *vs.* 70.3% respectively, $P=0.005$); and lower in the NOA group than the OA group (63.0% *vs.* 71.48% respectively, $P<0.001$). The high-quality embryo rate and implantation rate in the donor sperm group (52.8% and 55.6%, respectively) were significant higher, compared with the NOA group (44.4%, $P=0.014$ and 34.3%, $P<0.001$, respectively) and the OA groups (46.5%, $P=0.014$ and 43.1%, $P=0.009$, respectively). The clinical pregnancy rates and live birth rates were significant lower in the NOA group than the donor sperm group (49.1% *vs.* 71.3%, $P=0.014$ and 24.6% *vs.* 41.3%, $P=0.043$, respectively), but not significantly lower than the OA group; whereas biochemical pregnancy rate, miscarriage rate, and ongoing pregnancy rate did not differ among the groups. The clinical pregnancy rates and live birth rates were similar between the OA group and the donor sperm group.

Of the 18 children delivered after ICSI using testicular spermatozoa from male patients with NOA, there were 10 singletons and 8 twins. The premature birth rate and low birthweight

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 1. Baseline characteristics of the study groups.

	NOA	OA	*P*-value
Patients (n)	99	126	
Male age	30.6±4.5 (23–42)	31.0±6.0 (22–53)	0.538
Testicular volume (ml)	6.2±3.7 (1.0–21.0)	13.9±3.5 (6.0–27.5)	<0.001*
FSH (mIU/ml)	24.6±14.7 (1.7–67.8)	4.9±3.0 (0.6–19.7)	<0.001*
LH (mIU/ml)	13.0±8.4 (0.2–47.6)	4.4±2.5 (1.3–14.0)	<0.001*
Testosterone (nmol/l)	11.4±7.0 (1.3–39.5)	13.6±7.2 (2.2–39.6)	0.026*
Inhibin B (pg/ml)	44.2±54.0 (0–243.4)	182.5±84.5 (64.5–268.6)	<0.001*
Sperm retrieval rate (%)	38.4	100	<0.001*
Etiologies			
47, XXY	17	–	
AZFc microdeletions	3	–	
Didymits	4	–	
Cryptorchidism	8	–	
Idiopathic form	67	–	
CBAVD	–	12	
Genital tract Infection	–	26	
Mullerian/utricular cysts	–	2	
Vasectomy	–	3	
Undiagnosed	–	83	

* Statistically significant. FSH – follicle-stimulating hormone; LH – luteinizing hormone; NOA – nonobstructive azoospermia; OA – obstructive azoospermia; CBAVD – congenital bilateral absence of the vas deferens; AZF – azoospermia factor.

Table 2. Baseline characteristics in nonobstructive azoospermia men with positive SRR and negative SRR.

	Positive SRR	Negative SRR	P-value
Patients (n)	38	61	
Male age	30.6±4.6 (23–41)	30.5±4.4 (23–42)	0.953
Testicular volume (ml)	6.2±4.4 (1.0–21)	6.2±3.2 (1.0–12.5)	0.988
FSH (mIU/ml)	24.7±14.2 (1.7–58.7)	24.5±15.1 (4.5–67.8)	0.953
LH (mIU/ml)	13.5±8.1 (0.2–35.3)	12.7±8.7 (3.2–47.6)	0.655
Testosterone (nmol/l)	10.5±5.8 (3.5–26.3)	18.7±52.0 (1.3–39.5)	0.335
Inhibin B (pg/ml)	44.6±48.9 (0.8–198.3)	42.1±57.3 (0–243.4)	0.676
Histopathology			
Sertoli-cell only (%)	16.7 (7/42)	83.3 (35/72)	–
Maturation arrest (%)	28.6 (2/7)	71.4 (5/7)	–
Hypospermatogenesis (%)	100 (15/15)	0 (0/15)	–
Hyalinization (%)	51.9 (14/27)	48.1 (13/27)	–

FSH – follicle-stimulating hormone; LH – luteinizing hormone; SRR – sperm retrieval rate.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 3. ICSI outcome in NOA men with successful and failed sperm retrieval (donor sperm), and obstructive azoospermia.

	Nonobstructive azoospermia		Obstructive azoospermia
	Positive sperm retrieval rate	Donor sperm	
Number of cycles	44	62	145
Female age	29.3±4. 3(21–39)	28.7±3.7 (21–38)	28.9±4.5 (21–44)
Oestradiol on HCG administration day (pg/mL)	2952.6±1461.9 (741–6765)	2949.6±1660.4 (892–8181)	3304.3±2198.0 (242–15200)
Progesterone on HCG administration day (ng/mL)	1.1±0.4 (0.2–2.0)	1.1±1.5 (0.3–12.2)	1.0±0.4 (0.2–2.9)
MII oocytes (n)	12.6±5.0 (3–23)	13.4±6.7 (2–36)	11.2±5.9 (1–28)[c]
Two-pronuclear zygote (n)	7.9±4.4 (1–21)	9.4±5.0 (1–23)	8.0±5.0 (1–22)
Nomal fertilization rate (%)	63.0[a]	70.3[b]	71.5
High quality embryo rate (%)	44.4[a]	52.8	46.5[c]
Implantation rate (%)	34.3[a]	55.6	43.1[c]
Biochemical pregnancy rate (%)	12.3	11.3	7.9
Clinical pregnancy rate (%)	49.1[a]	71.3	59.7
Miscarriage rate (%)	20.7	15.8	16.7
Ongoing pregnancy rate (%)	15.8	16.3	9.4
Live birth rate (%)	24.6[a]	41.3	38.2

[a] Statistically significant between positive SRR and donor sperm groups ($P<0.05$; Chi-square test); [b] Statistically significant between positive SRR and OA groups ($P<0.05$; Chi-square test); [c] Statistically significant between donor sperm and OA groups ($P<0.05$; Chi-square test).

Table 4. Outcome of neonates born after ICSI in NOA men with successful and failed sperm retrieval (donor sperm), and obstructive azoospermia.

	Nonobstructive azoospermia		Obstructive azoospermia
	Positive sperm retrieval rate	Donor sperm	
Delivers (n)	18	46	104
Singleton	10 (55.6%)	20 (43.5%)	46 (44.2%)
Twin	8 (44.4%)	26 (56.5%)	58 (55.8%)
Premature birth (%)	3 (16.7%)	9 (19.6%)	28 (26.9%)
Singleton	1 (10%)	1 (5.0%)	6 (13.0%)
Twin	2 (25%)	8 (30.8%)	26 (44.8%)
Low birthweight	0	4 (6.3%)	18 (17.3%)
Singleton	0	1 (5%)	2 (4.3%)
Twin	0	3 (12%)	16 (27.6%)
Malformations	0	0	1 (1%)
Stillbirths	0	1 (2.2%)	4 (3.8%)

ICSI – Intracytoplasmic sperm injection; NOA – nonobstructive azoospermia.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

rate in the NOA group (16.7% and 0%, respectively) were not higher than the OA group (26.9% and 17.3%, respectively) or the donor sperm group (19.6% and 6.3%, respectively). No baby was stillborn or had malformations in the NOA group. One baby (2.2%) was stillborn due to megabladder in the donor sperm group. In the OA group, 2 pair of twins (3.8%) died shortly after their premature birth (gestational age of 24 weeks and 28 weeks, respectively) and 1 baby (1.0%) had hypospadias (Table 4).

Discussion

Overall, ICSI makes it possible for male patients with NOA to achieve successful and healthy pregnancies. Understanding more about the impact of patients with NOA on ICSI outcomes will not only help us predict patient outcomes but it can provide better counsel for our patients. The main concern of the male patients with NOA involved 2 aspects: first was the SRR and the ICSI outcomes, and second was the risk of congenital anomaly in children born from a patient with testicular failure.

In the literature, there are many reports focused on predicting the chance of retrieving sperm in male patients with NOA. Paternal age, testicular volume, and FSH have limited value for predicting successful micro-TESE [23–25]. Testicular histological and body mass index seem to be a useful predictor for successful TESE, although the SRR varies greatly [26,27]. Almost all males with hypospermatogenesis pattern can obtain spermatozoa after micro-TESE; the SRR in patients with Sertoli cell-only and patients with maturation arrest had a wide range [27–29]. But there are no differences in pregnancy and live birth rates among different pathological patterns [29].

Our data showed a significantly lower normal fertilization rate using sperm from NOA male patients compared with OA male patients, but not significantly lower rates in pregnancy outcomes. This agreed with previous reports [9–11]. Having similar pregnancy outcomes does not mean that males with testicular failure have no effect on ICSI outcomes. In the literature, there have been few studies comparing ICSI outcomes between testicular sperm and donor sperm in NOA patients [13]. In the present study, donor sperm were used on the day of oocyte retrieval when no spermatozoa were identified by micro-TESE on the day before oocyte retrieval. In this study, male patients with NOA who used testicular sperm had significant lower fertilization rate, clinical pregnancy rate, and live birth rate than NOA patients who used donor sperm.

However, previous studies have shown conflicting results. Some studies have reported a significantly lower fertilization rat and clinical pregnancy rate for NOA patients [13,14], and other studies found similar fertilization and pregnancy outcomes [15,17]. The possible reasons for the differences might be that a successful ICSI requires only a few motile sperm and normal sperm morphology [30]. Kanto et al. observed first that fresh motile testicular sperm retrieved from NOA patients might have the same potential to achieve fertilization and pregnancy as sperm retrieved from OA patients [16]. Second, only the best quality embryos morphologically would be selected for transfer by the embryologist. Third, increased aneuploidy rate in testicular spermatozoa from patients with NOA carries deficiencies involving genetic material to affect fertilization and embryo development [20,21]. Magli et al. showed that the high incidence of chromosomal abnormalities in NOA patients was mainly due to mosaicism and gonosomal aneuploidy, but that analysis was performed on day-3 embryos and with the use of 9-chromosome fluorescent *in situ* hybridization technique [31]. Mazzilli et al. also showed severe male factor impaired early embryonic competence regarding fertilization rate and developmental potential. However, the euploidy rate and implantation potential of the obtained blastocysts were independent from sperm quality [32]. Therefore, although the best sperm for ICSI and the best embryos for transfer can eliminate differences between OA and NOA groups, ICSI outcomes are still uncertain and diverse due to the impaired sperm and unknown underlying causes of the NOA patients.

An additional concern is the health of the newborn from patients with NOA. Similar to previous literature reports [19], no difference was observed in our study regarding premature birth rate, low birth weight rate, congenital malformation rate, or still birth rate among groups. One baby in our study donor group died during the first 7 days because of megabladder. In the OA group, the stillbirth rate was high because one pair of twins died due to very premature birth (gestational age of 24 weeks) and the other twins died due to the premature birth (gestational age of 28 weeks). However, there was no malformations or still birth born infants in the NOA group. We concluded that neonatal parameters after ICSI did not seem to be affected by testicular failure.

The limitations of this present study were the low number of cases in the NOA group and the long-term outcomes of children. Future studies should focus on the long-term follow-up of the neonates, especially malformed babies.

Conclusions

The use of testicular sperm from male patients with NOA negatively affected fertilization and pregnancy outcomes. NOA patients achieved better fertilization and pregnancy outcomes by using donor sperm, but the chance of becoming a biological father was lost. Once a live birth was achieved, there was no difference in neonatal outcomes.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Conflict of interest

None.

References:

1. Jarow JP, Espeland MA, Lipshultz LI: Evaluation of the azoospermic patient. J Urol, 1989; 142: 62–65
2. Willott GM: Frequency of azoospermia. Forensic Forensic Sci Int, 1982; 20: 9–10
3. Palermo G, Joris H, Devroey P, Van Steirteghem AC: Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. Lancet, 1992; 340: 17–18
4. Craft I, Bennett V, Nicholson N: Fertilizing ability of testicular spermatozoa. Lancet, 1993; 342: 864
5. Devroey P, Liu J, Nagy Z et al: Pregnancies after testicular sperm extraction and intracytoplasmic sperm injection in non-obstructive azoospermia. Hum Reprod, 1995; 10: 1457–60
6. Silber SJ, Van Steirteghem AC, Lui J et al: High fertilization and pregnancy rates after intracytoplasmic sperm injection with spermatozoa obtained from testicular biopsy. Hum Reprod, 1995; 10: 148–52
7. Schlegel PN: Testicular sperm extraction: Microdissection improves sperm yield with minimal tissue excision. Hum Reprod, 1999; 14: 131–35
8. Amer M, Ateyah A, Hany R, Zohdy W: Prospective comparative study between microsurgical and conventional testicular sperm extraction in obstructive non-azoospermia: Follow-up by serial ultrasound examinations. Hum Reprod, 2000; 15: 653–56
9. Palermo GD, Schlegel PN, Hariprashad JJ et al: Fertilization and pregnancy outcome with intracytoplasmic sperm injection for azoospermic men. Hum Reprod, 1999; 14: 741–48
10. Nicopoullos JDM, Gilling-Smith C, Almeida PA, Ramsay JWA: The results of 154 ICSI cycles using surgically retrieved sperm from azoospermic men. Hum Reprod, 2004; 19: 579–85
11. Bocca S, Moussavi V, Brugh V et al: ICSI outcomes in men undergoing TESE for azoospermia and impact of maternal age. Andrologia, 2017; 49: e12617
12. Vernaeve V, Tournaye H, Osmanagaoglu K et al: Intracytoplasmic sperm injection with testicular spermatozoa is less successful in men with non-obstructive azoospermia than in men with obstructive azoospermia. Fertil Steril, 2003; 79: 529–33
13. Esteves SC, Prudencio C, Seol B et al: Comparison of sperm retrieval and reproductive outcome in azoospermic men with testicular failure and obstructive azoospermia treated for infertility. Asian J Androl, 2014; 16: 602–6
14. Yalcin I, Berker B, Sukur YE et al: Comparison of intracytoplasmic sperm injection with testicular spermatozoa success in infertile men with obstructive and non-obstructive azoospermia; A retrospective analysis. Hum Fertil, 2017; 20: 186–91
15. Devroey P, Nagy P, Tournaye H et al: Outcome of intracytoplasmic sperm injection with testicular spermatozoa in obstructive and non-obstructive azoospermia. Hum Reprod, 1996; 11: 1015–18
16. Kanto S, Sugawara J, Masuda H et al: Fresh motile testicular sperm retrieved from nonobstructive azoospermic patients has the same potential to achieve fertilization and pregnancy via ICSI as sperm retrieved from obstructive azoospermic patients. Fertil Steril, 2008; 90(5): 2010.e5-7
17. Tsai YR, Huang FJ, Lin PY et al: Clinical outcomes and development of children born to couples with obstructive and nonobstructive azoospermia undergoing testicular sperm extraction-intracytoplasmic sperm injection: A comparative study. Taiwan J Obstet Gynecol, 2015; 54: 155–59
18. Vernaeve V, Bonduelle M, Tournaye H et al: Pregnancy outcome and neonatal data of children born after ICSI using testicular sperm in obstructive and non-obstructive azoospermia. Hum Reprod, 2003; 18: 2093–97
19. Belva F, De Schrijver F, Tournaye H et al: Neonatal outcome of 724 children born after ICSI using non-ejaculated sperm. Hum Reprod, 2011; 26: 1752–58
20. Bernardini L, Gianaroli L, Fortini D et al: Frequency of hyperhypohaploidy and diploidy in ejaculate, epididymal and testicular germcells of infertile patients. Hum Reprod, 2000; 15: 2165–72
21. Martin RH, Greene C, Rademaker A et al: Chromosome analysis of spermatozoa extracted from testes of men with non-obstructive azoospermia. Hum Reprod, 2000; 15: 1121–24
22. Brinsden PR: A textbook of *in vitro* fertilization and assisted reproduction. New York, The Parthenon Publishing Group Inc., 1999; 196
23. Ramasamy R, Lin K, Gosden LV et al: High serum FSH levels in men with nonobstructive azoospermia does not affect success of microdissection testicular sperm extraction. Fertil Steril, 2009; 92: 590–93
24. Bryson CF, Ramasamy R, Sheehan M et al: Severe testicular atrophy does not affect the success of microdissection testicular sperm extraction. J Urol, 2014; 191: 175–78
25. Ramasamy R, Trivedi NN, Reifsnyder JE et al: Age does not adversely affect sperm retrieval in men undergoing microdissection testicular sperm extraction. Fertil Steril, 2014; 101: 653–55
26. Ramasamy R, Bryson C, Reifsnyder JE et al: Overweight men with nonobstructive azoospermia have worse pregnancy outcomes after microdissection testicular sperm extraction. Fertil Steril, 2013; 99: 372–76
27. Abdel Raheem A, Garaffa G, Rushwan N et al: Testicular histopathology as a predictor of a positive sperm retrieval in men with non-obstructive azoospermia. BJU Int, 2013; 111: 492–99
28. Tsujimura A, Matsumiya K, Miyagawa Y et al: Conventional multiple or microdissection testicular sperm extraction: A comparative study. Hum Reprod, 2002; 17: 2924–29
29. Guler I, Erdem M, Erdem A et al: Impact of testicular histopathology as a predictor of sperm retrieval and pregnancy outcome in patients with non-obstructive azoospermia: Correlation with clinical and hormonal factors. Andrologia, 2016; 48: 765–73
30. Bartoov B, Berkovitz A, Eltes F et al: Pregnancy rates are higher with intracytoplasmic morphologically selected sperm injection than with conventional intracytoplasmic injection. Fertil Steril, 2003; 80: 1413–19
31. Magli MC, Gianaroli L, Ferraretti AP et al: Paternal contribution to aneuploidy in preimplantation embryos. Reprod Biomed Online, 2009; 18: 536–42
32. Mazzilli, R, Cimadomo, D, Vaiarelli A et al: Effect of the male factor on the clinical outcome of intracytoplasmic sperm injection combined with preimplantation aneuploidy testing: Observational longitudinal cohort study of 1219 consecutive cycles. Fertil Steril, 2017; 108: 961–72

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]