

Original Article

Intracytoplasmic sperm injection (ICSI) outcomes of azoospermia with different causes: 107 cases report

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Abstract: This retrospective analysis compared the outcomes of fertilization and pregnancy rates of 107 azoospermia patients treating with intracytoplasmic sperm injection (ICSI). Sperms were obtained by testicular biopsy surgery, with which we used in ICSI subsequently. The outcomes were compared by different kinds of causes leading to azoospermia in the 107 cases. 69 cases of obstructive azoospermia and 38 cases non-obstructive, the fertilization rates were 61.94% and 53.47% respectively, and pregnancy rates were 67.65% and 52.63%. 78 cases with normal volume testes and 29 cases with small testes, the fertilization rates were 70.93% and 48.80% respectively, and pregnancy rates were 66.25% and 50.00%. There was significant difference in fertilization rates between obstructive, non-obstructive and normal volume testes, small testes ($P < 0.05$), but no significant difference in pregnancy rates ($P > 0.05$). The pregnancy rate was significant difference between female age < 32 and ≥ 32 whatever the cause of azoospermia was ($P < 0.05$). Our study reveals that obstructive azoospermia and normal volume testes have higher fertilization rates in ICSI, but the pregnancy rates are only related to female age.

Keywords: Azoospermia, male infertility, different causes, ICSI outcomes

Introduction

The true incidence of male infertility is unknown due to great variability in the prevalence of infertility, however, male factor accounts for 40-50% of infertility cases [1]. 10-20% in the male factors is azoospermia [2, 3]. Classically azoospermia is categorized as non-obstructive and obstructive, but in clinic we also see azoospermia with different testis volume, small or normal volume. Small testis and non-obstructive azoospermia reveal a lower spermatogenesis function, while normal volume and obstructive considered being normal spermatogenesis [4, 5]. We retrospectively reviewed the ICSI outcomes of 107 infertility couples due to azoospermia in our Reproductive Medical Centre between 2012 and 2013 to see if any differences between different causes of azoospermia.

Materials and methods

Object of study

We performed a retrospective study of ICSI patients due to azoospermia from the Reproductive Medical Centre of 2nd affiliated hospital of Chengdu University of Traditional Chinese Medicine during January of 2012 to December of 2013. 107 couples were included and couples with female uterus and ovary factors were excluded.

Patients and grouping

The male ages arrange from 24 to 61 years old, mean as 35.11 ± 7.97 years old, and female ages arrange from 20 to 45 years old, mean as 30.72 ± 6.36 years old. Periods of infertility arrange from 1 to 24 years, mean as 4.45 ± 4.74 years.

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Azoospermia with normal volume testis and small testis. 78 with normal volume testis in 107, ages arrange from 24 to 59, mean as 34.71 ± 1.80 years old, periods of infertility arrange from 1 to 24 years, mean as 4.77 ± 1.77 years. The rest 29 with small testis, ages arrange from 24 to 61, mean as 35.93 ± 2.98 years old, periods of infertility arrange from 1 to 17 years, mean as 5.37 ± 1.48 years. 19 of lower spermatogenesis, 2 chromosome micro-deletions, 2 endocrine abnormalities, 5 cryptorchid and 1 testis injury in small testis group. 47 of obstructive, 11 absences of the vas deferens, 14 lower spermatogenesis and 6 orchiditis in normal volume testis group.

Non-obstructive and obstructive azoospermia. There were 69 obstructive azoospermia patients with ages arrange from 24 to 56, which mean as 35.03 ± 1.92 years old, mean period of infertility was 6.24 ± 1.76 years. And 38 non-obstructive azoospermia ones with ages arrange from 24 to 61, which mean as 35.92 ± 1.43 years old, mean period of infertility was 5.23 ± 1.21 years.

A lot of studies have confirmed that the ICSI outcomes were affected by the female's age [6]. So we also analyzed the ICSI outcomes according to different ages of the female, combined with different causes of azoospermia of male.

Size of testis

World Health Organization standardized examination of male infertility diagnosis and treatment manual (2007 edition) points out that testicular volume reveals insufficient of testis seminiferous epithelium. For the white race, less than 15 ml are considered small testicular volume, while other races are not. Testis volume varies greatly between the different races. As general speaking, volume less than 3 ml are mostly considered to be Klinefelter's Syndrome. The testis volume of Low Gonadotrophin-Hypogonadism patients arrange from 5 to 12 mL. In our study we considered the small testis volume are ≤ 12 mL [7].

Testicular biopsy sperm retrieval surgery

3-4 ml of 2% Lidocaine for nerve block anesthesia of spermatic cord and subcutaneous of incision on the operational side; Hold the testis

and epididymis, and keep the epididymis far away from the incision in case of injury, then fasten the skin, cut open the skin, dartos and perididymis; Cut open the albuginea for 2-3 mm, squeeze and cut down proper amount of tissues of the testis with the eye scissors, put it in the sperm culture media; Discerp the tissue and look for sperm under inverted microscope; Sew the incision.

Fertilization, cleavage and pregnancy diagnosis

Pronuclear formation was monitored after 12-24 h of ICSI operation. Embryo transplantation was operated 72 h after getting the eggs, the level of HCG in urinary and blood were checked 2 weeks later and color Doppler ultrasound detected 4 weeks later, pregnancy diagnosed while we found the fertilized egg [8].

Fertilization rate and rate of pregnancy

Fertilization rate = numbers of fertilized ovum/ numbers of eggs for ICSI operation.

Rate of pregnancy = amount of pregnancy cycle/amount of embryo transplantation [9].

Statistical analysis

The statistical analysis was performed with SPSS for Mac OS (version 20.0, SPSS Inc., Chicago, IL, USA). Sample averages of male ages, female ages, infertile ages and the size of testis were given as mean \pm standard deviation (SD). Chi-square test was applied to analyze fertilization rates and rate of pregnancy. P value < 0.05 indicates that a statistical difference.

Results

There were 69 obstructive azoospermia patients of male infertility, the amount of fertilization eggs were 454 in 733 (61.94%), and 68 ICSI cycles were done in 69 obstructive azoospermia couples, with a result of 46 pregnancies (67.65%), in which 19 twins and 27 singles, 4 got diapauses and 2 induced labor afterward. There were 38 non-obstructive azoospermia patients of male infertility couples, the amount of fertilization eggs were 185 in 346 (53.47%), and 38 cycles done in non-obstructive group, with a result of 20 got pregnant (52.63%) in which 12 twins and 8 singles, 1 diapauses and 1 ectopic pregnancy. See **Table 1**.

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Table 1. ICSI outcomes of obstructive and non-obstructive azoospermia

	N	Amount of eggs for ICSI	Fertilization amount	Rate of fertilization (%)	Embryo Transfer Cycles	The number of pregnancy	Rate of pregnancy (%)
Obstructive	69	733	454	61.94**	68	46	67.65*
Non-obstructive	38	346	185	53.47**	38	20	52.63*

*P > 0.05. Compared with the rates of pregnancy on obstructive and non-obstructive azoospermia, there had no significant difference. **P < 0.05. Compared with the rates of fertilization on obstructive and non-obstructive azoospermia, significant difference existed.

Table 2. ICSI outcomes of normal testis and micro-orchidia azoospermia

	N	Size of testis (ml)	Amount of eggs for ICSI	Fertilization amount	Rate of fertilization (%)	Embryo Transfer Cycles	The number of pregnancy	Rate of pregnancy (%)
Micro-orchidia	29	10.18±1.08	250	122	48.80*	26	13	50.00**
Normal testis	78	18.09±0.85	829	588	70.93*	80	53	66.25**

*P < 0.05. The rates of fertilization of the two groups were significant difference. **P > 0.05. The rates of pregnancy on normal size testis azoospermia patients and micro-orchidia there had no significant difference.

We got the results between the rate of fertilization and pregnancy in two groups were 61.94% v.s. 53.47% with the value $P < 0.05$, and 67.65% v.s. 52.63% with the value $P > 0.05$.

In the 107 cases of infertile couples, 29 cases of males were been tested with micro-orchidia, while the testicular size of rest 78 males were normal. The amount of fertilization eggs in the former was 122 in 250 (48.80%), and 588 in 829 (70.93%) in the latter. And 80 embryo transfer cycles done in normal testis group and 53 couples got pregnancy (66.25%) with 22 twins and 31 singles, 3 got diapauses and 2 induced labors due to fetus abnormalities, 1 ectopic pregnancy. Micro-orchidia group completed 26 cycles and 13 were pregnancy (50.00%) with 7 twins and 6 singles, 1 diapause. See **Table 2**.

The conclusion were been drawn with SPSS 20.0 software between the rate of fertilization and pregnancy in two groups were 48.80% v.s. 70.93% with the value $P < 0.05$, and 50.00% v.s. 66.25% with the value $P > 0.05$.

ICSI outcomes according to different female ages and testis condition were listed in **Tables 3** and **4**. Based on the age of the female, we classified 107 women into two groups: < 32 year-old and ≥ 32 year-old. So there were 4 groups in all for analyzing the rate of fertilization and pregnancy based on different women ages and testes conditions.

In the group of the female age < 32, the amount of fertilization eggs in normal testis male patients was 457 in 622 (73.47%), and 78 in 172 (45.35%) in micro-orchidia patients, the value $P < 0.05$ between them. And 74 embryo transfer cycles were done in normal testis group and 40 couples had pregnancy (54.05%), while the number of pregnancy eggs in normal testis male patients was 12 in 24 (50.00%), and the value $P > 0.05$. In the group of the female age ≥ 32 , the rate of fertilization and pregnancy between normal testis and micro-orchidia male patients were 63.29% v.s. 53.85%, $P > 0.05$ and 34.29% v.s. 18.18% $P > 0.05$ in respectively.

In the group of normal testis azoospermia, the amount of fertilization eggs in the female age of < 32 was 457 in 622 (73.47%), and 131 in 207 (63.29%) in the female age of ≥ 32 couples, the value $P < 0.05$ between them. And 74 embryo transfer cycles were done in the female age of < 32 and 40 couples had pregnancy (54.05%), while the number of pregnancy eggs in the female age of ≥ 32 was 12 in 35 (34.29%), and the value $P < 0.05$. In the group of micro-orchidia azoospermia, the rate of fertilization and pregnancy between the female age of < 32 and ≥ 32 were 45.35% v.s. 53.85%, $P > 0.05$ and 50.00% v.s. 18.18% $P < 0.05$ in respectively.

Discussion

About 50% infertile couples are caused by male factors, and we call it male infertility. Average

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Table 3. ICSI outcomes of normal testis and micro-orchidia azoospermia (different ages of the female)

Ages of the female	Size of testis grouping	N	Amount of eggs for ICSI	Fertilization amount	Rate of fertilization (%)	Embryo Transfer Cycle	The number of pregnancy	Rate of pregnancy (%)
< 32	Normal testis	47	622	457	73.47*	74	40	54.05*
	Micro-orchidia	15	172	78	45.35*	24	12	50.00*
≥ 32	Normal testis	31	207	131	63.29**	35	12	34.29**
	Micro-orchidia	14	78	42	53.85**	22	4	18.18**

*In the group of female age < 32, the rates of fertilization on normal testis azoospermia patients and micro-orchidia had significant difference (P < 0.05), while the rates of pregnancy had no significant difference (P > 0.05). **In the group of female age ≥ 32, the rates of fertilization on normal testis azoospermia patients and micro-orchidia had no significant difference (P > 0.05), also the rates of pregnancy had no significant difference (P > 0.05).

Table 4. ICSI outcomes of different partner ages (normal testis and micro-orchidia azoospermia)

Size of testis grouping	Ages of the female	N	Amount of eggs for ICSI	Fertilization amount	Rate of fertilization (%)	Embryo Transfer Cycle	The number of pregnancy	Rate of pregnancy (%)
Normal testis	< 32	47	622	457	73.47*	74	40	54.05*
	≥ 32	31	207	131	63.29*	35	12	34.29*
Micro-orchidia	< 32	15	172	78	45.35**	24	12	50.00**
	≥ 32	14	78	42	53.85**	22	4	18.18**

*In the group of normal testis azoospermia, the rates of fertilization on the female age < 32 and ≥ 32 had significant difference (P < 0.05), and the rates of pregnancy also had significant difference (P < 0.05). **In the group of micro-orchidia azoospermia, the rates of fertilization on the female age < 32 and ≥ 32 had no significant difference (P > 0.05), but the rates of pregnancy had significant difference (P < 0.05).

account of sperm in one ejaculation in reproductive aged man reached 96 million, but azoospermia was always been considered as lack of sperm in an ejaculation with unknown etiology, which accounts for 1% in males, 2/3 of them has spermatogenic failure, which is incurable and considered to be the most serious type of male infertility [10]. ICSI treatment is the most effective way to get a child for these azoospermia males through testicular sperm extraction. The count and quality of sperms we take from testis directly affect the outcomes of ICSI.

Azoospermia is classically classified by obstructive and non-obstructive, the former always tells the normal spermatogenesis, while the later impaired or disordered spermatogenesis [11].

The volume of testis in micro-orchidia patients is always less than 12 ml, which indicates lack of seminiferous epithelium, low ability of fertility and sperm quality, some even show as azoospermia. Hormone treatment is most widely used for micro-orchidia patients to enhance sperm production, but hardly have satisfied

effect. ICSI would be a better choice for these patients at last if we can find sperms in their testis.

A sperm is directly injected into an egg by ICSI, so it is easy to fertilize. More and more studies have also shown that sperm vitality and quantity is not so important with the rates of fertilization and pregnancy in ICSI. Sperms from different origins are used in ICSI now, not only the ejaculated, but also from the epididymis and testis, and the pregnancy outcomes have no difference if the sperms were appropriated treated [6]. The outcomes in different testis volume groups also tell that the pregnancy rate is not related with testis volume in ICSI. The motility, morphology, maturity of the sperm and spermatogenesis of the testis may not have relations to ICSI pregnancy rate. New researches has showed that sperm DNA fragments maybe more important in ICSI.

The rates of fertilization have obvious statistical difference between obstructive-non-obstructive and normal size-micro orchidia testis groups (61.94% v.s. 53.47%, P < 0.05 and 70.93% v.s. 48.80%, P < 0.05). This may be

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caused by low ability of activating the eggs due to sperm maturity damage, but the accurate mechanism is still unknown.

Female age is negatively related to ICSI pregnancy rate, which has been confirmed by many studies [12]. In our study we also got the same result, the pregnancy rates had no statistical difference between normal testis and micro-orchidia patients (54.05% v.s. 50.00%, $P > 0.05$; 34.29% v.s. 18.18%, $P > 0.05$) if the female age is in the same period (< 32 or ≥ 32), however, the fertilization rates were not inconsistent, showing us both sides were not correlation (73.47% v.s. 45.35%, $P < 0.05$; 63.29% v.s. 53.85%, $P > 0.05$). Another result tells that the older females have a low ICSI pregnancy rate, shows the normal testis patients with female age < 32 and ≥ 32 have different outcomes (54.05% v.s. 34.29%, $P < 0.05$), the same in micro-orchidia patients (50.00% v.s. 18.18%, $P < 0.05$).

As a conclusion, compared with obstructive and non-obstructive, normal testis and micro-orchidia azoospermia, the former have better fertilization rates but the ICSI pregnancy rates are not different. Female age is important in ICSI, the older the low pregnancy is. ICSI would be the only hope for some of the azoospermia patients especially these with low spermatogenesis function in the future; younger partner is more likely to get pregnancy no matter the cause of azoospermia is.

Disclosure of conflict of interest

None.

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References

[1] Shuai HL, Ye Q, Huang YH, Xie BG. Comparison of conventional in vitro fertilization and intracytoplasmic sperm injection outcomes in patients with moderate oligoasthenoazoospermia. *Andrologia* 2015; 47: 499-504.

- [2] Xian ZY, Li F, Zou YG, Gou WB, Li JM, Mao XM, Tan WL, Zheng SB. Outcomes of azoospermia with different spermatogenesis ability treated by intracytoplasmic injection with sperm from epididymis and testis. *Zhong Guo Nan Ke Xue Za Zhi* 2010; 24: 35-38.
- [3] Gudeman SR, Townsend B, Fischer K, Walters RC, Crain D. Etiology of Azoospermia in a Military Population. *J Urol* 2015; 193: 1318-1321.
- [4] Liu QL, Cai ZM. Research Progress in Pathogenic Factors of Azoospermia. *Mod Diagn Treat* 2004; 19: 1318-1321.
- [5] Zheng JF, Chen XB, Zhao LW, Gao MZ, Peng J, Qu XQ, Shi HJ, Jin XL. ICSI treatment of severe male infertility can achieve prospective embryo quality compared with IVF of fertile donor sperm on sibling oocytes. *Asian J Androl* 2015; 17: 845-849.
- [6] Ashrafi M, Jahanian Sadatmahalleh S, Akhoond MR, Ghaffari F, Zolfaghari Z. ICSI Outcome in Infertile Couples with Different Causes of Infertility: A Cross-Sectional Study. *Int J Fertil Steril* 2013; 7: 88-95.
- [7] Li Z, translated. World Health Organization standardized examination of male infertility diagnosis and treatment manual. Beijing: People's medical publishing house; 2007. pp. 15-16.
- [8] Yan J, Huang G, Sun Y, Zhao X, Chen S, Zou S, Hao C, Quan S, Chen ZJ. Birth defects after assisted reproductive technologies in China: analysis of 15,405 offspring in seven centers (2004 to 2008). *Fertil Steril* 2011; 95: 458-60.
- [9] Xu ZY, Ye BL, Huang XF, Lin WQ, Lin JJ, Zhao JZ. Sperm origin and quality effect on pregnancy outcome after intracytoplasmic sperm injection. *Zhong Guo Nan Ke Xue Za Zhi* 2007; 27: 41-44.
- [10] Esteves SC, Agarwai A. The azoospermic male: current knowledge and future perspectives. *Clinics (Sao Paulo)* 2013; 68 Suppl 1: 1-4.
- [11] Esteves SC. Clinical management of infertile men with non-obstructive azoospermia. *Asian J Androl* 2015; 17: 459-470.
- [12] Korkmaz C, Tekin YB, Sakinci M, Ercan CM. Effects of maternal ageing on ICSI outcomes and embryo development in relation to oocytes morphological characteristics of birefringent structures. *Zygote* 2015; 23: 550-555.