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Original Article

Relationship of paternal age with outcome of percutaneous epididymal sperm aspiration and testicular sperm aspiration: intracytoplasmic sperm injection with obstructive azoospermia

Yang Yu, Qun Wang, Hongguo Zhang, Linlin Li, Dongliang Zhu, Ruizhi Liu

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

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Abstract: *Objective:* The study aimed to compare intracytoplasmic sperm injection (ICSI) outcome of patients with epididymal sperm and testicular sperm in different paternal age with obstructive azoospermia. *Methods:* We retrospectively studied the records of 177 men with obstructive azoospermia who underwent sperm retrieval for ICSI. 71 cases were performed with testicular sperm aspiration (TESA), 106 cases with percutaneous epididymal sperm aspiration (PESA). Patients were divided into three groups according to age: < 30 y (n = 81); 31-34 y (n = 56); and ≥35 y (n = 40). We compared ICSI outcomes in each group with different sperm source. *Results:* There was no difference in maternal age, E_2, and P on HCG administration day, the number of retrieved oocytes, and the number of 2PN in each group. A comparison between TESA versus PESA revealed significant lower high-quality embryo rate in paternal age < 30 years (45.31% *vs.* 54.85%; *P* = 0.031) and in paternal age ≥35 years (36.22% *vs.* 50.46%; *P* = 0.035). Paternal age in the 31-34 years showed a TESA cleavage rate that was significantly lower than the PESA group (93.96% *vs.* 98.58%; *P* = 0.025). However, for the paternal age ≥35 years group, the implantation rate (55.56% *vs.* 29.73%; *P* = 0.034) with TESA was significantly higher than that with PESA; the pregnancy rate (70.59% *vs.* 42.1%; *P* = 0.106) was also higher but there was no significant difference. *Conclusions:* When paternal age is ≥35 years, using testicular spermatozoa has a higher developmental potential than those obtained using epididymal spermatozoa.

Keywords: ICSI, paternal age, PESA, TESA, obstructive azoospermia

Introduction

Azoospermia is the complete absence of sperm in the ejaculate after centrifugation. It is classified into obstructive azoospermia (OA), which is caused by congenital or acquired obstruction, and nonobstructive azoospermia (NOA), which is caused by testicular failure [1]. The incidence rate of azoospermia is about 1% of all men and 10% to 15% of infertile men [2]. The reconstruction of the seminal tract enables the man with OA to achieve pregnancy without assisted reproductive technology (ART) [3]. However, it is not entirely feasible, for example, with CBAVD [4], and the reconstruction has already failed. We can also choose ARTs such as testicular sperm aspiration (TESA) [5], percutaneous epididymal sperm aspiration (PESA) [6], and testicular sperm extraction (TESE) [7] to retrieve sperm for intracytoplasmic sperm injection (ICSI). The method that will produce a better outcome for in vitro fertilization is still under investigation.

For older couples, pregnancy rates decrease and the risk of pregnancy increases as age increases; hence, it is very important to define the influence of paternal age with OA on assisted reproduction outcomes. There are few studies on the effect of paternal age on assisted reproduction. Some studies showed a negative correlation between male age and the rate of pregnancy after limiting women's age [8, 9]. Other studies showed that there was no significant correlation between paternal age and the pregnancy rate in ICSI treatment [10]. For

patients with obstructive azoospermia in ICSI, most of the studies have shown that there is no significant difference in the clinical outcome of ICSI treatment with testicular or epididymis sperm [11, 12]. However, with the increase in the age of the male, the functioning of the epididymis decreases gradually, and the content of DNA fragments increase during the process of sperm storage [13]. A study has shown that with the epididymal sperm in OA, along with age increase, the pregnancy rate and "take-home baby" rate decreased significantly in ICSI [14]. This may be related to the increase in reactive oxygen species in the epididymis and the prolonged contact with the active oxygen species [15]. It is also possible that older men are more likely to harbor damaged sperm [16]. However, there is no study on the effect of paternal age and sperm origin on ART outcome. Thus for the paternal age with OA, outcomes and differences of testicular and epididymal sperm source in ICSI are clinical problems that should be addressed. The present study aimed to assess relative results of ICSI using sperm from the epididymis or testicle in patients with OA from different paternal age ranges.

Materials and methods

Subject

This prospective cohort study included 177 cases with OA from 2011 to 2015 in the Reproductive Medicine Center of the First Clinical Hospital of Jilin University. A total of 71 TESA cases (Group A) were performed, 106 PESA cases (Group B) were performed. The obstructive azoospermia patients were divided three groups by age: men < 30 years old (group A1, n = 26; group B1, n = 55); men 30-34 years old (group A2, n = 24; group B2, n = 32); men ≥35 years old (group A3, n = 21; group B3, n = 19). By performing the examinations such as chromosome, AZF gene, semen analysis, and hormone and testicular biopsy, the patients were diagnosed as having obstructive azoospermia. Female partners had a normal fertility profile (established by evidence from hysterosalpingogram, follicular ultrasound, FSH, antimüllerian hormone). The exclusion criteria were as follows: varicocele, genital tract infection, systemic diseases, abnormal karyotype, Y chromosome microdeletions, and testicular failure cases (abnormal serum FSH, LH, or T level) (Reference range: FSH: 1.5-12.4 mIU/mL, LH: 1.7-8.6 mIU/mL, T: 9.9-27.8 nmol/L).

Percutaneous epididymal sperm aspiration procedure

Percutaneous epididymal sperm aspiration procedure was performed under local anesthesia with 2% lidocaine. A 7-G needle attached to a 5-mL syringe was inserted through the scrotal skin into the epididymal caput. Negative pressure was created by pulling the syringe plunger while the tip of the needle was moved gently in and out of the epididymal duct until a clear fluid entered the syringe. The aspirate was flushed with warm sperm medium for immediate microscopic examination.

Testicular sperm aspiration procedure

Testicular sperm aspiration procedure was performed under local anesthesia with 2% lidocaine. A 21-G 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.

IVF protocol

The long downregulation gonadotropin-releasing hormone agonist protocol was used in the luteal phase. Ovarian stimulation was begun when the following parameters were achieved: serum estradiol (E_2) < 50 pg/mL, FSH < 5 mIU/mL, LH < 5 mIU/mL, endometrium thickness < 5 mm, and follicular diameter < 5 mm. Ovulation was triggered by the administration of HCG when at least two follicles were 18 mm in diameter. Oocytes were retrieved 36-38 h after the administration of HCG, and the embryos were transferred 2 or 3 days later. Serum β-HCG was tested 14 days after transfer. If the β-HCG test was positive, ultrasound was performed 14 days later.

Statistical analysis

All statistical data were analyzed with SPSS, version 17.0 (SPSS Inc.). For quantitative data such as maternal age, retrieved oocytes, 2PN, E_2, and P levels on the day of HCG administration, independent-sample *t* test was used to compare two groups. The qualitative variables such as 2PN cleavage rate, high-quality embryo

Table 1. Outcome of TESA-ICSI of and PESA-ICSI cycle in paternal age < 30 years old group

	TESA	PESA	*P* value
Cycles (n)	26	55	
Maternal age (y)	25±2.40	25.44±2.15	0.444
E_2 on HCG administration day (pg/mL)	3313.45±1949.19	4394.88±2390.97	0.036
P on HCG administration day (ng/mL)	0.897±0.30	0.987±0.37	0.285
Retrieved oocytes (n)	12.69±5.09	13.89±6.62	0.374
2PN (n)	7.5±4.73	8.4±5.22	0.443
2PN cleavage rate (%)	98.97 (192/194)	98.27 (454/462)	0.731
High-quality embryo rate (%)	45.31 (87/192)	54.85 (249/454)	0.031*
Implantation rate (%)	44 (22/50)	41.74 (48/115)	0.864
Clinical pregnancy rate (%)	60 (15/25)	62.71 (37/59)	0.811

*$P < 0.05$ was considered statistically significant.

Table 2. Outcome of TESA-ICSI of and PESA-ICSI cycle in paternal age 30-35 years old group

	TESA	PESA	*P* value
Cycles (n)	24	32	
Maternal age (y)	29.58±3.48	29.59±3.3	0.991
E_2 on HCG administration day (pg/mL)	3729.23±2258.12	4324.32±2409.3	0.365
P on HCG administration day (ng/mL)	1.34±0.98	1.13±0.53	0.358
Retrieved oocytes (n)	13.17±6.93	10.41±3.98	0.9
2PN (n)	7.21±5.13	6.56±3.33	0.594
2PN cleavage rate (%)	93.96 (171/182)	98.58 (209/212)	0.025*
High-quality embryo rate (%)	42.69 (73/171)	48.8 (102/209)	0.256
Implantation rate (%)	48.89 (22/45)	45.45 (30/66)	0.847
Clinical pregnancy rate (%)	58.33 (14/24)	63.64 (21/33)	0.785

*$P < 0.05$ was considered statistically significant.

rate, implantation rate, clinical pregnancy rate were evaluated by the chi-square or Fisher's exact test. $P < 0.05$ was considered statistically significant.

Results

The study cohort consisted of 177 ICSI cycles, TESA used in 71 ICSI cycles (66 ET cycles) and PESA in 106 ICSI cycles (111 ET cycles). Paternal age was < 30 years in 81 cycles (84 ET cycles), 30-34 years in 56 cycles (57 ET cycles), and ≥35 years in 40 cycles (36 ET cycles). In each group, there were no significant differences in terms of maternal age, E_2, and P levels on the day of HCG administration; the number of retrieved oocytes; and 2PN between TESA and PESA cycles.

The high-quality embryo rate was significantly lower when TESA was used than when PESA was used in paternal age < 30 years (45.31% *vs.* 54.85%; $P = 0.031$) (**Table 1**). Similarly, the 2PN cleavage rate was significantly lower after ICSI when TESA was used than when PESA was used in paternal age 30-34 years (93.96% *vs.* 98.58%; $P = 0.025$) (**Table 2**). For paternal age < 30 years and 30-34 years, there were no significant differences in terms of implantation rate and the clinical pregnancy rate (**Tables 1** and **2**). For paternal age of ≥35 years, a comparison between TESA versus PESA indicated a significantly lower high-quality embryo rate (36.22% *vs.* 50.46%; $P = 0.035$), but a significantly higher implantation rate (55.56% *vs.* 29.73%; $P = 0.034$) and a higher clinical pregnancy rate (70.59% *vs.* 42.1%; $P = 0.106$) (**Table 3**), although there were no significant differences (**Figure 1**).

Discussion

With the increase in age, male semen quality is reduced [17]. However, it remains controversial whether the decline in semen quality can affect fertility and pregnancy outcomes of assisted

Table 3. Outcome of TESA-ICSI of and PESA-ICSI cycle in paternal age ≥35 years old group

	TESA	PESA	*P* value
Cycles (n)	21	19	0.444
Maternal age (y)	34.9±4.58	33.53±3.12	0.27
E_2 on HCG administration day (pg/mL)	3542.85±3664.14	3167.14±1785.3	0.52
P on HCG administration day (ng/mL)	1.08±0.35	1.24±0.94	0.491
Retrieved oocytes (n)	8.83±7.58	8.32±3.83	0.78
2PN (n)	5.13±4.05	5.68±3.0	0.614
2PN cleavage rate (%)	96.21 (127/132)	100 (109/109)	0.066
High-quality embryo rate (%)	36.22 (46/127)	50.46 (55/109)	0.035*
Implantation rate (%)	55.56 (20/36)	29.73 (11/37)	0.034*
Clinical pregnancy rate (%)	70.59 (12/17)	42.1 (8/19)	0.106

*$P < 0.05$ was considered statistically significant.

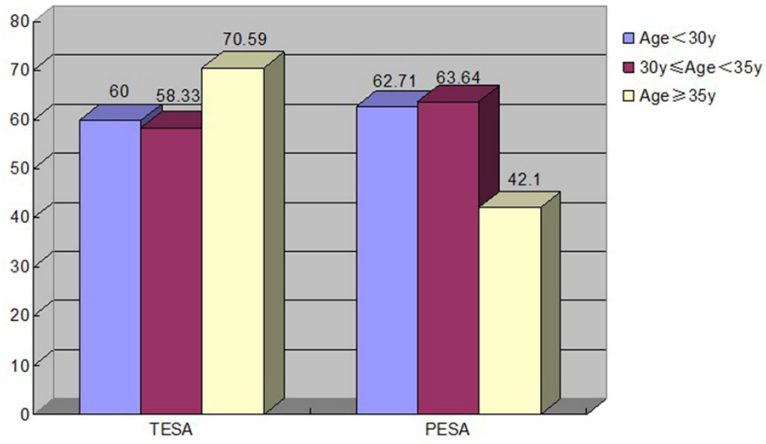

Figure 1. The clinical pregnancy rate (%) of TESA-ICSI and PESA-ICSI cycle in three paternal age groups.

reproduction. There is also no definite evidence to demonstrate a negative effect of paternal age on ART outcomes [18, 19]. The possible reason is that the outcome of ICSI treatment is not dependent on semen quality [20]. A successful ICSI requires only a few active sperm and normal sperm morphology [21]. Through a 400× or 1000-6000× microscope, the best sperms among the sperms with severe morphological abnormalities are screened for ICSI treatment [22]. However, Ferreira *et al*. [23] indicated that paternal age negatively influences the implantation and pregnancy rates in couples in whom the man's sperm concentration was < 20×10^6/mL. John *et al*. [24] found that even with normal donor eggs, if the man's age is greater than 50 years, the pregnancy outcomes and blastocyst formation rates are affected. Wu *et al.*'s [25] analysis of 9,991 *in vitro* fertilization cycles in the 30-34 years maternal age group found that the implantation rate was decreased with increased paternal age and the pregnancy rates were significantly higher in the < 30 and 30-32 paternal age groups than in those in the 36-38 and 39-41 paternal age groups. Our study showed that PESA for the ≥35 paternal age group compared with the < 30 group and 30-34 age group had the following results: lower implantation rate (29.73% *vs.* 41.74% *vs.* 45.45%) and clinical pregnancy rates (42.1% *vs.* 62.71% *vs.* 63.64%). However, TESA did not decrease with increased paternal age, respectively (55.56% *vs.* 44% *vs.* 44.89%; 70.59% *vs.* 60% *vs.* 58.33%) (**Figure 1**). This may be due to the increased retention time of ejaculated sperm and epididymis sperm in the epididymis.

Because of the different sources of sperm, in earlier studies, there was no significant difference between OA obtained by testicular or epididymal sperm for IVF outcomes [10, 11]. Some studies suggest that the sperm of the epididymis is better than that of the testicle. Semião *et al.* [26] noted a lower normal fertilization rate and a higher abortion rate among men in the TESA group than in men in the PESA group who had OA. However, in other studies, as the paternal age increases, the sperm obtained from the epididymis decreased in CBAVD, and the clinical pregnancy rate and "take-baby home" rate

also decreased [14]. This may be due to the advanced age and that epididymal function decline leads to increased DNA fragmentation. This is not only reflected in OA. Mehta et al. [27] hat after repeated failure of ICSI in patients with severe oligozoospermia, which was associated with DNA fragmentation, testicular sperm was used to obtain a 50% pregnancy rate. In our study, a comparison between TESA versus PESA revealed that the high-quality embryo rate significantly decreased with paternal age in < 30 years and ≥35 years. However, a significantly higher implantation rate was observed for the ≥35 years paternal age group. The clinical pregnancy rate of TESA was also better than that of PESA, but there was no significant difference.

In conclusion, we have found in our present study that the choice of TESA over PESA is probably prudent when paternal age is ≥35 years, as testicular spermatozoa have higher developmental potentials than those obtained using epididymal spermatozoa. Because of the limited of the number of cases, there was no further division of paternal age ≥35 years, nor analysis of the "take-baby home" rate and the abortion rate. Further studies should refine the age group to evaluate the effect of advanced paternal age on ICSI.

Disclosure of conflict of interest

None.

Address correspondence to: Ruizhi Liu, Centre for Reproductive Medicine, Centre for Prenatal Diagnosis, First Hospital, Jilin University, 71 Xinmin Street, Changchun 130021, Jilin Province, P. R. China. Tel: +86-431-88782896; Fax: +86-431-85654528; E-mail: lrz410@126.com

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