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Cite this article: Jixiang Z *et al.* (2022) Relationship of sperm motility with clinical outcome of percutaneous epididymal sperm aspiration–intracytoplasmic sperm injection in infertile males with congenital domestic absence of vas deferens: a retrospective study. *Zygote*. **30**: 234–238. doi: 10.1017/S0967199421000587

Received: 19 January 2021
Revised: 12 June 2021
Accepted: 16 June 2021
First published online: 27 July 2021

Keywords:
Congenital bilateral absence of vas deferens; Intracytoplasmic sperm injection; Live birth rate; Percutaneous epididymal sperm aspiration; Sperm motility

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Relationship of sperm motility with clinical outcome of percutaneous epididymal sperm aspiration–intracytoplasmic sperm injection in infertile males with congenital domestic absence of vas deferens: a retrospective study

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Summary

Congenital domestic absence of vas deferens (CBAVD) is a common factor in male infertility, and percutaneous epididymal sperm aspiration (PESA) combined with intracytoplasmic sperm injection (ICSI) is a primary clinical treatment, but the effect of the sperm obtained on pregnancy outcome remains to be explored. This study aimed to investigate the relationship between sperm motility with clinical outcome of PESA–ICSI in infertile males with CBAVD. A cohort of 110 couples was enrolled. In total, 76 infertile males were included in the high motility group, while the remaining 34 males were placed in the low motility group. Clinical pregnancy, embryo implantation rate and live birth rate were included as the primary outcome. After all follow-ups, we found that the high motility group achieved higher normal fertilization rates, cleavage rates, transplantable embryo rates and high-quality embryo rates than those in low motility group (normal fertilization rate, 78.2 ± 11.7% vs. 70.5 ± 10.2%, $P = 0.003$; cleavage rate, 97.1 ± 2.9% vs. 92.3 ± 3.0%, $P = 0.000$; transplantable embryo rate, 66.8 ± 14.9% vs. 58.6 ± 12.6%, $P = 0.009$ and high-quality embryo rate, 49.9 ± 10.5% vs. 40.5 ± 11.2%, $P = 0.000$). Additionally, compared with the low motility group, the clinical pregnancy rates, embryo implantation rates, and live birth rates in the high motility group were significantly increased (pregnancy rate, 61.8% vs. 26.5%, $P = 0.009$; embryo implantation rate, 36.5% vs. 18.0%, $P = 0.044$; live birth rate, 55.3% vs. 17.6%, $P = 0.000$). We concluded that the motility of sperm obtained by PESA affected the clinical outcome of ICSI in infertile males with CBAVD.

Introduction

Male factors accounted for *c.* 30% of all causes of infertility and CBAVD is one of the main factors leading to male infertility (Seo and Ko 2001; Leaver, 2016). Under normal conditions, spermatozoa can be expelled from the body through the vas deferens, and then united with eggs after entering the female genital tract to form an embryo after which the embryo implants in the uterus to form a fetus (Buch, 1994). Due to the absence of vas deferens, patients with CBAVD are unable to excrete, resulting in azoospermia in semen and the lack of ability to complete natural pregnancy (Son *et al.*, 1996). However, PESA or testicular sperm aspiration (TESA) tests could detect sperm in the epididymis or testes in patients with normal levels of sex hormones (Tsirigotis *et al.*, 1996; Gorgy *et al.*, 1998; Abdul-Jalil *et al.*, 2001). At present, PESA and TESA have become the most commonly used and effective methods for the treatment of CBAVD.

The motility of sperm obtained by PESA has often been poor in patients with CBAVD (Emiliani *et al.*, 2000). However, there have been few reports on the effect of sperm motility obtained by PESA or TESA on ICSI outcome. Therefore, the purpose of this study was to investigate the relationship between sperm motility and clinical outcome of PESA–ICSI in infertile men with CBAVD.

Materials and methods

Study population

From October 2012 to December 2017, 110 couples were enrolled in this study. All men were diagnosed as having CBAVD. No sperm was found in the semen after two routine semen examinations. See inclusion and exclusion criteria in Table 1. All patients underwent a PESA test to determine the presence of sperm before entering the treatment cycle. All patients were treated for the first ICSI attempt. This study was approved by the Ethics committee of the hospital, and all patients and their families signed informed consent forms.

Table 1. Inclusion and exclusion criteria

Criteria	Women	Men
Inclusion	Age, <40 years	Age, <50 years
	Negative pregnancy test Negative serum anti-sperm antibody test Normal serum thyrotropin and follicle-stimulating hormone values during the first 5 days of the cycle	CBAVD
Exclusion	Previously use fertilization *in vitro* or other assisted reproductive technology History of chemotherapy or radiation to the abdomen or pelvis History of ovary-related surgery Extensive tubal adhesions Chromosomal abnormality	Varicocelectomy within 6 months before the study Chromosomal abnormality

PESA procedures

PESA was defined as percutaneous epididymal sperm aspiration. All operations were performed by an experienced male surgeon. All patients were placed in the supine position. After routine disinfection and local anaesthesia with 1% lidocaine, the epididymal head was fixed, and a 12-point needle connected with a 5ml syringe was inserted into the epididymal head to extract epididymal fluid. After seeing the yellow turbid epididymal fluid, the needle was withdrawn, and epididymal fluid was slowly pumped into a small dish; sperm were then examined under an inverted microscope. (Abdul-Jalil *et al.*, 2001).

Grouping

The sperm obtained by PESA from all patients were incubated at 37°C in a CO_2 in air incubator for 2 h and then sperm motility was analyzed. Sperm motility were analyzed using a computer-assisted semen analysis system (CASA, CASAS-QH-III, Qing Hua Tong Fang, Beijing, China) under ×200 magnification by one doctor and in accordance with the 5th edition of World Health Organization (WHO) laboratory manual for the examination and processing of human semen. When the sperm count was too low to be analyzed by computer, it was manually counted and analyzed by the same experienced examiner. At least 200 sperm were analyzed for each sample. The samples with forward motility sperm were classified as belonging to the high motility sperm group, while the samples only with non-forward motility sperm or no motility sperm were classified as being in the low motility group.

Ovulation induction

Ovulation induction treatment was conducted using the conventional central ovulation induction programme, and follicle development were monitored by b-mode ultrasound. The follicles were retrieved 36 h after injection of 10,000 IU human chorionic gonadotrophin (hCG, Ovidrelle, Merck Serono, UK), when at least one or two follicles had reached 18 mm in diameter or over three follicles had reached 17 mm in diameter.

ICSI procedures

Hyaluronidase and mechanical methods were used to remove the peripheral granulosa cells 4 h after egg collection, and MII eggs with polar bodies were selected for microinjection. The sperm obtained by PESA from all patients were incubated at 37°C in a CO_2 in air incubator for 2 h and then started ICSI operation. All ICSI procedures were performed by the same doctor (Tsirigotis *et al.*, 1996).

Embryo scoring and transplantation

Fertilization was observed 16–18 h after injection, and normal fertilization was observed for diplokaryotes, cleavage was observed 48 h later, and embryos were graded 72 h later according to the Istanbul consensus criteria for embryo score at cleavage stage in 2011 (Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology, 2011). High-quality embryos were selected for transplantation, and the remaining high-quality embryos were cryopreserved.

Clinical pregnancy diagnostic criteria

Blood HCG > 5 U/l was diagnosed as biochemical pregnancy 14 days after transplantation, and vaginal b-ultrasonography 35 days after transplantation was diagnosed as clinical pregnancy.

Follow-up and outcome measurements

All patients completed 12 months of follow-up by telephone or outpatient records after pregnancy. The rates of live birth, miscarriage or multiple births were recorded in all patients. The general situation, embryo development and pregnancy outcome of the two groups of patients were compared. The general situation included male age, female age, infertility time, the average number of eggs acquired, the average number of embryo transfer, embryo development, including fertilization rate, cleavage rate, pregnancy outcome including clinical pregnancy rate, abortion rate and live birth rate.

Statistical analyses

SPSS 18.0 software was used for statistical analysis. Continuous variables are presented as mean ± standard deviation (SD) and compared by independent sample *t*-test. The chi-squared test or Fisher's exact chi-squared was used for categorical variables. A P-value < 0.05 was considered significant.

Results

Patient characteristics

In total, 110 patients with CBAVD and their spouses were enrolled in this study. Among them, 76 couples were assigned to the high motility group, while the remaining 34 couples were assigned to the low motility group. There were no significant differences between

https://doi.org/10.1017/S0967199421000587 Published online by Cambridge University Press

Table 2. Comparison of general data

Characteristics	High motility group ($n = 76$)	Low motility group ($n = 34$)	*P*-value[a]
Male age (years)	28. 7 ± 2. 2	28. 3 ± 2. 2	0.245
Female age (years)	25. 5 ± 1. 4	25. 4 ± 1. 4	0.393
Infertility duration (years)	2.1 ± 0.5	2.2 ± 0.6	0.257
Testicular volume (ml)	29. 8 ± 2. 0	29. 7 ± 2. 1	0.137
Testosterone (ng/l)	52.2 ± 9.4	51.7 ± 8.9	0.416
Male FSH (IU/l)	6.7 ± 1.9	6.5 ± 1.8	0.742
Number of sperms retrieved ($\times 10^6$/ml)	24.6 ± 8.9	22.9 ± 8.6	0.716
Sperm of normal morphology (%)	8.9 ± 1.3	8.7 ± 1.5	0.672
Number of oocytes aspirated	10.4 ± 1.2	10.6 ± 1.1	0.249
Number of MII oocytes	9.4 ± 1.0	9.4 ± 1.1	0.363
Number of embryos transferred	1.8 ± 0.1	1.8 ± 0.2	0.419

Data are presented as mean ± standard deviation (SD).
[a]Student's *t*-test.

Table 3. Comparison of relevant laboratory indicators

Characteristics	High motility group ($n = 76$)	Low motility group ($n = 34$)	*P*-value[a]
Normal fertilization rate (%)	78.2 ± 11.7	70.5 ± 10.2	0.003
Cleavage rate (%)	97.1 ± 2.9	92.3 ± 3.0	0.000
Transferable embryo rate (%)	66.8 ± 14.9	58.6 ± 12.6	0.009
High-quality embryo rate (%)	49.9 ± 10.5	40.5 ± 11.2	0.000

Data are presented as number (%).
[a]Chi-squared test.

Table 4. Comparison of clinical pregnancy outcome indicators

Characteristics	High motility group ($n = 76$)	Low motility group ($n = 34$)	*P*-value[a]
Clinical pregnancy rate (%)	61.8 (47/76)	26.5 (9/34)	0.009
Embryo implantation rate (%)	36.5 (50/137)	18.0 (11/61)	0.044
Early abortion rate (%)	10.6 (5/47)	33.3 (3/9)	0.174
Live birth rate (%)	55.3(42/76)	17.6(6/34)	0.000

Data are presented as number (%)
[a]Chi-squared test.

the two groups in male age, female age, infertility years, testicular volume, testosterone, male follicle-stimulating hormone (FSH), number of sperms retrieved, sperm of normal morphology, the average number of eggs obtained, numbers of MII eggs and numbers of embryos transplanted ($P > 0.05$). The baseline information is shown in Table 2. All pregnant patients had completed follow-up for 12 months up to December 2018.

Laboratory outcomes

Laboratory outcomes were analyzed for all patients. The normal fertilization rate, cleavage rate, transplantable embryo rate and high-quality embryo rate in the high motility group were significantly higher than those in the low motility group (normal fertilization rate, 78.2 ± 11.7% vs. 70.5 ± 10.2%, $P = 0.003$; cleavage rate, 97.1 ± 2.9% vs. 92.3 ± 3.0%, $P = 0.000$; transplantable embryo rate, 66.8 ± 14.9% vs. 58.6 ± 12.6%, $P = 0.009$ and high-quality embryo rate, 49.9 ± 10.5% vs. 40.5 ± 11.2%, $P = 0.000$), as shown in Table 3.

Clinical outcomes

Clinical pregnancy rate, embryo implantation rate and live birth rate in the high motility group were significantly higher than that in the low motility group (pregnancy rate, 61.8% vs. 26.5%, $P = 0.009$; embryo implantation rate, 36.5% vs. 18.0%, $P = 0.044$; live birth rate, 55.3% vs. 17.6%, $P = 0.000$). Early abortion rate in the high motility group (10.6%) was similar to that in the low motility group (33.3%), as shown in Table 4.

Discussion

With rapid development of assisted reproductive technologies, ICSI technology has became the main method to solve the infertility problem caused by male factors (Plachot *et al.*, 2002; O'Neill *et al.*, 2018). Fertilization is caused by the fusion of sperm and oocyte. However, this process may be affected by several factors such as sperm motility, sperm morphology, or sperm DNA index (García-Roselló *et al.*, 2006). In this study, 110 patients with CBAVD were treated with PESA combined with ICSI. The epididymis is an important part of sperm capacitation and maturation, and the epididymis sperm is more mature. In clinical treatment, it is often found by PESA that a small number of patients have poor sperm motility or that inactivity of sperm cannot be observed under the microscope (Oum *et al.*, 1997). At present, the effects of epididymal and testicular sperm on embryo quality and pregnancy are still controversial (Desai and Goldfarb, 2007; Wood *et al.*, 2003).

We analyzed the clinical outcomes of a series of embryos and pregnancies with different motilities of sperm obtained using PESA after ICSI treatment, and found that the patients in the high motility group achieved higher normal fertilization rates, cleavage rates, transplantable embryo rates, high-quality embryos rates, clinical pregnancy rates, embryo implantation rates and live birth rates. There have been few reports on the effect of different motility epididymal sperm ICSI on fertilization rate and pregnancy rate. Some studies found that normal fertilization rates of epididymal motile sperm were significantly higher than that of epididymal inactivity sperm (Pasqualotto *et al.*, 2002; Sadeghi *et al.*, 2011). The epididymis is the capacitive part of spermatozoa, and epididymis motility sperm have high fertilization ability and developmental potential. However, long-term sperm retention in the epididymis may affect sperm motility (Olds-Clarke, 1996). Sperm with inactivity or poor motility had poor fertilization ability and, even if they could be fertilized, the embryos had poorer developmental potential (Giwercman *et al.*, 2003; Singh *et al.*, 2015). Also, it has been suggested that the DNA fragmentation index (DFI) of sperm with poor motility was higher (Chi *et al.*, 2017). There is a negative correlation between the level of DFI and the

https://doi.org/10.1017/S0967199421000587 Published online by Cambridge University Press

pregnancy rate and the embryo implantation rate of ICSI (Dar *et al*., 2013; Zhang Z *et al*., 2015; Zhang J *et al*., 2019). In addition, excessive production of reactive oxygen species (ROS) in the male reproductive tract can cause a state of oxidative stress. Sperm are extremely sensitive to oxidative attack caused by excessive ROS, which is due to the lack of a cytoplasmic antioxidant enzyme system in sperm, and the damage caused by excessive ROS cannot be repaired in time (Tunc and Tremellen, 2009; Wang *et al*., 2017). The toxic effects of ROS on sperm are mainly reflected in damage to the sperm membrane, DNA and mitochondria, and an increased incidence of various sperm deformities, therefore affecting the fertilization ability of sperm (Donnelly *et al*., 2000; Gavriliouk and Aitken, 2015; Treulen *et al*., 2015).

Sperm motility is associated with increased levocarnitine in the epididymal cavity and levocarnitine in sperm cells (Tang *et al*., 2008). Spermatozoa produced in the testis cannot move and fertilize before entering the epididymis, and the content of levocarnitine is very low. Postgonadal maturation of spermatozoa mainly occurs in the head of the epididymis. During movement of sperm from the head to the tail of the epididymis, with the continuous increase in the content of L-carnitine in the sperm, the sperm gradually matures and its motor ability continuously increases. Studies have shown that exogenous L-carnitine supplementation can down-regulate the levels of TNF-α and ROS in the epididymis in patients with infertility, thereby improving the epididymis environment, sperm quality and ICSI pregnancy outcome (Ng *et al*., 2004; Wu *et al*., 2012; Korosi *et al*., 2017). In this study, due to the limited sample size, it was necessary to accumulate more cases continuously in the follow-up studies. Furthermore, the influence of epididymal sperm motility on outcome of ICSI should be further analyzed for the molecular mechanism.

In conclusion, the motility of sperm obtained by PESA affected the clinical outcome of ICSI. We need to intervene early before entering the ovulation cycle for patients with CBAVD diagnosed by PESA.

Financial support. This research received no specific grant from any funding agency, commercial or not-for-profit sectors.

Conflicts of interest. None.

Ethical standards. The treatment process and data use for all patients were performed under the guidelines of the Ethics Committee of Jiangsu Huaian Maternity and Children Hospital, China.

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