Int J Clin Exp Med 2019;12(8):10712-10720
www.ijcem.com /ISSN:1940-5901/IJCEM0094941

Original Article

Pregnancy and neonatal outcomes after ICSI with testicular, epididymal, or ejaculated sperm: analysis of 2,512 cycles during an 8-year period

Taoran Deng, Na Guo, Longjie Gu, Xiaoqiong Yuan, Yaoyao Du, Xiang Hua, Xuemei Teng, Yangcheng Yao, Yufeng Li

Reproductive Medicine Center, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, Hubei, China

Received April 6, 2019; Accepted July 11, 2019; Epub August 15, 2019; Published August 30, 2019

Abstract: The current study investigated the effects of spermatozoa from different sources on pregnancies and neonatal outcomes in intracytoplasmic sperm injection (ICSI) cycles via a retrospective cohort study. A total of 2,512 ICSI cycles, from 2007 to 2015, were classified into the testicular sperm aspiration group (TESA group, n = 148), percutaneous epididymal sperm aspiration group (PESA group, n = 1,031), and ejaculated sperm group (control group, n = 1,333). Higher abnormal fertilization rates, decreased implantation rates, and elevated early miscarriage rates were observed in the PESA group, compared with the control group. Additionally, lower embryo utilization rates in the PESA group were found, compared with the TESA group and control group. Non-ejaculated sperm produced lower good-quality embryo rates than ejaculated sperm. There were no significant differences in clinical pregnancy rates (CPR), live birth rates (LBR), late miscarriage rates, induced labor rates, cumulative CPR, cumulative LBR, neonatal outcomes, and major congenital birth defect risks between the three groups. The current study revealed some less than satisfactory results caused by PESA-ICSI, compared to ejaculated sperm-ICSI or even TESA-ICSI, possibly due to the retrieval of distal senescent sperm by blind aspiration. Hence, microsurgical epididymal sperm aspiration may be reconsidered as an adequate alternative, retrieving high quality motile sperm from the proximal-most epididymal site.

Keywords: Sperm, ICSI, pregnancy outcomes, neonatal outcome, congenital malformation

Introduction

Intracytoplasmic sperm injection (ICSI) technology has been applied for male infertility over two decades [1]. Sperm can be taken from varied sites for ICSI, such as ejaculated semen, the testis, epididymis, and vas deferens. Currently, most physicians and embryologists prefer percutaneous epididymal sperm aspiration (PESA) to testicular sperm aspiration (TESA) whenever possible. This is due to the better quantity, motility, and maturity of sperm acquired via PESA [2]. Despite the advantages of PESA regarding convenient performance for ICSI, whether epididymal sperm provides better clinical outcomes than testicular sperm remains unknown.

Spermatozoa from different sources differ in maturity, malformations, imprinted genes, chromosomal abnormalities, and many other aspects [2-4]. Therefore, extensive studies have been conducted comparing clinical outcomes after ICSI according to sperm origins. However, results have been inconsistent.

Many single-center studies have reported poorer pregnancy outcomes after using testicular sperm. Clinical pregnancy rates of ICSI with epididymal and testicular sperm are 22%-68% and 24%-57%, respectively [5-10]. Epididymal spermatozoa has been generally considered to be more effective than testicular sperm in terms of pregnancy chances [5-8]. Several studies have demonstrated a higher miscarriage risk of ICSI with testicular sperm than with epididymal sperm [9-11]. However, when all reported results were pool-studied via meta-analyses, most pregnancy outcomes tended to be comparable among ICSI cycles with different sources of

sperm. This might be due to variations in the baseline characteristics of included studies, including female factors, male infertility diagnosis, sperm retrieval techniques, and ICSI procedures [5, 12, 13]. Embryologists should be cautious when referring to the conclusions of systematic reviews.

Moreover, most previous were limited. The studied population was mixed with obstructive azoospermia (OA) and non-obstructive azoospermia (NOA) in some reports. Therefore, a single-center study with a large sample size and clear participant inclusion criteria is necessary, comparing sperm origin. The current retrospective study included ICSI cycles during 8 years of using testicular or epididymal sperm in men with OA, as well as ejaculated sperm in men with severe oligozoospermia, asthenozoospermia, and teratozoospermia. The current study compared embryonic development and pregnancy/neonatal outcomes between ICSI using sperm retrieved from different origins in patients with OA. The aim of the current study was to evaluate the validity of current strategies of sperm retrieval for men with OA.

Materials and methods

Patients

The current study included patients undergoing ICSI at the Reproductive Medicine Center of Tongji Hospital in Wuhan, China, from January 2007 to September 2015. Men with OA were included in TESA and PESA groups. Diagnosis criteria for OA were as follows [14]: (1) At least one testicle volume > 15 mL; (2) FSH < 10 mIU/mL; (3) Seminal plasma biochemistry tests indicating no abnormal spermatogenesis function; and (4) Without diseases affecting testicular function, such as varicocele and enorchia. PESA was the first surgical choice for retrieving sperm in patients with OA. If no motile sperm were found, then TESA was performed. After a fine dissection of testicular tissues with syringes or scissors, the tissue fluid was examined via microscopy, confirming sperm presence and motility. If the spermatozoa retrieved by TESA were non-motile, the tissue fluid was cultured in G-IVFTM PLUS medium at 37°C and 5% CO_2 for several hours. Sperm motility was then evaluated again. Ejaculated sperm were collected from men with severe oligozoospermia, asthenozoospermia, and teratozoospermia, defined as total sperm count < 5 million/mL and/or < 5% rapid progressive type A motility and/or < 4% morphologically normal sperm. Controlled ovarian hyperstimulation (COH) protocol was carried out based on individual infertility history and sex hormone levels. Couples in the TESA group (n = 148) and PESA group (n = 1,031) shared similar baseline characteristics, including female and male age, basic FSH, number of oocytes retrieved, infertility type, and duration. Because there were the fewest patients in TESA group, maternal infertility influences were minimized by matching each TESA case with 9 control cases (using ejaculated sperm), according to female age (± 2 years), basic FSH (± 1 mIU/mL), number of oocytes retrieved (± 3), and duration of infertility (± 2 years). Eventually, a total of 1,333 cycles with ejaculated sperm were enrolled. The current study was approved by the Ethical Committee of Tongji Hospital. Written informed consent concerning ICSI and follow-ups was obtained from all participants.

Outcome measures

Pronuclei (PN) were observed for 16-18 hours after ICSI. The percentage of 2PN among MII (mature) oocytes was calculated as the normal fertilization rate. The total number of 1PN and multi-PN divided by the count of MII oocytes was defined as the abnormal fertilization rate. Cleavage conditions were assessed 24 hours after ICSI. The percentage of cleaved embryos among MII oocytes was defined as the cleavage rate. The proportion of good-quality embryos developed from 2PN of the cleaved embryos was calculated as the good-quality embryo rate. Usually, no more than two good-quality embryos were chosen for transfer on day 3. Excess embryos were cryopreserved or continuously cultured to the blastocyst stage. Embryo utilization rate was identified as the total number of embryos transferred and cryopreserved on day 3 and those cultured past day 3 per number of cleaved embryos. Clinical pregnancy was defined as the visualization of a gestational sac with fetal heart activity on ultrasound examinations 28 days after ET. The percentage of aborted fetuses of the total number of fetuses defined the miscarriage rate. Follow-up information concerning neonatal outcomes, including live birth, birth weight, sex, and congenital malformations, was gathered by telephone interviews with patients. Birth weight was divid-

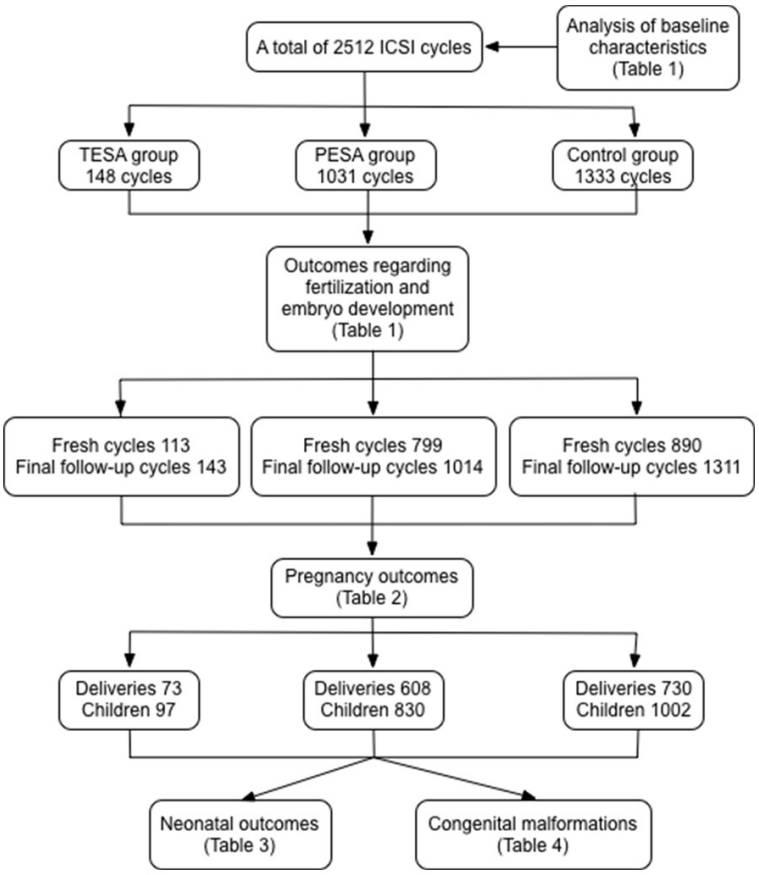

Figure 1. Flow chart of the study design.

ed into four degrees, including very low birth weight (VLBW, < 1,500 g), low birth weight (LBW, 1,500-2,499 g), normal birth weight (NBW, 2,500-4,000 g), and high birth weight ((HBW, > 4,000 g). Preterm birth (PTB) was defined as delivery before gestational week 37, while very preterm birth (VPTB) was defined as delivery before gestational week 32. Infant death was defined as the death of babies under the age of 1 year. Major congenital malformations were analyzed in this study. They were defined as ones causing structural and/or functional impairments of the body and/or organs, affecting viability and quality of life and requiring surgical correction.

Statistical analysis

Data analyses were performed using SPSS version 22.0. Continuous data are presented as mean ± standard deviation (SD) and were analyzed using one-way analysis of variance. Categorical data were analyzed using R × C χ^2-tests between the three groups. Fisher's exact tests were applied for 2 × 2 χ^2-tests if the expected count was less than 5. Statistical significance is indicated by $P < 0.05$. For analysis between two groups, continuous data were analyzed using t-tests. Categorical data were analyzed using χ^2-tests. In addition, statistical significance was adjusted using Bonferroni's method, determined as $P < 0.0167$.

Results

Baseline data and fertilization

A total of 2,512 ICSI cycles (TESA group, n = 148; PESA group, n = 1,031; control group, n = 1,333) were included in this study. A flow chart of the study design is shown in **Figure 1**. This study first compared baseline characteristics of ICSI cycles using testicular, epididymal, or ejaculated sperm. Female age, baseline FSH, number of oocytes retrieved, infertility type, and duration of infertility were comparable between the three groups (**Table 1**). Male ages were similar in the three groups. Furthermore, lower rates of normal fertilization (60.40% versus 68.06%, 68.36%) and cleaved embryos (63.98% versus 71.46%, 71.79%) were found in the TESA group, compared to PESA and control groups. Higher abnormal fertilization rates were observed in the PESA group, compared to the control group (4.79% versus 3.76%). Surgically retrieved sperm produced a lower good-quality embryo rate than ejaculated sperm (65.02%, 64.34% versus 70.83%). Moreover, there was a decreasing trend in good-quality embryo rates for PESA-ICSI, compared to TESA-ICSI, though not statistically significant. Additionally, the embryo utilization rate was decreased in the PESA group, compared to the TESA group and control group (92.03% versus 88.46%, 92.07%).

Pregnancy outcomes

Table 2 shows pregnancy outcomes of ICSI cycles using testicular, epididymal, and ejaculated sperm. There were 1,802 fresh cycles included in the present study (TESA group, n =

Table 1. Clinical characteristics of ICSI cycles using testicular, epididymal, and ejaculated sperm

	TESA[1]	PESA[2]	Control[3]	*P*-value			
				1 vs 2 vs 3	1 vs 2	1 vs 3	2 vs 3
Cycles	148	1031	1333				
Female age (y)	29.57 ± 6.14	29.38 ± 4.50	29.50 ± 4.28	0.752	0.705	0.888	0.487
Female bFSH (mIU/ml)	6.91 ± 2.51	6.87 ± 1.90	7.03 ± 1.84	0.139	0.872	0.571	0.050
No. of oocytes retrieved	12.09 ± 7.10	13.01 ± 6.17	12.58 ± 6.50	0.122	0.136	0.425	0.101
Primary infertility, n (%)	104 (70.27)	811 (78.66)	1016 (76.22)	0.055	0.022	0.110	0.160
Duration of infertility (y)	4.62 ± 4.06	4.48 ± 3.52	4.39 ± 3.14	0.671	0.683	0.514	0.545
Male age (y)	32.01 ± 6.14	32.00 ± 5.43	32.19 ± 5.22	0.694	0.996	0.733	0.405
Total No. of MII oocytes	1452	11426	14085				
Normal fertilization rate, n (%)	877 (60.40)	7777 (68.06)	9628 (68.36)	< 0.001[a]	< 0.001[b]	< 0.001[b]	0.618
Abnormal fertilization rate, n (%)	54 (3.72)	547 (4.79)	530 (3.76)	< 0.001	0.069	0.933	< 0.001[b]
Cleaved embryos, n (%)	929 (63.98)	8165 (71.46)	10111 (71.79)	< 0.001[a]	< 0.001[b]	< 0.001[b]	0.566
Good-quality embryos, n (%)	604 (65.02)	5253 (64.34)	7162 (70.83)	< 0.001[a]	0.681	< 0.001[b]	< 0.001[b]
Utilizable embryos, n (%)	855 (92.03)	7223 (88.46)	9309 (92.07)	< 0.001[a]	< 0.001[b]	0.971	< 0.001[b]

a. There were significant differences among the three groups. Statistical significance is determined by P < 0.05. b. There were significant differences between the two groups. Statistical significance was adjusted using Bonferroni's method and determined as P < 0.0167.

Table 2. Pregnancy outcomes of fresh ICSI cycles using testicular, epididymal, and ejaculated sperm

	TESA[1]	PESA[2]	Control[3]	*P*-value			
				1 vs 2 vs 3	1 vs 2	1 vs 3	2 vs 3
Fresh cycles	113	799	890				
Transferred embryos	218	1575	1748				
D3 (%)	218 (100)	1563 (99.24)	1740 (99.54)				
D5/D6/D7 (%)	0	12 (0.76)	8 (0.46)				
Implantation rate, n (%)	71 (32.57)	525 (33.33)	661 (37.81)	0.017[a]	0.822	0.131	0.007[b]
Clinical pregnancy, n (%)	51 (45.13)	422 (52.82)	470 (52.81)	0.286	0.126	0.124	0.998
Live birth, n (%)	46 (40.71)	371 (46.43)	371 (41.69)	0.116	0.253	0.842	0.050
Miscarriage (≤ 3 m), n (%)	8 (11.27)	83 (15.81)	55 (8.32)	< 0.001[a]	0.318	0.400	< 0.001[b]
Miscarriage (> 3 m), n (%)	0	9 (1.90)	11 (1.66)	0.497	0.257	0.235	0.947
Induced abortion, n (%)	0	2 (0.38)	4 (0.61)	0.636	1.000	1.000	0.699
Ectopic pregnancy rate, n (%)	0	14 (1.75)	10 (1.12)	0.235	0.238	0.614	0.276
Loss of follow-up	5	17	22				
Cycles with completed follow-up	143	1014	1311				
Cumulative clinical pregnancy rate, n (%)	94 (65.73)	728 (71.79)	945 (72.08)	0.274	0.135	0.110	0.878
Cumulative live birth rate, n (%)	78 (54.55)	607 (59.86)	728 (55.53)	0.089	0.226	0.822	0.036

a. There were significant differences among the three groups. Statistical significance is determined as P < 0.05. b. There were significant differences between the two groups. Statistical significance was adjusted using Bonferroni's method and determined as P < 0.0167.

113; PESA group, n = 799; control group, n = 890). More than 99% of female patients received embryo transfers on day 3. This study observed lower implantation rates (33.33% versus 37.81%) and elevated early miscarriage rates (15.81% versus 8.32%) of the PESA group, compared with the control group. There was an increasing trend of early miscarriage rates in PESA-ICSI cycles, compared to TESA-ICSI cycles, without significant difference. Rates of clinical pregnancies, live births, late miscarriages, induced abortions, and ectopic pregnancies were similar between the three groups. A total of 2,468 cycles, including completed information about pregnancy outcomes (TESA group, n = 143; PESA group, n = 1,014; control group, n = 1,311), were analyzed for cumulative clinical pregnancy rates (CPR) and cumulative live birth rates (LBR). There were no significant differences between TESA, PESA, and control groups.

Neonatal outcomes

Table 3 shows neonatal outcomes of ICSI cycles using testicular, epidydimal, and ejaculated sperm. A total of 1,411 deliveries were achieved. Delivery method, sex ratios, and twin proportions were similar in the three groups. There were 97 babies born from the TESA group, 830 babies from the PESA group (1 stillbirth and 4 infant deaths), and 1,002 babies

Table 3. Neonatal outcomes of ICSI cycles using testicular, epididymal, and ejaculated sperm

	TESA[1]	PESA[2]	Control[3]	*P*-value			
				1 vs 2 vs 3	1 vs 2	1 vs 3	2 vs 3
Deliveries, n	73	608	730				
Male, n (%)	43 (44.33)	444 (53.49)	510 (50.90)	0.179	0.087	0.217	0.268
Twins, n (%)	24 (32.88)	222 (36.50)	272 (37.50)	0.753	0.541	0.459	0.778
Cesarean section, n (%)	62 (84.93)	546 (89.80)	648 (88.77)	0.432	0.204	0.329	0.543
Children, n	97	830	1002				
Stillbirth, n (%)	0	1 (0.12)	2 (0.20)	0.843	1.000	1.000	1.000
Infant death, n (%)	0	4 (0.48)	3 (0.30)	0.674	1.000	1.000	0.708
All children							
Gestational week	37.9 ± 2.0	37.8 ± 2.0	37.8 ± 2.0	0.860	0.527	0.588	0.775
PTB, n (%)	11 (15.07)	111 (13.37)	150 (14.97)	0.450	0.575	0.334	0.330
VPTB, n (%)	2 (2.06)	11 (1.33)	12 (1.20)	0.769	0.559	0.469	0.807
Birth weight loss of newborns, n	1	12	14				
Mean birth weight (kg)	2.94 ± 0.61	2.85 ± 0.61	2.87 ± 0.63	0.392	0.148	0.255	0.543
VLBW, n (%)	1 (1.04)	12 (1.47)	19 (1.92)	0.182	1.000	1.000	0.474
LBW, n (%)	17 (17.71)	200 (24.45)	233 (23.58)	0.338	0.142	0.192	0.668
NBW, n (%)	74 (77.08)	580 (70.90)	694 (70.24)	0.371	0.204	0.159	0.759
HBW, n (%)	4 (4.17)	26 (3.18)	42 (4.25)	0.483	0.546	1.000	0.233
Singletons							
Gestational week	38.6 ± 2.1	38.6 ± 1.5	38.7 ± 1.5	0.882	0.636	0.564	0.940
Preterm deliveries, n (%)	3 (6.12)	29 (7.51)	38 (8.30)	0.823	0.725	0.596	0.675
Birth weight loss of newborns, n	0	6	8				
Mean birth weight (kg)	3.30 ± 0.57	3.27 ± 0.47	3.30 ± 0.53	0.780	0.745	0.989	0.353
VLBW, n (%)	1 (2.04)	0	1 (0.22)	0.019[a]	0.114	0.187	1.000
LBW, n (%)	2 (4.08)	19 (5.00)	27 (6.00)	0.744	1.000	1.000	0.530
NBW, n (%)	42 (85.71)	335 (88.16)	380 (84.44)	0.304	0.622	0.815	0.123
HBW, n (%)	4 (8.16)	26 (6.84)	42 (9.33)	0.427	0.764	1.000	0.192
Twins							
Gestational week	36.7 ± 1.3	36.4 ± 2.3	36.3 ± 2.0	0.521	0.797	0.826	0.902
Preterm deliveries, n (%)	13 (54.17)	94 (42.34)	124 (45.59)	0.483	0.267	0.419	0.470
Birth weight loss of newborns, n	1	6	6				
Mean birth weight (kg)	2.57 ± 0.40	2.49 ± 0.49	2.51 ± 0.49	0.397	0.216	0.374	0.515
VLBW, n (%)	0	12 (2.74)	18 (3.35)	0.407	0.617	0.386	0.585
LBW, n (%)	15 (31.91)	181 (41.32)	206 (38.29)	0.358	0.212	0.387	0.335
NBW, n (%)	32 (68.09)	245 (55.94)	314 (58.36)	0.504	0.299	0.465	0.446
HBW, n (%)	0	0	0	-			

a. There were significant differences among the three groups. However, after adjustment by Fisher's exact test, there were no significant differences found between any two of the groups.

from the control group (2 stillbirths and 3 infant deaths). For all babies, no significant differences concerning gestational week, mean birth weight, and rates of PB, VPB, VLBW, LBW, NBW, and HBW were found between the three groups. For singletons, the three groups did not differ in gestational week, preterm deliveries, and mean birth weights. Likewise, comparable neonatal outcomes in twins were found.

Incidence of major congenital birth defects

Table 4 shows incidence rates of major congenital birth defects of babies delivered from ICSI cycles using testicular, epididymal, and ejaculated sperm. No major congenital malformations were present in the TESA group, while there were 14 and 23 major congenital birth defects, respectively, observed in PESA and control groups. However, there were no significant differences in incidence of major congenital malformations between the three groups.

Discussion

Many previous studies have revealed the importance of transition in sperm from the testicle to the epididymis, playing a key role in sp-

Table 4. Major congenital malformations of babies delivered from ICSI cycles using testicular, epididymal, and ejaculated sperm

	TESA[1]	PESA[2]	Control[3]	*P*-value			
				1 vs 2 vs 3	1 vs 2	1 vs 2	2 vs 3
Total, n (%)	0	14 (1.69)	23 (2.30)	0.235	0.384	0.254	0.357
Nervous	0	4	2				
Cardiovascular	0	2	9				
Eye, ear, face	0	1	2				
Digestive system	0	1	2				
Urogenital	0	1	2				
Musculoskeletal	0	1	1				
Respiratory	0	2	3				
Chromosomal	0	0	0				
Others	0	2	2				

erm maturation and normal development [7, 15, 16]. In contrast, prolonged presence in the reproductive tract could possibly cause senescent and detrimental changes in the sperm [17-19]. Differences in clinical outcomes after ICSI using testicular, epididymal, and ejaculated sperm remain inconsistent. The present study aimed to explore association levels of fertilization, embryo development, and pregnancy/neonatal outcomes with the sources of spermatozoa.

Although there existed discordant data about fertilization after ICSI with different sources of spermatozoa, some general trends were verified. In male partners with OA, lower normal fertilization rates and higher abnormal fertilization rates were found in the testicular sperm group, compared to the epididymal sperm group [12, 16, 20, 21]. Compared with ejaculated sperm from men with severe oligo-/asthenozoospermia, testicular sperm in patients with OA provided lower normal fertilization rates [20, 22]. The current study found similar results to published reports. Compared to epididymal and ejaculated sperm, poorer motility and immaturity levels of testicular sperm rendered them less likely to fertilize an injected oocyte [12, 16].

Inconsistent results about cleavage rates have been reported in prior studies, possibly caused by the various definitions of cleave embryo rates [7, 9, 10, 20-23]. In the center, embryos with transferable quality developed from 0PN, 1PN, and 2PN could all be considered utilizable. Hence, the current study evaluated cleavage rates from all oocytes receiving ICSI (not only from normally fertilized oocytes), similar to the analysis in the study of Buffat et al. [9]. Lower cleavage rates were observed in ICSI cycles with testicular sperm, compared to those with epididymal and ejaculated sperm. The most possible explanation might be that the low normal fertilization rate of TESA-ICSI caused adverse impact for subsequent cleavage.

Another area where the use of sperm from different origins may be of consequence is embryo development. Paternal influence on embryonic development usually occurs at 4- to 8-cell stage, after activation of the embryonic genome [24]. Published data did not show significant influence of sperm sources on the probability to develop into good-quality embryos on day 3 [7, 9, 10, 21-23]. This may be because the paternal effects not necessarily appeared in this early genomic transition stage. However, compared with testicular or ejaculated sperm, it was observed that epididymal sperm seemed to produce trends of fewer good-quality embryos and utilizable embryos, as well as less implantation. The sperm factor gradually manifested itself during embryonic development. Early embryo quality may further affect pregnancy outcomes.

Several studies have demonstrated better pregnancy outcomes after ICSI with epididymal sperm, compared to testicular sperm, including lower miscarriage rates, higher CPR, and higher LBR [7, 9, 16, 20]. However, contrary results have also been reported, showing a lower ongoing pregnancy rate in the PESA group, compared to the TESA group [25]. When systematically reviewing previous data, miscarriage rates (included 348 cycles) and CPR (included 1,499 cycles) turned out to be similar between ICSI cycles using testicular or epididymal sperm [12]. Kawwass et al. [26] evaluated surgical acquired sperm use for 347,078 ICSI cycles in USA. They reported that perinatal outcomes were statistically significant but clinically similar between cycles involving non-ejaculated sp-

erm versus ejaculated sperm. Sperm retrieval techniques were not restricted, including microsurgical epididymal sperm aspiration (MESA) and testicular sperm extraction (TESE). In the current study, no differences in CPR and LBR were observed between the 2,512 ICSI cycles with sperm from different locations. However, epididymal sperm tended to be associated with increased early miscarriage rates, compared to ejaculated sperm and testicular sperm.

Unsatisfactory embryo development and pregnancy outcomes of ICSI using epididymal sperm might be predominantly due to detrimental changes during sperm transit. The prolonged presence of sperm in the epididymis causes elevated levels of reactive oxygen species (ROS), resulting in increased sperm DNA fragmentation, leading to post-testicular harm [18]. Moreover, disulfide bonds excess, a manifestation of aged sperm found in the epididymis, has been associated with sperm nucleus de-condensation delay after fertilization and decreases embryo viability [27, 28]. Other experimental data has demonstrated that aged sperm in the epididymis causes structural chromosomal aberrations without affecting fertilization [29, 30]. It is difficult for PESA to find the proximal-most least senescent sperm in clinical surgical performance. Since a majority of early miscarriages are caused by chromosomal imbalances [31], the higher early miscarriage risks of the PESA group could be explained.

Additionally, genital inflammation can affect sperm quality. Frequently, a great number of pyocytes could be found in semen at the time of PESA, indicating the presence of reproductive system infections. In clinical practice, epididymitis is more often found than orchitis. Inflammation and swelling usually begins in the tail of epididymis. They may spread to the rest of the epididymis and testicular tissues [32]. Epididymitis causes depression of epididymal secretion ability that supports the sperm maturation process, resulting in significantly more malformations of sperm acrosome and tail. This has negative effects on embryo development [33].

In this study, testicular and epididymal sperm were comparable in pregnancy/neonatal outcomes, despite some differences in early embryo parameters. TESA-ICSI showed similar early miscarriage rates to ICSI using ejaculated sperm. Considering the high risk of early miscarriages in PESA-ICSI, TESA is a proper back-up treatment for PESA. MESA is an approach used to find the proximal-most sperm in the epididymis for ICSI. Comparing the least senescent epididymal sperm with testicular sperm, MESA-ICSI produces remarkably better pregnancy outcomes [7]. However, some units have replaced MESA with PESA. This may be because PESA is simpler, less traumatic and expensive, and effective at most times. According to present findings, if the expenses can be accepted by patients or the initial PESA-ICSI cycle ends up with poor outcomes, MESA may still be considered as an alternative.

Many studies have demonstrated that birth parameters and congenital malformation risks of children conceived with surgically retrieved sperm were similar to those with ejaculated sperm [34-37], although methodological shortcomings still need to be considered. Most studies have found no differences in neonatal outcomes or birth defect risks between ICSI cycles using testicular, epididymal, and ejaculated sperm [34, 35, 38-40]. Only Tsai et al. [40] found a significant sex-ratio imbalance toward males in the testicular sperm group, compared to the ejaculated sperm group. Likewise, in the current study, neonatal outcomes and incidence of major congenital malformations were found to be comparable between the three groups. Sperm origin does not influence physiological parameters of newborns conceived by ICSI.

The strength of the current study was that comprehensive outcomes through the complete ICSI cycle were evaluated in the OA population of a large sample size. Limitations of the current study included the retrospective single-center study design, varied COH treatments according to individual infertility conditions, causes of OA not fully recorded, and pregnancy/neonatal outcomes obtained via telephone reviews.

Conclusion

TESA is an effective back-up approach if PESA fails. Since the predominant reason for unsatisfactory outcomes in the PESA-ICSI group was the possibility of retrieving distal senescent sperm by blind aspiration, MESA could be used an alternative to retrieve high quality

motile sperm from the proximal-most epididymal site.

Acknowledgements

The authors would lie to thank each of the doctors, nurses, and embryologists in the Reproductive Medicine Center of Tongji Hospital for assistance in collecting data. This work was supported by grants from the National Natural Science Foundation of China (No. 81571508; No. 81771654).

Disclosure of conflict of interest

None.

Address correspondence to: Yufeng Li, Reproductive Medicine Center, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, No. 1095 Jiefang Avenue, Wuhan 430030, Hubei, China. Tel: +86-18627188668; E-mail: tjlyf-66@126.com

References

[1] Tournaye H, Devroey P, Liu J, Nagy Z, Lissens W and Van Steirteghem A. Microsurgical epididymal sperm aspiration and intracytoplasmic sperm injection: a new effective approach to infertility as a result of congenital bilateral absence of the vas deferens. Fertil Steril 1994; 61: 1045-51.

[2] Shin DH and Turek PJ. Sperm retrieval techniques. Nat Rev Urol 2013; 10: 723-30.

[3] Qiu Y, Wang LG, Zhang LH, Li J, Zhang AD and Zhang MH. Sperm chromosomal aneuploidy and DNA integrity of infertile men with anejaculation. J Assist Reprod Genet 2012; 29: 185-94.

[4] Tesarik J. Paternal effects on cell division in the human preimplantation embryo. Reprod Biomed Online 2005; 10: 370-5.

[5] Nicopoullos JD, Gilling-Smith C, Almeida PA, Norman-Taylor J, Grace I and Ramsay JW. Use of surgical sperm retrieval in azoospermic men: a meta-analysis. Fertil Steril 2004; 82: 691-701.

[6] Pasqualotto FF, Rossi-Ferragut LM, Rocha CC, Iaconelli A Jr and Borges E Jr. Outcome of in vitro fertilization and intracytoplasmic injection of epididymal and testicular sperm obtained from patients with obstructive and nonobstructive azoospermia. J Urol 2002; 167: 1753-6.

[7] van Wely M, Barbey N, Meissner A, Repping S and Silber SJ. Live birth rates after MESA or TESE in men with obstructive azoospermia: is there a difference? Hum Reprod 2015; 30: 761-6.

[8] Wang J, Sun H, Hu Y, Wang B, Zhang N, Chen H, Zhu W. Outcome of intracytoplasmic injection of epididymal and testicular sperm obtained from azoospermic patients. Zhonghua Nan Ke Xue 2004; 10: 751-4.

[9] Buffat C, Patrat C, Merlet F, Guibert J, Epelboin S, Thiounn N, Vieillefond A, Adda-Lievin A, Lebon C and Jouannet P. ICSI outcomes in obstructive azoospermia: influence of the origin of surgically retrieved spermatozoa and the cause of obstruction. Hum Reprod 2006; 21: 1018-24.

[10] Semiao-Francisco L, Braga DP, Figueira Rde C, Madaschi C, Pasqualotto FF, Iaconelli A Jr and Borges E Jr. Assisted reproductive technology outcomes in azoospermic men: 10 years of experience with surgical sperm retrieval. Aging Male 2010; 13: 44-50.

[11] Borges E Jr, Rossi-Ferragut LM, Pasqualotto FF, dos Santos DR, Rocha CC and Iaconelli A Jr. Testicular sperm results in elevated miscarriage rates compared to epididymal sperm in azoospermic patients. Sao Paulo Med J 2002; 120: 122-6.

[12] Zhan XX, Wan CC, Li HB, Gou J, Cai HC, Zhao J, Yan CF, Diao ZY and Shang XJ. ICSI with testicular or epididymal sperm for patients with obstructive azoospermia: a systematic review. Zhonghua Nan Ke Xue 2016; 22: 1122-1130.

[13] Holte TO, Hofmann B, Lie RT, Norderhaug IN, Romundstad P, Saeterdal I, Orstavik KH and Tanbo T. In male infertility: intracytoplasmic sperm injection (ICSI) using surgically retrieved sperm from the testis or the epididymis. Oslo, Norway: Knowledge Centre for the Health Services at The Norwegian Institute of Public Health (NIPH); 2007.

[14] Jungwirth A, Diemer T, Dohle GR, Kopa Z, Krausz C, Tournaye H; Infertility EAUWGoM. EAU guidelines on male infertility. Eur Urol 2016.

[15] Devroey P, Nagy P, Tournaye H, Liu J, Silber S and Van Steirteghem A. Outcome of intracytoplasmic sperm injection with testicular spermatozoa in obstructive and non-obstructive azoospermia. Hum Reprod 1996; 11: 1015-8.

[16] Hameed N and Ozturk O. Testicular versus epididymal spermatozoa in intracytoplasmic sperm injection treatment cycles. J Ayub Med Coll Abbottabad 2010; 22: 159-63.

[17] Cho CL, Esteves SC and Agarwal A. Novel insights into the pathophysiology of varicocele and its association with reactive oxygen species and sperm DNA fragmentation. Asian J Androl 2016; 18: 186-93.

[18] Esteves SC, Roque M, Bradley CK and Garrido N. Reproductive outcomes of testicular versus ejaculated sperm for intracytoplasmic sperm injection among men with high levels of DNA fragmentation in semen: systematic review

and meta-analysis. Fertil Steril 2017; 108: 456-467, e1.
[19] Rubes J, Selevan SG, Sram RJ, Evenson DP and Perreault SD. GSTM1 genotype influences the susceptibility of men to sperm DNA damage associated with exposure to air pollution. Mutat Res 2007; 625: 20-8.
[20] Dong RN GY, Sun YP, Su YC, Li J. Clinical pregnancy outcome of 3106 ICSI cycles using spermatozoa from difference sources. Sheng Zhi Yu Bi Yun 2013; 33: 233-238.
[21] Xie D, Qiu Z, Luo C, Chu Q and Quan S. Effect of spermatozoa from different sources on normal fertilization of oocytes and embryo quality and development in intracytoplasmic sperm injection cycles. Nan Fang Yi Ke Da Xue Xue Bao 2014; 34: 857-61.
[22] Goker EN, Sendag F, Levi R, Sendag H and Tavmergen E. Comparison of the ICSI outcome of ejaculated sperm with normal, abnormal parameters and testicular sperm. Eur J Obstet Gynecol Reprod Biol 2002; 104: 129-36.
[23] Guo HB, Zhang Y, Zhang CL, Li HS, Gu BX, Yin BL, Song XB and Xie JK. Outcomes of ICSI with sperm from different sources: a retrospective study of 431 cycles. Zhonghua Nan Ke Xue Za Zhi 2009; 15: 925-928.
[24] Balaban B, Urman B, Isiklar A, Alatas C, Mercan R, Aksoy S and Nuhoglu A. Blastocyst transfer following intracytoplasmic injection of ejaculated, epididymal or testicular spermatozoa. Hum Reprod 2001; 16: 125-129.
[25] Dozortsev D, Neme R, Diamond MP, Abdelmassih S, Abdelmassih V, Oliveira F and Abdelmassih R. Embryos generated using testicular spermatozoa have higher developmental potential than those obtained using epididymal spermatozoa in men with obstructive azoospermia. Fertil Steril 2006; 86: 606-11.
[26] Kawwass JF, Chang J, Boulet SL, Nangia A, Mehta A and Kissin DM. Surgically acquired sperm use for assisted reproductive technology: trends and perinatal outcomes, USA, 2004-2015. J Assist Reprod Genet 2018; 35: 1229-1237.
[27] Usui N and Yanagimachi R. Behavior of hamster sperm nuclei incorporated into eggs at various stages of maturation, fertilization, and early development. The appearance and disappearance of factors involved in sperm chromatin decondensation in egg cytoplasm. J Ultrastruct Res 1976; 57: 276-88.
[28] Dozortsev D, De Sutter P and Dhont M. Behaviour of spermatozoa in human oocytes displaying no or one pronucleus after intracytoplasmic sperm injection. Hum Reprod 1994; 9: 2139-44.
[29] Martin-DeLeon PA and Boice ML. Sperm aging in the male and cytogenetic anomalies. An animal model. Hum Genet 1982; 62: 70-7.
[30] Martin-Deleon PA, Shaver EL, Gammal EB. Chromosome abnormalities in rabbit blastocysts resulting from spermatozoa aged in the male tract. Fertil Steril 1973; 24: 212-9.
[31] Philipp T, Philipp K, Reiner A, Beer F and Kalousek DK. Embryoscopic and cytogenetic analysis of 233 missed abortions: factors involved in the pathogenesis of developmental defects of early failed pregnancies. Hum Reprod 2003; 18: 1724-32.
[32] National guideline for the management of epididymo-orchitis. Clinical effectiveness group (Association of Genitourinary Medicine and the Medical Society for the Study of Venereal Diseases). Sex Transm Infect 1999; 75: S51-3.
[33] Gan C ZM. The clincial significance of spermatozoa malformation for diagnosis of chronic epididymitis. Journal of Clinical and Experimental Medicine 2013; 12: 1074-1075.
[34] Fedder J, Loft A, Parner ET, Rasmussen S and Pinborg A. Neonatal outcome and congenital malformations in children born after ICSI with testicular or epididymal sperm: a controlled national cohort study. Hum Reprod 2013; 28: 230-40.
[35] Xu ZY, Ye BL, Huang XF, Lin WQ, Lin JJ and Zhao JZ. Sperm origin and quality effect on pregnancy outcome after intracytoplasmic sperm injection. Zhongguo Nan Ke Xue Za Zhi 2007; 21: 41-44.
[36] Woldringh GH, Besselink DE, Tillema AH, Hendriks JC and Kremer JA. Karyotyping, congenital anomalies and follow-up of children after intracytoplasmic sperm injection with non-ejaculated sperm: a systematic review. Hum Reprod Update 2010; 16: 12-9.
[37] Van Steirteghem A, Bonduelle M, Devroey P and Liebaers I. Follow-up of children born after ICSI. Hum Reprod Update 2002; 8: 111-6.
[38] Guo YH, Dong RN, Su YC, Li J, Zhang YJ and Sun YP. Follow-up of children born after intracytoplasmic sperm injection with epididymal and testicular spermatozoa. Chin Med J (Engl) 2013; 126: 2129-33.
[39] Woldringh GH, Horvers M, Janssen AJ, Reuser JJ, de Groot SA, Steiner K, D'Hauwers KW, Wetzels AM and Kremer JA. Follow-up of children born after ICSI with epididymal spermatozoa. Hum Reprod 2011; 26: 1759-67.
[40] Tsai CC, Huang FJ, Wang LJ, Lin YJ, Kung FT, Hsieh CH and Lan KC. Clinical outcomes and development of children born after intracytoplasmic sperm injection (ICSI) using extracted testicular sperm or ejaculated extreme severe oligo-astheno-teratozoospermia sperm: a comparative study. Fertil Steril 2011; 96: 567-71.