Journal of **R**eproduction & **I**nfertility
Volume 26, Issue no. 1
https://doi.org/10.18502/jri.v26i1.18776

Original Article

Check for updates

Time-Lapse Evaluation of Embryos in Non-Obstructive Azoospermia (NOA): High Rate of 1PN Fertilization and Rapid Embryo Development in TESE Compared to Ejaculated Sperm

Tulay Irez [1*], Yagmur Ayse Yazla Ozturk [2], Fidan Mammadova [2], Sirin Kinetli [2], Mine Erguven [3], Nurten Dayioglu [4], Hakan Ozornek [5]

1- Department of Histology and Embryology, Faculty of Medicine, Istanbul Yeni Yuzyil University, Istanbul, Turkey
2- Health Science Institute, Istanbul Yeni Yuzyil University, Istanbul, Turkey
3- Department of Medical Biochemistry, Faculty of Medicine, Istanbul Aydin University, Istanbul, Turkey
4- Department of Medical Statistics, Faculty of Medicine, Istanbul Yeni Yuzyil University, Istanbul, Turkey
5- Department of Obstetrics and Gynecology, Faculty of Medicine, Koç University, Istanbul, Turkey

Abstract

Background: Non-obstructive azoospermia (NOA), marked by impaired spermatogenesis, poses challenges in assisted reproduction. Limited data exist comparing chromosomal integrity of testicular versus ejaculated sperm. This study aimed to compare embryo morphokinetics, fertilization abnormalities, and PGT-A outcomes between embryos from ejaculated and testicular sperm in NOA cases.

Methods: This retrospective study analyzed 397 patients from two IVF centers (2015–2023), with 317 using ejaculated sperm and 80 using testicular sperm from NOA patients. Fertilization patterns (2PN, 1PN, ≥3PN), embryo morphokinetics, and aneuploidy rates were assessed. Logistic regression examined factors influencing aneuploidy including male and female age, recurrent implantation failure (RIF), hormone levels, and oocyte quality, while chi-square and t-tests compared groups, with significance at $p<0.05$.

Results: Embryos derived from testicular sperm developed faster than those from ejaculated sperm ($p<0.05$). The 2PN fertilization rate was significantly lower, while the 1PN rate was higher in the testicular sperm group (10.1% *vs*. 16.4%, $p=0.020$). The rates of ≥3PN anomalies and embryo aneuploidy were similar between groups ($p>0.05$). Logistic regression identified male age ($p=0.001$), female age ($p=0.007$), and RIF ($p=0.047$) as significant predictors of aneuploidy.

Conclusion: Our study identified advanced parental age and RIF as key predictors of embryo aneuploidy. PGT-A may improve outcomes, especially for older patients or those with RIF, regardless of sperm origin. Embryos from testicular sperm in NOA patients develop faster morphokinetically but show a higher rate of 1PN fertilization than those from ejaculated sperm.

** Corresponding Author:*
Tulay Irez, Istanbul Yeni Yuzyil University, Faculty of Medicine, Department of Histology & Embryology Yilanli Ayazma cad 26, 34010, Zeytinburnu Topkapi, Istanbul,Turkey
E-mail:
tulay.irez@yeniyuzyil.edu.tr

Received: 31, Dec. 2024
Accepted: 11, May 2025

Keywords: 1PN fertilization, Embryo development, Micro-TESE, Non-obstructive azoospermia, PGT-A, Time-lapse imaging.
To cite this article: Irez T, Ozturk YAY, Mammadova F, Kinetli S, Erguven M, Dayioglu N, et al. Time-Lapse Evaluation of Embryos in Non-Obstructive Azoospermia (NOA): High Rate of 1PN Fertilization and Rapid Embryo Development in TESE Compared to Ejaculated Sperm. J Reprod Infertil. 2025;26(1):3-10. https://doi.org/10.18502/jri.v26i1.18776.

Introduction

It is known that approximately 5-10% of men evaluated for infertility are azoospermic (1). NOA is usually associated with testicular dysfunction, often resulting from disorders in the hypothalamic-pituitary axis (2). NOA is believed to account for one percent of azoospermic cases

Copyright © 2025, Journal of Reproduction & Infertility

This work is licensed under a Creative Commons Attribution–NonCommercial 4.0 International License.

and the use of ICSI has increased the success rates in these cases (3, 4).

Microdissection testicular sperm extraction (micro-TESE) is recognized as the most appropriate technique for surgical sperm retrieval in NOA cases. Schlegel reported that micro-TESE removed significantly less testicular tissue and obtained a higher rate of sperm compared to conventional TESE (5). Meta-analysis studies also showed that micro-TESE was more successful (6). Sperm retrieval rates in micro-TESE cases are influenced by testicular histology, with the highest success rates typically observed in cases of hypospermatogenesis (7-9). NOA is thought to account for one percent of male infertility (10). Cytogenetic evaluation with karyotyping shows that approximately 5% of men with NOA have cytogenetic abnormalities (11). Non-mosaic Klinefelter syndrome (47, XXY) is the most frequently detected cytogenetic abnormality (12, 13). Other cytogenetic abnormalities detected in azoospermic men include Robertsonian translocations, reciprocal translocations, and chromosomal inversions (14). Some of these genetic disorders are thought to predispose sperm and embryos to aneuploidy, which may affect the genetic health of offspring developed with assisted reproductive technology (ART). Men with NOA, associated with primary testicular failure, may also carry Y chromosome microdeletions. Hypogonadotropic hypogonadism is also found in some cases, and these patients can be treated with exogenous hormones (15). Tests within this scope require mutation analyses, parental genetic structure, and PGT-A screening for embryo aneuploidy.

Testicular sperm, despite having the ability to fertilize an oocyte after intracytoplasmic sperm injection (ICSI), are immature due to the lack of epididymal passage. Testicular sperm are thought to differ from ejaculated sperm in terms of chromatin maturity, frequency of DNA damage, and RNA content. Furthermore, the biological effects of using testicular sperm on fertilization, preimplantation embryo development, and post-implantation development are not fully understood. In our previous study examining time-lapse embryo development in cases of obstructive azoospermia, faster developmental kinetics were observed in embryos derived from TESE-obtained sperm (16). It has been shown that the risk of chromosomal abnormalities is high in embryos obtained after fertilization with testicular sperm (17, 18). However, the fact that similar pregnancy rates and live birth outcomes were achieved when ejaculated sperm was used after treatment highlights the importance of proper embryo selection. Considering that sperm obtained with micro-TESE develop in different environments, it is assumed that hormonal disorders, deficiencies in the pituitary-hypothalamic axis, and biological differences may influence the processes of embryo development (19).

Time-lapse embryo observation accounts for a higher embryo development rate than traditional methods. The findings related to this technique have increased the success of embryo selection based on high implantation ability, particularly regarding the timing of cell cycles associated with embryo compaction and blastulation. Understanding the effects of sperm origin and morphological or physiological characteristics on cell cycles is crucial in unraveling the paternal effect. Sperm origin and paternal influences have not been considered in previous studies.

In this study, an attempt was made to investigate the differences in human preimplantation embryo development after ICSI using testicular sperm (micro-TESE-ICSI) and ejaculated sperm in non-obstructive azoospermia cases and to compare all parameters from fertilization to aneuploid embryo development. The study primarily focused on cases of non-obstructive azoospermia, comparing the growth rates and developmental stages of pronuclei (PN), morulae, and blastocysts.

Methods

Study design and participants: Data from couples undergoing a micro-TESE-ICSI cycle or an ICSI cycle with ejaculated sperm, combined with time-lapse embryo culture, were retrospectively analyzed at the Bahçeci IVF Center and Eurofertil IVF Center in Istanbul from 2015 to 2023. All procedures were performed in accordance with relevant laws and institutional regulations, and approval was obtained from the Istanbul Yeni Yüzyıl University's Clinical Research Ethics Committee (approval number: 06.02.2024/11). Indications for ICSI using ejaculated sperm included male factor infertility or a previous IVF cycle that resulted in total fertilization failure (TFF), both of which were part of the inclusion criteria. Only cycles containing autologous, fresh oocytes, and eight or more embryos were included in the study, with 3 to 4 transferable embryos from each case subjected to PGD. Data from couples who underwent more than one cycle

during the study period were included, starting with their first available treatment cycle. Cases of PCOS and endometriosis were excluded from the study. In our study, 317 cases involving ejaculated sperm and 80 NOA cases with a single cycle, including PGD, were examined, and a total of 1598 embryos were evaluated with time-lapse results.

Testicular sperm retrieval, NOA, and ejaculated sperm processing: Testicular sperm extraction was performed under local anesthesia with a standard open surgical biopsy technique. A 2-centimeter transverse scrotal incision was made. The tunica albuginea was incised for 1 *cm*, and a small fragment of testicular tissue was excised with sharp scissors and placed in HEPES-buffered human tubal fluid (HTF) culture medium supplemented with 5% (*w/v*) human serum albumin (HSA; Sigma-Aldrich, Germany) (20). Testicular tissue consisting of seminiferous tubules was dissected using sterile insulin syringes. Then, the spermatogenic cell suspension was washed with HTF followed by centrifugation at 1500 *rpm* for 10 *min*. The pellet was resuspended in 1 milliliter (*ml*) HTF and subsequently diluted 1:1 with cryoprotectant and cryopreserved in straws (Cryo Bio System, France) by placing them in liquid nitrogen vapor. Before use, testicular sperm cells were thawed and washed with 5 *ml* HTF, supplemented with 10% HSA and centrifuged at 1200 *rpm* for 5 *min*. The remaining pellet was resuspended in 1 *ml* HTF/10% HSA, carefully layered on top of 1 *ml* 40% PureSperm (Nidacon International AB, Sweden) and centrifuged at 2000 *rpm* for 15 *min*. The upper layer of the gradient was removed, leaving only the soft pellet. This was washed in 5 *ml* HTF and stored at 35°*C* for a minimum of 1.5 *hr*. An ICSI dish was prepared containing 50 *µl* drops of HTF under liquid paraffin oil, and the purified spermatogenic cells were pipetted into the drops. The best-quality sperm cells, based on motility and morphology, were selected under an inverted microscope (Nikon, Japan) at 200–400 times magnification.

Ejaculated semen samples were left to liquefy and sperm concentration and motility were determined. They were subsequently processed by density centrifugation, using a 40–80% PureSperm density gradient (Nidacon International AB, Sweden) according to the manufacturer's instructions. The resulting soft pellet was washed twice in HTF medium and isolated spermatozoa were stored at room temperature until the ICSI procedure. Sperm samples were categorized according to the World Health Organization's laboratory manual (21).

Ovarian stimulation, oocyte collection, and oocyte injection: Ovarian stimulation protocols have been standardized at our center, and the distribution of the Gonadotropin releasing hormone (GnRH)-agonist or antagonist protocol reflects policy changes over time, rather than patient selection. For controlled ovarian stimulation, recombinant follicle stimulating hormone (rFSH) (Gonal-f; Merck, Switzerland), a combination of rFSH and recombinant luteinizing hormone (rLH) (Luveris; Merck, Switzerland), or human menopausal gonadotropin (hMG) (Ferring, Switzerland) was used. GnRH antagonist (Cetrotide; Merck-Serono, Germany or Orgalutran; MSD, Germany) was administered when at least one follicle reached ≥14 *mm*. Follicular development was monitored by ultrasonography at regular intervals. When at least two dominant follicles measuring 17 *mm* were observed, transvaginal ultrasound-guided oocyte retrieval (OPU) was planned within 36 *hr* after the injection of human recombinant chorionic gonadotropin (hCG) (Pregnyl; Organon, the Netherlands), which was used as a trigger for final follicular maturation. Ovum pick-up was scheduled according to standardized criteria. For ICSI with ejaculated sperm, metaphase II (MII) oocytes were injected with motile spermatozoa. For ICSI with testicular sperm, MII oocytes were injected with either motile testicular spermatozoa or immotile but viable testicular spermatozoa, as selected by 1% pentoxifylline in HTF-Hepes.

Embryo culture, selection, and transfer: Injected oocytes were placed in separate wells of EmbryoSlide culture dishes (Vitrolife, Sweden) and cultured in SAGE 1-Step medium (Origio, Denmark) at 36.8°*C*, 7% O_2, and 5% CO_2 in an EmbryoScope time-lapse incubator (Vitrolife, Sweden). Embryo transfer was performed on day five after fertilization. According to the directives of the Republic of Türkiye Ministry of Health, it is a standard practice to perform single embryo transfer (SET). Only women aged 35 years and older without medical contraindications or women undergoing their third or subsequent treatment cycles may opt for double embryo transfer. Embryo selection for transfer was assisted by time-lapse imaging.

Time-lapse imaging and evaluation: EmbryoScope (Vitrolife, Sweden) automatically captures images

at seven focal planes every 10 *min*. This study included an analysis of the acquired video recordings. For both micro-TESE-ICSI with NOA and ICSI with ejaculated sperm, t=0 was defined as the time of injection of the last oocyte, and the entire procedure took between 20 and 50 *min*, depending on the number of oocytes. Manual annotations were recorded by trained laboratory personnel according to the instructions. The time of pronuclear appearance (tPNa), number of pronuclei (PN), the first frame in which both pronuclei faded (tPNf), and the timing of reaching the 2, 3, 4, 5, 6, 7, and 8 cell stages (t2, t3, t4, t5, t6, t7, t8, tM, and tB) were recorded. Additionally, the juxtaposition of the pronuclei was noted (pronuclei time 1).

Embryo biopsy and PGT analysis: On day 3, artificial hatching was performed in the zona pellucida using a diode laser (RI Saturn 3; CooperSurgical, UK). On day 5/6, five to eight cells from the trophectoderm were transferred into a 30 *mm* inner diameter biopsy pipette (Origio, Denmark). Next-generation sequencing (NGS) was utilized for PGT-A analysis with ReproSeq kit (Thermo Fisher Scientific, USA) and the Ion Torrent S5 System (Thermo Fisher Scientific, USA) following the manufacturer's instructions. Genetic analyses were conducted using the Ion Reporter software package v5.2 and v5.6 (Thermo Fisher Scientific, USA).

Statistical analysis: Logistic regression analysis was used to investigate the proportional distribution of fertilization outcomes and anomalies in patient data, the time from fertilization to the blastocyst stage, aneuploidy rates in embryos derived from NOA and ejaculated sperm, and factors influencing PGT-A results including male and female age, recurrent pregnancy loss, treatment protocols, hormone levels, oocyte quality, and time-lapse imaging data. Chi-square and t-tests were used to analyze the research data. Baseline data were tested for normality. If continuous data did not meet the normality assumption, the Mann–Whitney U test was performed, and estimates were reported as medians with interquartile ranges. Categorical data were analyzed using the Chi-square test or Fisher's exact test, and t-tests. Logistic regression analysis was employed to control for potential confounding factors. Odds ratios and 95% confidence intervals were calculated. All statistical analyses were performed using the SPSS software, *vs.* 24 (IMB, USA). Two-sided $p<0.05$ were considered statistically significant.

Results

Table 1 shows the distribution of ejaculated and testicular sperm cases according to the indication and PGD results. Fertilization rates and fertilization anomalies with ejaculated and testicular sperm are presented in table 2. According to these results, the 1PN fertilization rate was significantly higher in the testicular sperm group (p=0.006). Table 3 shows normal and abnormal PGD results depending on the age of men and women. Table 4 shows the time-lapse results. According to these results, the developmental processes of ejaculated sperm are longer than those of testicular sperm ($p<0.01$). When the cases were evaluated according to male and female ages, normal embryos belonged to younger cases in both groups (p= 0.001, p=0.001) (Table 3). Based on the analysis of 317 cases with PGD indication, comparison of the groups with and without genetic anomalies revealed that tPNa and pronuclei time 1 were significantly longer in the absence of aneuploidy (p=0.028 and p=0.037) (Table 5). The effects of oocyte morphology, embryo morphokinetic characteristics, female and male age, and female diagnosis on PGD results were examined using logistic regression analysis. When the backward logistic regression method was applied, only the woman's age was found to influence the PGD outcome. On the other hand, when the variables were applied separately using the Enter method of logistic regression analysis, female age, male age, and recurrent pregnancy loss were found to influence the PGD result (Tables 6 and 7). No re-

Table 1. Rates and comparisons of cases included in the study

	Ejaculated n (%)	Testicular n (%)	p-value *
Indication			
NORMO	72 (22.6%)	-	0.001
AT	174 (55.1%)	-	
OAT+OT	71 (22.3%)	-	
Azoospermia	-	80 (100.0%)	
PGD			
Abnormal	205 (64.6%)	65 (81.3%)	0.154
Normal	112 (35.4%)	15 (18.7%)	

* Chi-square test

Table 2. Comparison of patient-based fertilization rates and embryo cleavage outcomes

	Ejaculated (n=317)	Testicular (n=80)	p-value *
	Mean±SD	Mean±SD	
Women age	31.55±3.84	31.6±3.97	0.86
MII	6.88±5.20	6.34±4.55	0.347
2PN (%)	0.82±0.18	0.74±0.13	0.044
1PN (%)	0.16±0.10	0.21±0.09	0.006
3PN and more (%)	0.02±0.25	0.05±0.36	0.872
2PN cleavage (%)	0.87±0.19	0.83±0.23	0.17

* Mann-Whitney U test

Table 3. Evaluation of female and male age according to PGD results

	Normal (n=205)	Abnormal (n=117)	p-value *
	Mean±SD	Mean±SD	
Female age	35.39±4.28	38.66±4.51	0.001
Male age	38.01±5.44	40.67±6.59	0.001

* Independent samples t-test

lationship was found between oocyte morphology and embryo morphokinetic characteristics. Age and RIF were found to be associated with negative PGD results. The abnormality of PGD results increased by 1.161 times in women and 1.095 times in men, depending on age. Repeated implantation failure increased PGD abnormality by 2.675 times (Tables 6 and 7).

Discussion

In this study, the differences in human preimplantation embryo development were investigated following ICSI using testicular sperm obtained through TESE compared to ejaculated sperm. The study focused on cases of non-obstructive azoospermia, comparing the growth rates and developmental stages of pronuclei, morulae, and blastocysts. It is known that approximately 5-10% of men evaluated for infertility are azoospermic. NOA cases are often associated with testicular dysfunction resulting from disorders in the hypothalamic-pituitary axis (2). With the introduction of ICSI, testicular sperm is increasingly used in in vitro fertilization treatment and is known to increase success rate (3). Testicular sperm differs from ejaculated sperm in terms of chromatin maturity, DNA damage frequency, and RNA content (22). Testicular sperm can fertilize

Table 4. Time-lapse evaluation of ICSI results performed with ejaculated and testicular sperm

Time point	Ejaculated (n=317)	Testicular (n=80)	p-value *
	Mean±SD	Mean±SD	
tPNa	10.48±5.2	8.79±2.21	0.003
tPNf	25.8±7.04	23.83±4.05	0.013
t2	28.05±6.14	26.88±5.51	0.116
t3	36.72±7.86	34.16±7.46	0.020
t4	39.91±8.32	36.69±8.45	0.002
t5	48.26±10.4	44.28±10.96	0.002
t8	60.74±12.12	56.02±10.35	0.003
tM	92.28±9.95	87.38±10.98	0.003
tSB	99.99±9.5	95.65±6.98	0.009
Pronuclei time 1	15.94±4.87	14.51±2.98	0.008

* Independent samples t-test

Table 5. Means and comparisons of PGD normal and abnormal cases

Time points	Normal (n=205)	Abnormal (n=117)	p-value *
	Mean±SD	Mean±SD	
tPNa	9.89±1.57	9.02±2.04	0.028
tPNf	24.13±2.83	23.88±2.82	0.442
t2	26.63±2.95	26.39±3.22	0.512
t3	36.73±4.41	36.53±4.96	0.736
t4	38.87±4.46	38.36±4.52	0.334
t5	49.74±7.46	48.32±7.05	0.106
t8	59.33±8.55	58.61±8.02	0.477
tM	89.12±7.95	88.31±8.38	0.402
tSB	97.32±8.41	95.98±7.74	0.167
Pronuclei time 1	16.60±2.83	14.90±2.67	0.037

* Independent samples t-test

Table 6. Effect of age and female diagnosis on abnormal PGD report

	p-value	OR	95%CI for OR	
			Lower	Upper
Step 1				
Female age	0.001	1.150	1.071	1.235
Male age	0.681	1.013	0.953	1.077
Low ovarian reserve	0.138	-	-	-
Recurrent implantation failure	0.048	2.666	1.008	7.051
Unexplained infertility	0.295	1.344	0.773	2.339
Constant	0.001	0.004	-	-
Step 2				
Female age	0.001	1.161	1.100	1.227
Low ovarian reserve	0.136			
Recurrent implantation failure	0.047	2.675	1.012	7.072
Unexplained infertility	0.299	1.341	0.771	2.332
Constant	0.001	0.005	-	-
Step 3				
Female age	0.001	1.142	1.085	1.201
Constant	0.001	0.011	-	-

OR: Odds ratio

oocytes after ICSI, but they are often immature since they are retrieved before undergoing the epididymal transport process. Despite several studies, the biological effects of using testicular sperm on fertilization, preimplantation embryo development, and postimplantation development are not yet fully understood (16, 23, 24). In this study, time-lapse embryo culture was used to

Table 7. Effect of age and female diagnosis on abnormal PGD report

	p-value	OR	95%CI for OR	
			Lower	Upper
Female age	0.001	1.161	1.100	1.227
Male age	0.001	1.095	1.048	1.144
Low ovarian reserve	0.136	1.452	0.657	9.012
Recurrent implantation failure	0.047	2.675	1.012	7.072
Unexplained infertility	0.299	1.341	0.771	2.332

OR: Odds ratio

examine these possible differences. Embryos resulting from couples treated with micro-TESE-ICSI (n=80) were analyzed and compared with embryos derived from couples treated with ejaculated sperm ICSI (n=397). In our study, embryos treated with micro-TESE sperm had shorter developmental times. It was also found that micro-TESE embryos had more 1PN and fewer 2PN embryos. Our results provide insight into the biological differences between testicular and ejaculated sperm and their implications for human fertilization. The influence of sperm origin on embryonic development and pregnancy outcome has received considerable attention. There are many conflicting reports regarding the influence of male factor infertility and sperm origin on embryo growth potential following ICSI and post-fertilization. Among the studies employing time-lapse microscopy (TLM) to analyze the influence of azoospermia and sperm origin on embryo development, Lammers et al. analyzed 48 cycles (32 testicular and 14 epididymal cycles) using surgically retrieved sperm and compared the morphokinetic and clinical outcomes with those observed in 556 cycles involving ejaculated sperm (25). In their study, the mean timings for pronuclear stage (cc2), initial compression (tSC), beginning of blastulation (tSB), and blastocyst formation (tB) were significantly delayed in cycles using surgically retrieved sperm.

However, there was sufficient overlap in the distribution of timings and no individual markers were found to predict outcome. The researchers concluded that the morphokinetic analysis did not identify any clinically relevant differences. Our research is one of the studies that used morphokinetic criteria to characterize paternal influence on embryonic development. TLM showed that embryos derived from micro-TESE exhibited significantly different kinetic behaviors than embryos derived from ejaculated sperm without male factors. In this study, when non-obstructive azoospermia cases were compared with controls receiving ejaculated sperm, embryos derived from testicular sperm developed faster than embryos derived from ejaculated sperm. The same result was obtained in another study conducted in 2019 (16). The reason the results of Lammers et al. could not be obtained may be explained by the presence of asthenospermia in some of the ejaculated sperm cases (25). In contrast, oligoasthenoteratozoospermia was exhibited by others, along with the inhomogeneity of the ejaculated group. Furthermore, when the fertilization rates and anomalies were taken into account, it was observed that 1PN fertilization was high in NOA cases. It is hypothesized that 1PN zygotes result from parthenogenetic activation of either paternal or maternal material, or as a result of abnormalities in nuclear envelope formation, such as asynchrony of pronuclear appearance, delayed pronuclear formation, or fusion of male and female pronuclei (26). The formation of a haploid embryo in the oocyte would be led by parthenogenetic activation. In a study on haploids showing only 1PN, Dozortsev et al. found that an uncondensed sperm nucleus was detected alongside 1PN in 45–86% of 1PN zygotes (27, 28). In contrast, van der Heijden et al. showed that some 1PN zygotes are of paternal origin and result from the fusion of parental chromatin, usually occuring after sperm penetration (29). On the other hand, if asynchrony or fusion of male and female pronuclear cells occurs during pronuclear formation, fertilization is carried out, and the resulting embryos can become diploid (29). According to Desai et al., the developmental potential of 1PN zygotes is also much lower than 2PN zygotes

(24). It has been suggested that this may be attributed to the fact that many 1PN zygotes are haploid. In contrast, Liao et al. reported that 74.6% of 1PN zygotes derived from IVF and ICSI developed to the blastocyst stage and were diploid (30). Hirata et al. also examined the aneuploidy of 1PN zygotes. They observed that 1PN zygotes that developed to the blastocyst stage underwent a process similar to that of 2PN zygotes and exhibited identical pregnancy rates (31). Time-lapse technology has been used to monitor and track embryo development since 2011. The fertilization process starts with the extrusion of the second polar body (IIPB), followed by pronucleus formation, effacement, progression to the 2-cell stage, 4-8-cell stage, morula, and ultimately the blastocyst. Many models have been developed for these time intervals, and the implantation process has been predicted based on them (32). In a hierarchical model, morphological screening is based on a combination of the absence of exclusion criteria, the timing of cell divisions, and their synchronization. Subsequent studies have demonstrated that no single model can be universally applicable for embryoscopic follow-up. Other parameters can be added to artificial intelligence programs (33). So far, only a few studies have used TLM to assess embryo development in azoospermic patients (34). Desai et al., in a survey conducted in 2018, showed that no difference was found in the clinical pregnancy rate among the three groups after the transfer of embryos developed as a result of ICSI with TESE, PESA, and ejaculated sperm (control) (24). A non-significant decrease in implantation (30.9%) and live birth rate (43%) was observed in the TESE group compared to the PESA (35.3%, 58%) and control sperm (45.1%, 56%) groups. Compression failure was significantly higher in TESE-NOA embryos (35.2%; $p<0.001$) compared to TESE-OA (4%), PESA (9%), and control sperm (3.8%) embryos. The two points at which TESE-derived embryos (both NOA and OA) exhibited the most distinct behavior compared to PESA and control sperm embryos were cc2 (time from t3 to t2; time to the beginning of the second cell cycle) and tSB (time to the beginning of blastulation). A significantly lower percentage of TESE-derived embryos showed kinetics associated with high-quality embryos having the highest developmental potential. Additionally, the incidence of direct unequal cleavage (DUC) was significantly higher after ICSI with sperm from azoospermic men (24). Our study compared the abnormality rates in micro-TESE embryos using ejaculated sperm, as analyzed by PGD. Although some studies have reported higher aneuploidy rates in NOA cases, our study did not find statistically significant differences in abnormal embryo rates between NOA and micro-TESE cases (35, 36). Our study found that micro-TESE embryos had no significant normal and abnormal PGD results compared to controls in time-lapse examinations of embryos. Nevertheless, they exhibited faster developmental timelines in time-lapse analysis. In our study, it was observed that sperm origins did not affect aneuploidy rates. It is suggested that sperm from NOA patients exhibit higher rates of aneuploidy, altered DNA compaction, and both single- and double-stranded DNA breaks, which are attributed to severe spermatogenesis failure (35, 36). Many researchers confirmed that aneuploidy rates increase with parental age and repeated IVF failure (37-39). In this study, there was a positive correlation between parental age and aneuploidy rates. In addition, logistic regression analysis revealed that female age, male age, and repeated implantation failure increased the likelihood of abnormal PGD results 1.161, 1.095, and 2.675 times, respectively. It is helpful to evaluate this result proportionally in clinical practice. A limitation of our study is that the group using ejaculated sperm does not consist of a homogeneous population. Another significant limitation of this study was its retrospective nature. The etiology of azoospermic men included in this publication could not be adequately controlled. The inclusion of a control group using normospermic sperm for ICSI was also essential. Furthermore, due to lack of adequate implantation data, a comparison of clinical pregnancy and live birth outcomes with different sperm types could not be made, based on whether the transferred embryo(s) fell within optimal kinetic ranges. Repeating prospective randomized analyses in subsequent studies has been found to be advantageous.

Conclusion

Embryos derived from testicular sperm in NOA patients exhibit significantly faster morphokinetic development, but are associated with a higher rate of 1PN fertilization compared to those derived from ejaculated sperm. Despite these fertilization anomalies, aneuploidy rates were comparable between groups. Advanced maternal age and recurrent implantation failure were identified as

significant predictors of embryo aneuploidy, regardless of sperm origin. These findings suggest that while testicular sperm can be effectively used in NOA cases, special consideration should be given to maternal age and history of RIF during the treatment planning. For older patients and those with RIF, PGT-A may be particularly valuable to guide embryo selection and improve clinical outcomes, irrespective of sperm origin.

Conflict of Interest

Authors declare no conflict of interest.

References

1. Practice committee of the American society for reproductive medicine. Management of nonobstructive azoospermia: a committee opinion. Fertil Steril. 2018;110(7):1239-45.
2. Tao Y. Endocrine aberrations of human nonobstructive azoospermia. Asian J Androl. 2022;24(3): 274-86.
3. Chen X, Ma Y, Zou S, Wang S, Qiu J, Xiao Q, et al. Comparison and outcomes of nonobstructive azoospermia patients with different etiology undergoing microTESE and ICSI treatments. Transl Androl Urol. 2019;8(4):366-73.
4. Tharakan T, Luo R, Jayasena CN, Minhas S. Non-obstructive azoospermia: current and future perspectives. Fac Rev. 2021;10:7.
5. Schlegel PN. Testicular sperm extraction: microdissection improves sperm yield with minimal tissue excision. Hum Reprod. 1999;14(1): 31-5.
6. Bernie AM, Mata DA, Ramasamy R, Schlegel PN. Comparison of microdissection testicular sperm extraction, conventional testicular sperm extraction, and testicular sperm aspiration for nonobstructive azoospermia: A systematic review and meta-analysis. Fertil Steril. 2015;104(5): 1099-103.e1-3.
7. Caroppo E, Colpi EM, Gazzano G, Vaccalluzzo L, Scroppo FI, D'Amato G, et al. Testicular histology may predict the successful sperm retrieval in patients with non-obstructive azoospermia undergoing conventional TESE: a diagnostic accuracy study. J Assist Reprod Genet. 2017;34(1): 149-54.
8. McLachlan RI, Rajpert-De Meyts E, Hoei-Hansen CE, de Kretser DM, Skakkebaek NE. Histological evaluation of the human testis--approaches to optimizing the clinical value of the assessment: mini review. Hum Reprod. 2007;22(1):2-16.
9. Deruyver Y, Vanderschueren D, van der Aa F. Outcome of microdissection TESE compared with conventional TESE in non-obstructive azoospermia: a systematic review. Andrology. 2014;2(1):20-4.
10. Alkandari MH, Zini A. Medical management of non-obstructive azoospermia: a systematic review. Arab J Urol. 2021;19(3):215-20.
11. Koşar PA, Özçelik N, Koşar A. Cytogenetic abnormalities detected in patients with non-obstructive azoospermia and severe oligozoospermia. J Assist Reprod Genet. 2010;27(1):17-21.
12. Peña VN, Kohn TP, Herati AS. Genetic mutations contributing to non-obstructive azoospermia. Best Pract Res Clin Endocrinol Metab. 2020;34(6): 101479.
13. Cerván-Martín M, Castilla JA, Palomino-Morales RJ, Carmona FD. Genetic landscape of nonobstructive azoospermia and new perspectives for the clinic. J Clin Med. 2020;9(2):300.
14. Wang D, Chen R, Kong S, Pan QY, Zheng YH, Qiu WJ, et al. Cytogenic and molecular studies of male infertility in cases of Y chromosome balanced reciprocal translocation. Mol Med Rep. 2017;16 (2):2051-4.
15. Cioppi F, Rosta V, Krausz C. Genetics of azoospermia. Int J Mol Sci. 2021;22(6):3264.
16. Buran A, Tulay P, Dayıoğlu N, Bakircioglu ME, Bahceci M, İrez T. Evaluation of the morphokinetic parameters and development of pre-implantation embryos obtained by testicular, epididymal and ejaculate spermatozoa using time-lapse imaging system. Andrologia. 51(4):e13217.
17. Palermo GD, Colombero LT, Hariprashad JJ, Schlegel PN, Rosenwaks Z. Chromosome analysis of epididymal and testicular sperm in azoospermic patients undergoing ICSI. Hum Reprod. 2002;17 (3):570-5.
18. Weng SP, Surrey MW, Danzer HC, Hill DL, Chen PC, Wu TC. Chromosome abnormalities in embryos derived from microsurgical epididymal sperm aspiration and testicular sperm extraction. Taiwan J Obstet Gynecol. 2014;53(2):202-5.
19. Scarselli F, Casciani V, Cursio E, Muzzì S, Colasante A, Gatti S, et al. Influence of human sperm origin, testicular or ejaculated, on embryo morphokinetic development. Andrologia. 2018;50(8): e13061.
20. Shah R. Surgical sperm retrieval: techniques and their indications. Indian J Urol. 2011;27(1):102-9.
21. Practice committee of the American society for reproductive medicine. Diagnostic evaluation of the infertile male: a committee opinion. Fertil Steril. 2015;103(3):e18-25.
22. Van Marion ES, Speksnijder JP, Hoek J, Boellaard WP, Dinkelman-Smit M, Chavli EA, et al. Time-lapse imaging of human embryos fertilized with testicular sperm reveals an impact on the first

embryonic cell cycle. Biol Reprod. 2021;104(6): 1218-27.

23. Desai N, Ploskonka S, Goodman LR, Austin C, Goldberg J, Falcone T. Analysis of embryo morphokinetics, multinucleation and cleavage anomalies using continuous time-lapse monitoring in blastocyst transfer cycles. Reprod Biol Endocrinol. 2014;12:54.
24. Desai N, Gill P, Tadros NN, Goldberg JM, Sabanegh E, Falcone T. Azoospermia and embryo morphokinetics: testicular sperm-derived embryos exhibit delays in early cell cycle events and increased arrest prior to compaction. J Assist Reprod Genet. 2018;35(7):1339-48.
25. Lammers J, Reignier A, Splingart C, Catteau A, David L, Barriere P, et al. Does sperm origin affect embryo morphokinetic parameters? J Assist Reprod Genet. 2015;32(9):1325-32.
26. Kai Y, Moriwaki H, Yumoto K, Iwata K, Mio Y. Assessment of developmental potential of human single pronucleated zygotes derived from conventional in vitro fertilization. J Assist Reprod Genet. 2018;35(8):1377-84.
27. Dozortsev D, De Sutter P, Dhont M. Behaviour of spermatozoa in human oocytes displaying no or one pronucleus after intracytoplasmic sperm injection. Hum Reprod. 1994;9(11):2139-44.
28. Goud P, Goud A, Van Oostveldt P, Van der Elst J, Dhont M. Fertilization abnormalities and pronucleus size asynchrony after intracytoplasmic sperm injection are related to oocyte postmaturity. Fertil Steril. 1999;72(2):245-52.
29. Van der Heijden GW, Van den Berg IM, Baart EB, Derijck AA, Martini E, De Boer P. Parental origin of chromatin in human monopronuclear zygotes revealed by asymmetric histone methylation patterns, differs between IVF and ICSI. Mol Reprod Dev. 2009;76(1):101-8.
30. Liao Q, Zhang Q, Feng X, Huang H, Xu H, Tian B, et al. Development of deep learning algorithms for predicting blastocyst formation and quality by time-lapse monitoring. Commun Biol. 2021;4(1): 415.
31. Hirata K, Goto S, Izumi Y, Taguchi M, Hayashi A, Fujioka M, et al. Chromosome analysis of blastocysts derived from single pronuclear zygotes by array CGH and clinical outcomes by the transfer of single pronuclear zygotes. J Assist Reprod Genet. 2020;37(7):1645-52.
32. Meseguer M, Herrero J, Tejera A, Hilligsøe KM, Ramsing NB, Remohí J. The use of morphokinetics as a predictor of embryo implantation. Hum Reprod. 2011;26(10):2658-71.
33. Kirkegaard K, Hindkjaer JJ, Ingerslev HJ. Hatching of in vitro fertilized human embryos is influenced by fertilization method. Fertil Steril. 2013;100(5):1277-82.
34. Berntsen J, Rimestad J, Lassen JT, Tran D, Kragh MF. Robust and generalizable embryo selection based on artificial intelligence and time-lapse image sequences. PLoS One. 2022;17(2):e0262661.
35. Arumugam M, Shetty DP, Kadandale JS, Nalilu SK. Association of sperm aneuploidy frequency and DNA fragmentation index in infertile men. J Reprod Infertil. 2019;20(3):121-26.
36. Garolla A, Ghezzi M, Cosci I, Sartini B, Bottacin A, Engl B, et al. FSH treatment in infertile males candidate to assisted reproduction improved sperm DNA fragmentation and pregnancy rate. Endocrine. 2017;56(2):416-25.
37. Garrisi JG, Colls P, Ferry KM, Zheng X, Garrisi MG, Munné S. Effect of infertility, maternal age, and number of previous miscarriages on the outcome of preimplantation genetic diagnosis for idiopathic recurrent pregnancy loss. Fertil Steril. 2009; 92(1):288-95.
38. Won SY, Kim H, Lee WS, Kim JW, Shim SH. Preimplantation genetic diagnosis and pre-implantation genetic screening: two years experience at a single center. Obstet Gynecol Sci. 2018;61(1):95-101.
39. Mei Y., Wang Y, He L, Zheng J, Lin Y, Wang F. Performance of preimplantation genetic testing for aneuploidy for patients with unexplained recurrent pregnancy loss and repeated implantation failure. Heliyon. 2024;10(11):e31983.