Clinical Investigation / Araştırma

DOI: 10.4274/tjod.galenos.2026.44592

Turk J Obstet Gynecol

Comparison of embryological and clinical outcomes of ICSI cycles using ejaculated versus testicular sperm (TESA/mTESE) in men with cryptozoospermia: A retrospective cohort study

Kriptozoospermili erkeklerde ejakülat spermi ve testikül sperm (TESA/mTESE) kullanılan ICSI sikluslarının embriyolojik ve klinik sonuçlarının karşılaştırılması: Retrospektif bir kohort çalışması

Ayşen Yücetürk[1], Nuri Peker[1], Özge Karaosmanoğlu[1], Burak Elmas[1], İlke Özer Aslan[1], Tansu Güdelci[2], Bülent Tıraş[3]

[1]Acıbadem Maslak Hospital, Clinic of Obstetrics and Gynecology, Assisted Reproductive Technologies Unit, İstanbul, Türkiye

[2]Acıbadem Maslak Hospital, Clinic of Urology, İstanbul, Türkiye

[3]Acıbadem Mehmet Ali Aydınlar University, Department of Obstetrics and Gynecology, İstanbul, Türkiye

Abstract

Objective: To compare the embryological and clinical outcomes of intracytoplasmic sperm injection (ICSI) cycles using ejaculated versus testicular sperm (TESA/mTESE) in men with cryptozoospermia.

Materials and Methods: This retrospective cohort study included 127 ICSI cycles performed in couples with male cryptozoospermia at the Acıbadem Maslak Hospital Assisted Reproductive Technology Center between 2017 and 2026. Cycles were grouped according to sperm source as ejaculated (n=58) or testicular (n=69; mTESE, n=51; TESA, n=18). The primary outcome was the pregnancy rate per embryo transfer. Multivariable logistic regression was used to adjust for confounders.

Results: Baseline characteristics were similar, except for a higher number of previous IVF attempts in the testicular sperm group (p=0.032) and a more frequent history of miscarriage in the ejaculated sperm group (15.5% vs. 4.5%, p=0.037). The testicular sperm group had more day 5 blastocysts (median 5 vs. 3, p=0.004) and a higher blastocyst formation rate (57.2% vs. 34.3%, p<0.001), but also more grade 3 blastocysts (p<0.001). Per embryo transfer, pregnancy (80.0% vs. 54.5%, p=0.006) and clinical pregnancy rates (78.0% vs. 47.3%, p=0.001) were higher in the ejaculated sperm group, whereas live birth rates were similar (48.0% vs. 45.5%, p=0.794). After adjustment for female age, previous IVF attempts, embryo stage at transfer and embryo quality, sperm source was not independently associated with pregnancy (adjusted odds ratio: 1.94, 95% confidence interval: 0.71-5.26, p=0.195); blastocyst-stage transfer and grade 1 embryo quality were the independent predictors.

Conclusion: In men with cryptozoospermia, testicular sperm increased blastocyst yield but did not improve pregnancy or live birth rates compared with ejaculated sperm. When ejaculated spermatozoa are available, routine surgical sperm retrieval does not appear to provide a clinical advantage.

Keywords: Cryptozoospermia, ICSI, TESA, mTESE, ejaculated sperm, testicular sperm

PRECIS: In cryptozoospermia, testicular sperm increased blastocyst yield but not pregnancy or live birth rates compared with ejaculated sperm; embryo stage and quality, not sperm source, independently predicted pregnancy.

Corresponding Author/Sorumlu Yazar: Nuri Peker MD,
Acıbadem Maslak Hospital, Clinic of Obstetrics and Gynecology, Assisted Reproductive Technologies Unit, İstanbul, Türkiye
E-mail: dr.nuripeker@gmail.com **ORCID ID:** orcid.org/0000-0002-4854-3851

Received/Geliş Tarihi: 12.08.2026 **Accepted/Kabul Tarihi:** 31.08.2026 **Epub:** 30.10.2026

Cite this article as: Yücetürk A, Peker N, Karaosmanoğlu Ö, Elmas B, Aslan İÖ, Güdelci T, et al. Comparison of embryological and clinical outcomes of ICSI cycles using ejaculated versus testicular sperm (TESA/mTESE) in men with cryptozoospermia: a retrospective cohort study. Turk J Obstet Gynecol. [Epub Ahead of Print]

Copyright© 2026 The Author(s). Published by Galenos Publishing House on behalf of Turkish Society of Obstetrics and Gynecology.
This is an open access article under the Creative Commons Attribution-NonCommercial 4.0 International (CC BY-NC 4.0) License.

Öz

Amaç: Kriptozoospermili erkeklerde ejakülat spermi ile testiküler sperm (TESA/mTESE) kullanılan intrasitoplazmik sperm enjeksiyonu (ICSI) sikluslarının embriyolojik ve klinik sonuçlarını karşılaştırmak.

Gereç ve Yöntemler: Bu retrospektif kohort çalışmaya, 2017-2026 yılları arasında Acıbadem Maslak Hastanesi Yardımcı Üreme Teknikleri Merkezi'nde erkek kriptozoospermisi olan çiftlere uygulanan 127 ICSI siklusu dahil edildi. Sikluslar sperm kaynağına göre ejakülat (n=58) ve testiküler (n=69; mTESE n=51, TESA n=18) olarak gruplandı. Birincil sonlanım, embriyo transferi başına gebelik oranıydı. Karıştırıcı faktörler çok değişkenli lojistik regresyon ile değerlendirildi.

Bulgular: Testiküler grupta önceki IVF deneme sayısının daha fazla, ejakülat grubunda ise düşük öyküsünün daha sık olması (sırasıyla p=0,032 ve p=0,037) dışında başlangıç özellikleri benzerdi. Testiküler grupta 5. gün blastokist sayısı (medyan 5'e karşı 3, p=0,004) ve blastokist oluşum oranı (%57,2'ye karşı %34,3, p<0,001) daha yüksekti; ancak grade 3 blastokist sayısı da daha fazlaydı (p<0,001). Embriyo transferi başına gebelik (%80,0'e karşı %54,5, p=0,006) ve klinik gebelik oranları (%78,0'e karşı %47,3, p=0,001) ejakülat grubunda daha yüksek, canlı doğum oranları ise benzerdi (%48,0'e karşı %45,5, p=0,794). Kadın yaşı, önceki IVF deneme sayısı, transfer edilen embriyonun evresi ve kalitesine göre düzeltme yapıldığında sperm kaynağı gebelik ile bağımsız olarak ilişkili bulunmadı (düzeltilmiş olasılık oranı: 1,94; %95 güven aralığı: 0,71-5,26; p=0,195); blastokist evresinde transfer ve grade 1 embriyo kalitesi bağımsız belirleyicilerdi.

Sonuç: Kriptozoospermili erkeklerde testiküler sperm kullanımı blastokist verimini artırmakla birlikte, ejakülat spermine kıyasla gebelik veya canlı doğum oranlarını iyileştirmemiştir. Ejakülatta sperm bulunduğunda rutin cerrahi sperm elde edilmesinin klinik bir avantaj sağlamadığı görülmektedir.

Anahtar Kelimeler: Kriptozoospermi, ICSI, TESA, mTESE, ejakülat spermi, testiküler sperm

Introduction

Cryptozoospermia is a condition characterized by an extremely low sperm count, in which spermatozoa can only be detected following centrifugation or upon detailed microscopic examination of the ejaculate[1]. Clinically, it is often confused with azoospermia due to the absence of spermatozoa on standard semen analysis; however, further investigation reveals the presence of a very small number of sperm cells. Since both sperm count and motility in the ejaculate are considerably low in these patients, the success rate of intracytoplasmic sperm injection (ICSI) may be adversely affected[2,3]. In contrast, in a healthy male with normal sperm count and motility, ejaculated spermatozoa have a high fertilization capacity, having completed their maturation process within the male reproductive tract, and are capable of achieving natural conception[4]. Although ICSI offers a ray of hope for cryptozoospermic patients to achieve parenthood, the use of these poor-quality ejaculated spermatozoa in ICSI may limit success. Therefore, the use of surgically retrieved testicular spermatozoa has been considered as an alternative approach for these patients[5], although fertilization rates, embryo quality, implantation rates, and pregnancy rates remain controversial whether testicular or ejaculated spermatozoa yield superior results[6,7].

The aim of this study is to compare the embryological and clinical outcomes of ICSI cycles performed using either ejaculated spermatozoa or testicular spermatozoa (TESA/mTESE) in patients diagnosed with cryptozoospermia.

Materials and Methods

Study Design

This retrospective cohort study was conducted at Acıbadem Maslak Hospital Assisted Reproduction Unit among patients diagnosed with cryptozoospermia who underwent ICSI using either ejaculated sperm or testicular sperm (TESA/mTESE). The study protocol was approved by the Acıbadem Mehmet Ali Aydınlar University Medical Research Ethics Committee (approval number: 2026-13/573, date: 23.07.2026). All procedures were performed in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments. Due to the retrospective nature of the study, the requirement for informed consent was waived by the ethics committee.

Study Population and Sample Size

The medical records of couples who underwent ICSI at the Assisted Reproduction Unit of Acıbadem Maslak Hospital between 2017 and 2026 were retrospectively reviewed. Cycles were eligible if the male partner had a diagnosis of cryptozoospermia, ICSI was performed using freshly obtained ejaculated or testicular sperm, and the female partner was aged 20-43 years. A total of 127 ICSI cycles met the eligibility criteria and were included in the analysis.

Exclusion criteria were a history of uterine anomaly (unicornuate, didelphic or bicornuate uterus), a history of uterine synechiae, an abnormal karyotype in the female partner and a diagnosis of azoospermia.

Study Groups and Data Collection

Cycles were divided into two groups according to sperm source: the ejaculated sperm group (n=58), in which ICSI was performed using ejaculated spermatozoa, and the testicular sperm group (n=69), in which spermatozoa were retrieved by TESA (n=18) or mTESE (n=51). Demographic characteristics [female and male age, body mass index (BMI) and duration of marriage], obstetric history (gravidity, parity, living children and previous miscarriage), previous in vitro fertilization (IVF) history, smoking history, and embryological and clinical outcomes were recorded. Male reproductive hormone levels

(FSH, LH and testosterone) were available for only a minority of cycles and were therefore not included in the comparative analysis. All data were anonymized prior to analysis.

Sperm Retrieval and Preparation

Ejaculated Sperm

Semen samples were obtained by masturbation on the day of oocyte retrieval following 2-5 days of sexual abstinence. Samples were collected in sterile containers and left to liquefy at room temperature for 20-30 minutes. Semen analysis was performed according to the World Health Organization (WHO) 2021 (sixth edition) laboratory manual for the examination and processing of human semen. The 5th edition was used for analyses performed before 2021; the definition of cryptozoospermia does not differ between editions. For the diagnosis of cryptozoospermia, semen samples were first examined under a microscope. When no spermatozoa were observed, the sample was centrifuged at 3000 rpm for 10-15 minutes, and the resulting pellet was re-examined for the presence of spermatozoa. Cryptozoospermia was defined as the presence of spermatozoa only after centrifugation, in accordance with WHO criteria.

Testicular Sperm Aspiration (TESA)

Testicular spermatozoa were obtained on the day of oocyte retrieval via percutaneous testicular sperm aspiration under local or general anesthesia. The aspirate was processed in the andrology laboratory, and spermatozoa were selected for use in ICSI.

Microdissection Testicular Sperm Extraction (mTESE)

The patient is placed on the operating table in the supine position. The scrotal skin is stretched over the anterior surface of the testis, and a single 2-3 cm midline scrotal incision is made. The testis, along with the spermatic cord, is delivered through the incision. The testicular parenchyma is then exposed under 6-8x microscopic magnification. The testicular parenchyma is dissected at 16-25x magnification, and dilated tubular structures are excised. The excised testicular tissue samples are sent to the IVF laboratory in a Petri dish containing sperm solution. The procedure is terminated in patients in whom sperm are found; in patients with no sperm found, biopsy samples are taken for histopathological diagnosis.

Embryological and Clinical Parameters

The fertilization rate was calculated as the number of zygotes with two pronuclei (2PN) divided by the number of metaphase II (MII) oocytes. Embryo development was recorded on days 3, 5 and 6, and the total number of embryos in culture, the number of embryos in each quality grade and the number of arrested embryos were documented on each day. Cleavage-stage embryos were graded as grade 1 (good), grade 2 (fair) or grade 3 (poor). Blastocysts were graded according to the Gardner and Schoolcraft system and grouped as grade 1 (AA, AB and BA), grade 2 (BB) and grade 3 (all other grades). The blastocyst formation rate was calculated as the number of day 5 blastocysts divided by the number of 2PN zygotes. Day 3 and day 6 assessments were not available for all cycles; analyses for these days were limited to cycles with available data.

The primary outcome was the pregnancy rate per embryo transfer, with pregnancy defined as a positive serum β-hCG test. Secondary outcomes were clinical pregnancy (an intrauterine gestational sac on transvaginal ultrasonography), biochemical pregnancy (a positive β-hCG test without a gestational sac), miscarriage (loss of a clinical pregnancy), live birth and ongoing pregnancy (a viable pregnancy that had not yet resulted in delivery at the time of data collection). The biochemical pregnancy rate was calculated per pregnancy and the miscarriage rate per clinical pregnancy; all other clinical outcomes were calculated per embryo transfer. Pregnancy, clinical pregnancy and live birth rates were also calculated per started cycle.

Statistical Analysis

Statistical analyses were performed using jamovi statistical software (version 2.6.44; The jamovi Project, Sydney, Australia) and GraphPad Prism 9.0 (GraphPad Software, San Diego, CA, USA). The normality of continuous variables was assessed using the Shapiro-Wilk test. Normally distributed variables were presented as mean ± standard deviation and compared using Student's t-test, whereas non-normally distributed variables were presented as median [interquartile range (IQR)] and compared using the Mann-Whitney U test. Categorical variables were presented as number (percentage) and compared using Pearson's chi-square test; Fisher's exact test (2×2 tables) or the Fisher-Freeman-Halton exact test (larger tables) was used when any expected cell count was less than 5. The ICSI cycle was the unit of analysis. Embryological variables were analysed in cycles with available data, and the number of cycles analysed is reported for each variable. To identify factors associated with pregnancy per embryo transfer, univariable binary logistic regression was performed first, and variables with $p<0.10$ were entered into a multivariable model. Results are presented as odds ratios (ORs) or adjusted ORs (aORs) with 95% confidence intervals (CIs). Multicollinearity was assessed using variance inflation factors (VIFs), calibration using the Hosmer-Lemeshow test and discrimination using the area under the receiver operating characteristic curve (AUC). Because the number of events per variable was below 10, the multivariable model was repeated using Firth's penalized likelihood logistic regression. Further sensitivity analyses were performed after restricting the cohort to frozen-thawed transfers, to women aged 20-40 years, to cycles without previous intratesticular treatment and, within the testicular sperm group, to mTESE cycles. All tests were two-sided, and a p-value <0.05 was considered statistically significant.

Results

A total of 127 ICSI cycles were included, of which 58 (45.7%) were performed with ejaculated sperm and 69 (54.3%) with testicular

Table 1. Baseline characteristics of the cycles according to sperm source

Variables	Ejaculated sperm (n=58)	Testicular sperm (n=69)	p
Female age (years)	30.3±4.7	31.6±5.0	0.163[a]
Male age (years)	32 (30-36)	35 (31-40)	0.075[b]
BMI (kg/m^2)	24.4 (22.5-29.1)	25.5 (22.6-28.1)	0.733[b]
Duration of marriage (years)	4.0 (2.0-7.9)	5.0 (3.0-10.0)	0.243[b]
Previous IVF attempts (n)	1 (0-2)	1 (0-3)	**0.032**[b]
Gravidity ≥1	14 (24.1)	16 (23.9)	0.973[c]
Parity ≥1	4 (6.9)	4 (6.0)	1.000[d]
Living children ≥1	3 (5.2)	3 (4.5)	1.000[d]
History of miscarriage ≥1	9 (15.5)	3 (4.5)	**0.037**[c]
Previous cycle with embryo arrest ≥1	3 (5.2)	7 (10.1)	0.343[d]
Previous fertilization failure ≥1	5 (8.6)	8 (11.6)	0.582[c]
Male smoking history	25 (45.5)	22 (36.1)	0.304[c]
Female smoking history	13 (23.6)	8 (12.9)	0.131[c]
Testicular sperm retrieval method			
TESA	-	18 (26.1)	-
mTESE	-	51 (73.9)	-

Data are presented as mean ± SD, median (IQR) or n (%). Statistically significant p-values are shown in bold
[a]: Student's t-test; [b]: Mann-Whitney U test; [c]: Pearson's chi-square test; [d]: Fisher's exact test. Smoking history was defined as >0 pack-years. Smoking data were missing for 3 cycles in the ejaculated sperm group and for 8 (male) and 7 (female) cycles in the testicular sperm group; obstetric history was missing for 2 cycles in the testicular sperm group
BMI: Body mass index, IQR: Interquartile range, IVF: In vitro fertilization, mTESE: Microdissection testicular sperm extraction, SD: Standard deviation, TESA: Testicular sperm aspiration

sperm (mTESE, n=51; TESA, n=18). Baseline characteristics are presented in Table 1. Female age, male age, BMI, duration of marriage, gravidity, parity and smoking history did not differ significantly between the groups. The number of previous IVF attempts was higher in the testicular sperm group [median 1 (IQR: 0-3) vs. 1 (IQR: 0-2), p=0.032], whereas a history of miscarriage was more frequent in the ejaculated sperm group (15.5% vs. 4.5%, p=0.037).

Embryological outcomes are shown in Table 2. The numbers of MII oocytes and 2PN zygotes and the fertilization rate were similar between the groups. On day 5, the testicular sperm group had a higher total number of embryos in culture (median 9 vs. 6, p=0.010), more blastocysts (median 5 vs. 3, p=0.004) and a higher blastocyst formation rate (57.2%±29.1% vs. 34.3%±21.4%, p<0.001). This difference was mainly attributable to grade 2 (p=0.046) and grade 3 blastocysts (p<0.001), whereas the number of grade 1 blastocysts did not differ (p=0.122). Day 3 data were available for 17 cycles in the ejaculated and 45 cycles in the testicular sperm group; in this subset, the testicular sperm group had more grade 2 (p=0.005) and arrested embryos (p=0.035) and fewer grade 3 embryos (p=0.015). Day 6 outcomes, available for 15 and 20 cycles, respectively, did not differ between the groups.

Cycle and clinical outcomes are presented in Table 3. The distribution of cycle outcomes, including cycle cancellation, did not differ between the groups (p=0.479). Embryo transfer was performed in 50 cycles (86.2%) in the ejaculated and 57 cycles (82.6%) in the testicular sperm group; the outcome was unknown for two transfers in the testicular sperm group. Cleavage-stage transfers tended to be more frequent (26.3% vs. 10.0%) and grade 1 embryo transfers less frequent (36.8% vs. 52.0%) in the testicular sperm group, although these differences were not statistically significant (p=0.077 and p=0.115, respectively). Per embryo transfer, the pregnancy rate (80.0% vs. 54.5%, p=0.006) and the clinical pregnancy rate (78.0% vs. 47.3%, p=0.001) were significantly higher in the ejaculated sperm group. The miscarriage rate per clinical pregnancy was numerically higher in the ejaculated sperm group (20.5% vs. 3.8%, p=0.074), and live birth rates were comparable (48.0% vs. 45.5%, p=0.794). Seven pregnancies (14.0%) in the ejaculated sperm group were ongoing at the time of data collection, compared with none in the testicular sperm group (p=0.004). Similar results were observed per started cycle: pregnancy 69.0% vs. 43.5% (p=0.004), clinical pregnancy 67.2% vs. 37.7% (p<0.001) and live birth 41.4% vs. 36.2% (p=0.553).

In univariable logistic regression (Table 4), ejaculated sperm (OR: 3.33, 95% CI: 1.39-7.98, p=0.007), fewer previous IVF attempts (OR: 0.72 per attempt, p=0.013), blastocyst-stage transfer (OR for cleavage-stage transfer: 0.14, 95% CI: 0.05-0.41, p<0.001) and grade 1 embryo quality (OR: 4.25, 95% CI: 1.69-10.65, p=0.002) were associated with pregnancy, and female age showed a borderline association (p=0.066). In the multivariable model, cleavage-stage transfer (aOR: 0.13, 95% CI: 0.03-0.47, p=0.002) and grade 1 embryo quality (aOR: 5.36, 95% CI: 1.78-16.12, p=0.003) remained independent predictors of pregnancy, whereas the association with sperm source was attenuated and

Table 2. Embryological outcomes according to sperm source

Variables	Ejaculated sperm (n=58)	Testicular sperm (n=69)	p
Oocytes and fertilization			
MII oocytes (n)	12 (7-20)	12 (7-17)	0.923[b]
2PN zygotes (n)	9 (5-14)	8 (5-13)	0.378[b]
Fertilization rate (%)	81.2 (70.0-90.0)	76.5 (57.1-92.9)	0.437[b]
Day 3 (available for 17 vs. 45 cycles)			
Total embryos (n)	4 (2-6)	6 (4-9)	0.093[b]
Grade 1 embryos (n)	1 (0-2)	0 (0-1)	0.151[b]
Grade 2 embryos (n)	1 (1-2)	3 (2-6)	**0.005**[b]
Grade 3 embryos (n)	0 (0-2)	0 (0-0)	**0.015**[b]
Arrested embryos (n)	0 (0-1)	1 (0-2)	**0.035**[b]
Day 5 (available for 52 vs. 53 cycles)			
Total embryos (n)	6 (3-9)	9 (5-12)	**0.010**[b]
Blastocysts (n)	3 (2-6)	5 (3-7)	**0.004**[b]
Blastocyst formation rate (%)	34.3±21.4	57.2±29.1	**<0.001**[a]
Grade 1 blastocysts (n)	1 (0-2)	0 (0-1)	0.122[b]
Grade 2 blastocysts (n)	2 (1-3)	3 (1-4)	**0.046**[b]
Grade 3 blastocysts (n)	0 (0-0)	1 (0-3)	**<0.001**[b]
Arrested embryos (n)	0 (0-5)	2 (0-5)	0.222[b]
Day 6 (available for 15 vs. 20 cycles)			
Total embryos (n)	2 (2-4)	2 (1-4)	0.973[b]
Blastocysts (n)	2 (1-2)	2 (1-2)	0.945[b]
Grade 1 blastocysts (n)	0 (0-0)	0 (0-1)	0.838[b]
Grade 2 blastocysts (n)	1 (0-2)	1 (0-1)	0.398[b]
Grade 3 blastocysts (n)	0 (0-0)	0 (0-0)	0.155[b]
Arrested embryos (n)	0 (0-0)	0 (0-3)	0.587[b]

Data are presented as median (IQR) or mean ± SD. [a]: Student's t-test; [b]: Mann-Whitney U test. Fertilization rate = 2PN zygotes/MII oocytes × 100; blastocyst formation rate = day 5 blastocysts/2PN zygotes × 100. Day 3 and day 6 data were available only for the indicated number of cycles; 2PN data were missing for one cycle and the blastocyst formation rate could be calculated for 51 cycles in the ejaculated sperm group. Statistically significant p-values are shown in bold
2PN: Two pronuclei, IQR: Interquartile range, MII: Metaphase II, SD: Standard deviations

no longer statistically significant (aOR: 1.94, 95% CI: 0.71-5.26, p=0.195). The model showed adequate calibration (Hosmer-Lemeshow p=0.568) and discrimination (AUC: 0.81), with no evidence of multicollinearity (all VIFs <1.3). Firth's penalized regression yielded a similar estimate for sperm source (aOR: 1.85, 95% CI: 0.70-4.93, p=0.206). In sensitivity analyses restricted to frozen-thawed transfers, women aged 20-40 years, cycles without previous intratesticular treatment or mTESE cycles, the crude pregnancy rate remained significantly higher in the ejaculated sperm group (all p≤0.016), whereas the adjusted association with sperm source remained non-significant (aOR range 1.96-2.27, all p≥0.13).

Discussion

In this study, we compared the embryological and clinical outcomes of ICSI cycles using ejaculated or testicular sperm in men with cryptozoospermia. Testicular sperm was associated with a higher blastocyst yield and blastocyst formation rate; however, this excess consisted mainly of grade 2 and grade 3 blastocysts, and the number of top-quality (grade 1) blastocysts was similar between the groups. In contrast, pregnancy and clinical pregnancy rates per embryo transfer were significantly higher with ejaculated sperm, while live birth rates were comparable. In multivariable analysis, the association between sperm source and pregnancy was attenuated after adjustment for the stage and quality of the transferred embryo, which emerged as the independent predictors of pregnancy. Cleavage-stage transfers were more frequent and grade 1 embryo transfers less frequent in the testicular sperm group, and these differences in the transferred embryo appear to explain a substantial part of the crude difference in pregnancy rates. Taken together, these findings indicate that the higher blastocyst yield obtained with testicular sperm did not translate into improved clinical outcomes. The miscarriage rate per clinical pregnancy was numerically higher in the ejaculated sperm group, although the difference was not statistically significant; this may partly

Table 3. Cycle and clinical outcomes according to sperm source

Variables	Ejaculated sperm	Testicular sperm	p
Per started cycle (n=58 vs. 69)			
Cycle outcome			
Frozen-thawed embryo transfer	48 (82.8)	53 (76.8)	0.479[e]
Fresh embryo transfer	2 (3.4)	4 (5.8)	
Cancelled: no euploid embryo after PGT-A	4 (6.9)	10 (14.5)	
Cancelled: embryo arrest on day 3	1 (1.7)	1 (1.4)	
Cancelled: embryo arrest on day 5	3 (5.2)	1 (1.4)	
PGT-A performed	16 (27.6)	24 (34.8)	0.384[c]
Pregnancy	40 (69.0)	30 (43.5)	**0.004**[c]
Clinical pregnancy	39 (67.2)	26 (37.7)	**<0.001**[c]
Live birth	24 (41.4)	25 (36.2)	0.553[c]
Per embryo transfer (n=50 vs. 57)			
Transfer type			
Frozen-thawed	48 (96.0)	53 (93.0)	0.683[d]
Fresh	2 (4.0)	4 (7.0)	
Stage of transferred embryo			
Cleavage-stage (day 3) embryo	5 (10.0)	15 (26.3)	0.077[c]
Blastocyst, untested	33 (66.0)	28 (49.1)	
Blastocyst, PGT-A tested	12 (24.0)	14 (24.6)	
Quality of transferred embryo			
Grade 1	26 (52.0)	21 (36.8)	0.115[c]
Grade 2	24 (48.0)	36 (63.2)	
Clinical outcomes per embryo transfer with known outcome (n=50 vs. 55)			
Pregnancy (positive β-hCG)	40 (80.0)	30 (54.5)	**0.006**[c]
Clinical pregnancy	39 (78.0)	26 (47.3)	**0.001**[c]
Ongoing pregnancy	7 (14.0)	0 (0.0)	**0.004**[d]
Live birth	24 (48.0)	25 (45.5)	0.794[c]
Live birth or ongoing pregnancy	31 (62.0)	25 (45.5)	0.090[c]
Biochemical pregnancy, per pregnancy (n=40 vs. 30)	1 (2.5)	4 (13.3)	0.157[d]
Miscarriage, per clinical pregnancy (n=39 vs. 26)	8 (20.5)	1 (3.8)	0.074[d]

Data are presented as n (%). [c]: Pearson's chi-square test; [d]: Fisher's exact test; [e]: Fisher-Freeman-Halton exact test. Pregnancy was defined as a positive serum β-hCG test and clinical pregnancy as an intrauterine gestational sac on ultrasonography. The outcome was unknown for two transfers in the testicular sperm group. Ongoing pregnancy refers to a viable pregnancy that had not yet resulted in delivery at the time of data collection. Statistically significant p-values are shown in bold
β-hCG: Beta human chorionic gonadotropin, PGT-A: Preimplantation genetic testing for aneuploidy

explain why the higher clinical pregnancy rate did not result in a higher live birth rate. In addition, seven pregnancies in the ejaculated sperm group were still ongoing at the time of data collection, and the live birth rate in this group may therefore be underestimated. Differences in sperm DNA integrity, embryonic chromosomal competence and other paternal factors may contribute to these observations; however, these factors could not be evaluated in the present study.

The optimal sperm source for IVF treatment in patients with cryptozoospermia remains controversial, with conflicting evidence regarding whether ejaculated sperm or surgically retrieved testicular sperm provides superior reproductive outcomes. In a study by Ben-Ami et al.[6], the effects of sperm source on IVF outcomes were evaluated in patients with the diagnosis of cryptozoospermia and reported pregnancy 42.5% in cycles using testicular sperm and 15.1% in those using ejaculated sperm. Live birth rates were 27.5% and 9.4% respectively[6]. Overall, testicular sperm use was associated with a significant increase in both pregnancy and live birth rates. Similarly, Ketabchi[8] compared ejaculated sperm with surgically retrieved sperm (TESE/PESA) in patients with cryptozoospermia and reported significantly higher fertilization rates, embryo quality, implantation rates, and clinical pregnancy rates in the surgically retrieved sperm group. Compared with the ejaculated sperm

Table 4. Univariable and multivariable logistic regression analysis of factors associated with pregnancy per embryo transfer

Variables	Univariable OR (95% CI)	p	Multivariable aOR (95% CI)	p
Sperm source (ejaculated vs. testicular)	3.33 (1.39-7.98)	**0.007**	1.94 (0.71-5.26)	0.195
Female age (years)	0.92 (0.85-1.01)	0.066	1.00 (0.90-1.11)	0.985
Male age (years)	0.99 (0.92-1.06)	0.777	-	-
BMI (kg/m^2)	0.97 (0.89-1.06)	0.522	-	-
Duration of marriage (years)	0.94 (0.87-1.01)	0.104	-	-
Previous IVF attempts (n)	0.72 (0.56-0.93)	**0.013**	0.81 (0.59-1.11)	0.192
Embryo stage at transfer (cleavage vs. blastocyst)	0.14 (0.05-0.41)	**<0.001**	0.13 (0.03-0.47)	**0.002**
Transferred embryo quality (grade 1 vs. grade 2)	4.25 (1.69-10.65)	**0.002**	5.36 (1.78-16.12)	**0.003**
Transfer type (fresh vs. frozen-thawed)	1.00 (0.17-5.74)	1.000	-	-
PGT-A tested embryo (yes vs. no)	1.67 (0.60-4.68)	0.327	-	-

The analysis included 105 embryo transfers with a known outcome (70 pregnancies). Variables with p<0.10 in univariable analysis were entered into the multivariable model. Hosmer-Lemeshow test p=0.568; Nagelkerke R^2=0.364; AUC: 0.81. Statistically significant p-values are shown in bold
aOR: Adjusted odds ratio, AUC: Area under the receiver operating characteristic curve, BMI: Body mass index, CI: Confidence interval, IVF: In vitro fertilization, PGT-A: Preimplantation genetic testing for aneuploidy

group, the surgically retrieved sperm group achieved higher fertilization (85.7% vs. 55.3%), implantation (77.1% vs. 39.5%), and clinical pregnancy rates (57.1% vs. 31.6%), as well as better embryo quality. Several previous studies have favored the use of testicular sperm in cryptozoospermic patients. Cui et al.[5] compared testicular and ejaculated sperm in cryptozoospermic patients undergoing ICSI and similarly reported significantly higher implantation, pregnancy, and live birth rates in the testicular sperm group. They found that the use of testicular sperm in ICSI for cryptozoospermic patients provided higher embryo implantation, pregnancy, and delivery rates compared to the use of ejaculated sperm. Saito et al.[9] reported significantly higher blastocyst formation and clinical pregnancy rates with testicular sperm, particularly in patients undergoing TESE after failed ICSI attempts with ejaculated sperm. Similarly, Ku et al.[10] demonstrated a significant advantage of testicular sperm in terms of take-home baby rates, especially among younger couples. These findings contrast with our results, in which testicular sperm did not provide better clinical outcomes than ejaculated sperm and live birth rates were comparable between the groups.

Conversely, the available evidence is not uniform, as several studies have reported comparable pregnancy and live birth rates between ejaculated and surgically retrieved sperm, while others have suggested a potential advantage of ejaculated sperm in terms of reproductive outcomes. In the meta-analysis by Abhyankar et al.[11] no statistically significant difference was found between testicular sperm and ejaculated sperm in terms of pregnancy and fertilization rates. Mantravadi et al.[12], reported a significantly higher fertilization and blastulation rates in the m-TESE group whereas no significant difference was found among the groups in terms of clinical pregnancy rates. Live birth rates were comparable as well. Similarly, Gnoth et al.[13] reported no statistically significant difference between testicular and ejaculated sperm. In the study, implantation rates, clinical pregnancy and live birth rates were found comparable. In addition, Hernandez-Nieto et al.[14] reported no significant differences between ejaculated and testicular sperm cohorts in terms of fertilization (61.7% vs. 64.9%, respectively; p=0.17), blastulation rates (55.8% vs. 55.3%; p=0.86). Furthermore, no statistically significant differences were observed between the two cohorts in terms of pregnancy, clinical pregnancy, ongoing pregnancy, multiple pregnancy, or pregnancy loss rates[14]. A recent review by Japari et al.[15] emphasized that the optimal sperm source in cryptozoospermic men remains controversial. While some meta-analyses advocate that testicular sperm provides better pregnancy and live birth rates, others demonstrate no difference in clinical outcomes between the two sources[15]. In another review with Saleh et al.[16] was stated that surgical sperm retrieval (SSR) may be considered as an option if the likelihood of finding sperm in the ejaculate on the day of oocyte retrieval (OPU) is uncertain, if the sperm count in the ejaculate is insufficient to fertilize all oocytes, or if previous ICSI attempts with ejaculated sperm have failed. The authors emphasize that stronger evidence is needed to conclude that testicular sperm is superior to ejaculated sperm as long as an adequate number of sperm is present in the ejaculate. Therefore, clinicians are advised to make decisions after thoroughly discussing the risks, costs, and potential benefits of the surgical procedure with patients[16].

Study Limitations

This study has several limitations. First, its retrospective and single-center design may have introduced selection bias and limits the generalizability of the findings; in particular, the choice of sperm source was not randomized and may have been influenced by previous treatment history, as reflected by the higher number of previous IVF attempts in the testicular sperm group. Second, the ICSI cycle was the unit of analysis,

and some couples contributed more than one cycle; these cycles were analysed as independent observations. Third, day 3 and day 6 embryo development data were available only for a subset of cycles, and male reproductive hormone levels were available for a minority of patients and could not be analysed. Fourth, the outcomes of seven ongoing pregnancies were not yet known at the time of data collection. Fifth, the sample size was not determined a priori; the study may have been underpowered to detect differences in live birth and miscarriage rates, and the number of events per variable in the multivariable model was limited. Finally, sperm function parameters such as sperm DNA fragmentation were not available.

Conclusion

In men with cryptozoospermia, the use of testicular sperm obtained by TESA or mTESE was associated with a higher blastocyst yield, consisting mainly of lower-quality blastocysts, but not with improved pregnancy or live birth rates compared with ejaculated sperm. Pregnancy and clinical pregnancy rates per transfer were higher with ejaculated sperm; however, this difference was largely explained by the stage and quality of the transferred embryo, and live birth rates were comparable between the groups. These findings suggest that when ejaculated spermatozoa are available, routine SSR may not provide additional clinical benefit. Larger prospective studies are needed to confirm these findings.

Ethics

Ethics Committee Approval: The study protocol was approved by the Acıbadem Mehmet Ali Aydınlar University Medical Research Ethics Committee (approval number: 2026-13/573, date: 23.07.2026).

Informed Consent: Due to the retrospective nature of the study, the requirement for informed consent was waived by the ethics committee.

Footnotes

Authorship Contributions

Surgical and Medical Practices: A.Y., N.P., Ö.K., İ.Ö.A., T.G., B.T., Concept: B.T., Design: A.Y., N.P., T.G., B.T., Data Collection or Processing: A.Y., N.P., Ö.K., T.G., Analysis or Interpretation: A.Y., N.P., Ö.K., T.G., B.T., Literature Search: A.Y., N.P., İ.Ö.A., T.G., Writing: A.Y., N.P., T.G., B.T.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

References

1. World Health Organization. WHO laboratory manual for the examination and processing of human semen. 6th ed. Geneva: World Health Organization; 2021. Available from: https://www.who.int/publications/i/item/9789240030787 ile değiştirilecektir.
2. Strassburger D, Friedler S, Raziel A, Schachter M, Kasterstein E, Ron-el R. Very low sperm count affects the result of intracytoplasmic sperm injection. J Assist Reprod Genet. 2000;17:431-6.
3. van der Westerlaken L, Helmerhorst F, Dieben S, Naaktgeboren N. Intracytoplasmic sperm injection as a treatment for unexplained total fertilization failure or low fertilization after conventional in vitro fertilization. Fertil Steril. 2005;83:612-7.
4. Weissman A, Horowitz E, Ravhon A, Nahum H, Golan A, Levran D. Pregnancies and live births following ICSI with testicular spermatozoa after repeated implantation failure using ejaculated spermatozoa. Reprod Biomed Online. 2008;17:605-9.
5. Cui X, Ding P, Gao G, Zhang Y. Comparison of the clinical outcomes of intracytoplasmic sperm ınjection between spermatozoa retrieved from testicular biopsy and from ejaculate in cryptozoospermia patients. Urology. 2017;102:106-10.
6. Ben-Ami I, Raziel A, Strassburger D, Komarovsky D, Ron-El R, Friedler S. Intracytoplasmic sperm injection outcome of ejaculated versus extracted testicular spermatozoa in cryptozoospermic men. Fertil Steril. 2013;99:1867-71.
7. Bendikson KA, Neri QV, Takeuchi T, Toschi M, Schlegel PN, Rosenwaks Z, et al. The outcome of intracytoplasmic sperm injection using occasional spermatozoa in the ejaculate of men with spermatogenic failure. J Urol. 2008;180:1060-4.
8. Ketabchi AA. Intracytoplasmic sperm injection outcomes with freshly ejaculated sperms and testicular or epididymal sperm extraction in patients with idiopathic cryptozoospermia. Nephrourol Mon. 2016;8:e41375.
9. Saito C, Mizuta S, Higashiyama R, Yamaguchi K, Takaya Y, Matsubayashi H, et al. Intracytoplasmic sperm injection outcomes with ejaculated and testicular sperm in patients with cryptozoospermia. Fertil Steril. 2018;110(Suppl):e65. (Oral Session).
10. Ku FY, Wu CC, Hsiao YW, Kang YN. Association of sperm source with miscarriage and take-home baby after ICSI in cryptozoospermia: a meta-analysis of testicular and ejaculated sperm. Andrology. 2018;6:882-9.
11. Abhyankar N, Kathrins M, Niederberger C. Use of testicular versus ejaculated sperm for intracytoplasmic sperm injection among men with cryptozoospermia: a meta-analysis. Fertil Steril. 2016;105:1469-75.e1.
12. Mantravadi K, Mittal G, Rao DG. Comparing pregnancy outcomes with ejaculated sperm and surgically retrieved sperm among individuals with cryptozoospermia. Fertil Steril. 2022;118(Suppl):e288-9. (Abstract).
13. Gnoth C, Markhinin V, Maxrath B, Skonieczny T, Friol K, Roos J, et al. Impact of sperm cell source on the results of intracytoplasmic sperm injection. Arch Gynecol Obstet. 2015;291:663-9.
14. Hernandez-Nieto C, Lee JA, Alkon T, Luna-Rojas M, Briton-Jones C, Bar-Chama N, et al. Sedimentation versus surgery: testicular and ejaculated sperm result in similar IVF outcomes in patients with cryptozoospermia. Fertil Steril. 2019;112(Suppl):e203-4. (Poster Session).
15. Japari A, Altay B, Christine W, Cayan S, Saleh R, Shah R, et al. Testicular sperm extraction for men with cryptozoospermia - are we jumping the gun? Arab J Urol. 2024;23:237-43.
16. Saleh R, Mostafa T, Çayan S, Shah R, Agarwal A. 'Controversies on risk factors, diagnosis and treatment of male infertility: Is there an end?'. Arab J Urol. 2025;23:167-8.