ISSN 2954-467X

The Journal of Reproduction

www.thejournalofreproduction.com

Surgical sperm extraction versus semen centrifugation: Method of spermatozoa recovery does not correlate with euploidy rates in patients with cryptozoospermia

Alkon-Meadows Tamar

Alkon-Meadows Tamar, MD, MSc [1], 0000-0001-8866-8379; Hernández-Nieto Carlos, MD[1]; Luna-Rojas Martha, MD[1]; Sandler Benjamin, MD[1].

ABSTRACT

Objective

The aim of this study is to evaluate the rate of embryonic euploidy in blastocysts derived from testicular versus ejaculated sperm in cryptozoospermic patients.

Design

Retrospective cohort analysis.

Material and methods

The study included couples who suffer from Cryptozoospermia and underwent an autologous in vitro fertilization (IVF) with preimplantation genetic testing (PGT-A) cycle(s) from 2014 to 2019. Only cases where oocyte insemination was conducted with intra-cytoplasmic sperm injection (ICSI) were evaluated. Cohorts were separated based on the source of sperm (Ejaculated vs. Testicular (TESE)). Demographic and clinical embryology parameters were compared among cohorts. Student's t-test, Wilcoxon' rank test, chi-square test, and multivariate logistic regression fitted with a GEE model were used for data analysis.

Results

A total of 573 blastocysts derived from 87 IVF/PGT-A cases were included in the study. 74 cases (n= 474 embryos) utilized ejaculated sperm and 13 cases (n= 99 embryos) utilized testicular sperm. No significant differences were found in demographic and stimulation parameters among cohorts. (Table 1) No differences among the ejaculated and testicular cohorts were found in fertilization rate (63.2%; 61.1%, p=0.32); blastulation rate (64.5%; 66.6%, p=0.69); and rate of embryo euploidy (49.7%; 52.1%, p=0.76) respectively. No differences were found in rate of cycle cancellation due to unavailable embryos for TE biopsy (18.9% vs 7.6%, p=0.32).

Conclusions

There is no genomic advantage to surgical sperm retrieval in cryptozoospermic patients.

[1] Reproductive Medicine Associates of New York -Mexico, 635 Madison Ave 10th Floor New York, New York, United States, 10022.

NOTE: The numbers following the affiliation markers are the author's ORCID iD.

ARTICLE HISTORY:
Received March 21, 2023.
Revised March 21, 2023.
Accepted March 22, 2023.
Available online April 26, 2023.

CONTACT:
Alkon-Meadows Tamar, MD, MSc.
talkon@rmany.com, tamaralkon@gmail.com
Paseo de la Reforma 2693, Lomas de Bezares, 11910, Ciudad de México, CDMX.
Phone: +52 55 2167 2515.

KEYWORDS: Cryptozoospermia, male infertility, intracytoplasmic sperm injection, testicular or epididymal sperm extraction, preimplantation genetic testing, aneuploidy.

MANUSCRIPT

Introduction

Since 1992, intracytoplasmic sperm injection (ICSI) has been used to overcome many causes of male factor infertility, including cryptozoospermia.[1] According to the World Health Organization (WHO) cryptozoospermia is defined as the absence of spermatozoa from fresh preparations but observed in a centrifuged pellet.[2] Due to low sperm count (<103 spermatozoa/mL) observed in the seminal fluid after centrifugation, cryptozoospermic patients require assisted reproductive technology (ART) to achieve a pregnancy.[3] Furthermore, men with cryptozoospermia may suffer from virtual azoospermia.[4] Because of this, many clinicians may consider the use of testicular sperm extraction (TESE) to achieve a better ART outcome.[5,6]

Controversy exists over the use of ejaculate versus testicular sperm for ICSI in cryptozoospermic patients.[5-9] Ejaculate sperm is thought to be more mature than testicular sperm.[10] Nevertheless, there are inherent concerns with the use of ejaculate sperm; the repeated centrifugations needed to identify viable sperm may increase the production of reactive oxidative species affecting the quality of the sperm.[11] Moreover, it has been proposed that sperm could suffer DNA damage due to oxidative stress after the release from Sertoli cells leading to low sperm quality and impaired clinical outcomes.[12-14] If DNA damage detected in ejaculated spermatozoa begins after the sperm is release from Sertoli cells, it can be hypothesized that sperm recovered directly from the testis could be less affected by this pathological process when compared with ejaculated sperm.

On the other hand, TESE has shown to have debatable benefits over ejaculate sperm,[5-9] and it carries risks of surgical complications and long-term adverse effects including hypoandrogenism.[15,16] Additionally, previous studies have described a correlation between testicular extracted sperm and spermatic aneuploidy in patients with non-obstructive azoospermia.[17] However, there are currently no peer reviewed publications associating higher aneuploidy rates with cryptozoospermia.

Given the lack of information regarding the possible causes for suboptimal outcomes patients with cryptozoospermia, and the possible relationship of embryonic aneuploidy in embryos derived form TESE, we sought to determine whether the embryonic euploidy rate differs in blastocysts derived from testicular versus ejaculated sperm in cryptozoospermic patients.

Materials and Methods

Study design and patient population

This retrospective, single center study included all cryptozoospermic patients who underwent in vitro fertilization (IVF) with ICSI and preimplantation genetic screening for aneuploidy (PGT-A) at RMA NY using next generation sequencing, from 2014 through December 2019. Cases of patients harboring chromosomal rearrangements, undergoing preimplantation genetic testing for monogenic defects (PGT-M) and/or using donor gametes were excluded from the analysis. Cohorts were segregated based on the source of sperm (ejaculated versus testicular). Demographic characteristics such as age, BMI (body mass index), ovarian reserve metrics were collected. Cycle characteristics and embryologic data, including number of mature oocytes (MII)fertilization rate, blastulation rate (total number of viable blastocysts over the total number of fertilized oocytes), embryo quality and ploidy rates (number of euploid/aneuploid/indeterminate blastocysts over the number of biopsied blastocysts) were compared between cohorts.

Stimulation protocol

Patients underwent controlled ovarian hyperstimulation (COH) for IVF as previously described.[18] Briefly, the COH protocol was selected at the discretion of the reproductive endocrinologist and involved the administration of follicle-stimulating hormone (FSH) and human menopausal gonadotropin (hMG) with a gonadotropin-releasing hormone (GnRH) agonist downregulation protocol with leuprolide acetate (Lupron, AbbVie Inc., North Chicago, IL), a GnRH antagonist protocol (Ganirelix Acetate, Organon USA Inc., Roseland, NJ or Cetrotide, EMD Serono, Rockland, MA), or a microflare protocol with leuprolide acetate (Lupron, AbbVie Inc., North Chicago, IL). These protocols have been described previously.[18] Follicular development was monitored using transvaginal ultrasonography. When at least two follicles reached 18 mm in diameter, final oocyte maturation was induced with either hCG (5000–10,000 IU, Novarel, Ferring Pharmaceuticals, Parsippany, NJ, USA), recombinant human chorionic gonadotropin (250–500 µg, Ovidrel, EMD Serono, Rockland, MA) or, in high responders at risk of ovarian hyperstimulation syndrome undergoing a GnRH antagonist protocol, a dual trigger with 2 mg of leuprolide acetate and 1000

IU of Hcg or leuprolide acetate alone. Thereafter, patients underwent vaginal oocyte retrieval under transvaginal ultrasound guidance 36h after oocyte maturation was triggered.

Methodology of the TESE Procedure

Patients with cryptozoospermia underwent testicular sperm retrieval as previously described.[20] Briefly, after stabilization of the testicle, a small incision in the testicle's midportion was performed, cutting through the scrotal skin, tunica vaginalis, and albuginea. A substantial piece of the extruding testicular tissue was cut with small scissors, washed with medium to remove blood traces, and placed in a Petri dish. Testicular tissue was vigorously fragmented and minced using two glass slides and immediately examined under the inverted microscope for the presence of spermatozoa in a wet preparation. Once spermatozoa were found, the surgical procedure was terminated. If spermatozoa were not observed, additional biopsies were taken from different areas of the same testicle and also from the contralateral one.

Laboratory procedures

Embryo Culture

All metaphase II (MII) oocytes underwent intracytoplasmic sperm injection (ICSI). Embryos were cultured to the blastocyst stage as previously described (Hernandez-Nieto, et al. 2019). On day 3 of embryo development, all embryos underwent laser-assisted zona hatching by creating a 25–30 µm opening in the zona pellucida with a 200– 300 ms pulse using ZILOS-tk Laser (Hamilton Thorne Biosciences, MA, USA) to facilitate posterior trophectoderm herniation. Blastocyst trophectoderm biopsies were performed on day 5-7 of development, contingent upon morphologic eligibility (Modified Gardner Scoring system).[19] Biopsy was performed as described previously.[18] The biopsy samples were placed in hypotonic wash buffer and submitted for analysis. Embryos were vitrified after the biopsies. Five to seven cells were analyzed by next generation sequencing (NGS) in order to determine chromosome, copy number and assigned to the following categories: euploid, aneuploid or inconclusive by the reference laboratory (during the study period mosaicism was not yet reported).

Outcome measures

The primary outcome was ploidy rate in blastocysts derived from testicular versus ejaculated sperm in cryptozoospermic patients, defined as the number of euploid and/or aneuploid blastocysts over the total number of biopsied blastocysts. Secondary outcome measures included fertilization rate, blastulation rate (total number of viable blastocysts over the total number of fertilized oocytes), and number of biopsied embryos.

Statistical analysis

Descriptive data was compared by Student's t-test, Wilcoxon' rank test and chi-square test when appropriate. The results were expressed as percentages, means and SDs with Clopper–Pearson binomial 95% CI. Adjusted odds ratios (OR) with 95% CI were calculated using multivariate logistic regression analyses to adjust for confounding variables. Logistic regression models were fitted with generalized estimating equations (GEE) to account for patients that underwent multiple cycles. Statistical analyses were performed using SAS version 9.4 (SAS institute Inc., Cary, NC, USA). All p-values were two-sided and were considered significant if less than 0.05.

Regulatory approval

This retrospective study was approved by the Icahn School of Medicine at Mount Sinai Institutional Review Board, Inc.

Results

A total of 573 blastocysts derived from 87 IVF PGT-A cases were included in the study. 74 cases (n= 474 embryos) utilized ejaculated sperm and 13 cases (n= 99 embryos) utilized testicular sperm. No significant differences were found in demographic and cycle characteristics among cohorts. (Table 1).

	Ejaculated sperm cycles		Testicular sperm cycles		
	N=74		N=13		
	Mean	SD	Mean	SD	
Male patient age (years)	39.77	7.48	42.82	7.26	0.17
Female patient age (years)	36.13	4.36	36.98	5.29	0.53
BMI (kg/m2)	24.73	4.53	23.91	5.10	0.55
Baseline FSH (IU/mL)	6.01	4.03	4.80	2.46	0.36
Antimullerian hormone (ng/ml)	2.60	3.45	3.05	2.35	0.69
Baseline Antral Follicle count	10.81	6.05	12.70	3.92	0.34
Surge E2 (pg/mL)	2055.10	1036.86	2490.62	1215.28	0.17
Mature MII oocytes	10.12	6.50	12.46	7.15	0.24
Fertilized oocytes	6.41	4.57	7.62	6.28	0.40
Total blastocysts / Cycle	4.14	4.01	5.08	5.16	0.45
Biopsied embryos /Cycle	3.65	2.95	5.75	4.83	0.26
Euploid embryos/ Cycle	1.81	2.06	3.00	3.07	0.16
Previous Oocyte Retrievals	0.69	1.80	1.00	1.22	0.55
	N	%	N	%	
Cancelled cycles / No embryos for Biopsy	14/74	18.9	1/13	7.6	0.32
Fertilization rate	474/749	63.2	99/162	61.1	0.61
Blastulation rate	306/474	64.5	66/99	66.6	0.69
Blastocyst biopsied/ Non biopsied Rate	201/306	65.6	46/66	69.6	0.53
Euploidy rate	100/201	49.7	24/46	52.1	0.76

Table 1. Demographic cycle characteristics and laboratory outcomes of couples who suffer from cryptozoospermia and underwent an autologous IVF with PGT-A**.**

Note: Data presented as mean, percentages and standard deviation, unless stated otherwise. Abbreviations: BMI, body mass index; LH, luteinizing hormone; FSH, follicle-stimulating hormone; MII, metaphase II. Significance established at p < .05.

No differences were found in fertilization rate (63.2%; 61.1%, p=0.32); blastulation rate (64.5%; 66.6%, p=0.69); and rate of embryo euploidy (49.7%; 52.1%, p=0.76) among cohorts. Finally, no differences were found in rate of cycle cancellation due to unavailable embryos for TE biopsy (18.9% vs 7.6%, p=0.32). (Table I)

After adjusting for female and male patient's age, BMI, AMH, and number of biopsied embryos, there were no association with surgical extracted sperm and lower odds of embryo euploidy (OR 0.69, CI95% 0.11-4.3, p=0.69).

Discussion

The results of this analysis suggest that there is no association with surgical extracted sperm and lower rates of embryonic euploidy. Whereas prior studies have investigated reproductive outcomes of fresh ejaculate or TESE and the transfer of unscreened embryos in patients with cryptozoospermia, this study is among the first to focus on the influence of the sperm source (from testicular versus ejaculated sperm) over the embryonic euploidy rate in cryptozoospermic patients.

When comparing ICSI outcomes using testicular versus ejaculate sperm in patients with

cryptozoospermia in fresh embryo transfers, studies have yield divergent result.[20,21] O'Connell et al. demonstrated ejaculated sperm to be more optimal than testicular sperms in cryptozoospermia patients and suggested that fertilization rates are related to sperm maturation.[20] Conversely, Cui X et al. demonstrated that the use of testicular sperm achieved better embryonic quality and IVF outcomes than ejaculate sperm.[21] Few studies have compared the fertility outcome of ejaculate with testicular sperm cells. Weissman et al reported a series of 4 couples with male factor infertility and multiple failed IVF/ICSI cycles with poor embryo quality and repeated implantation failure using motile ejaculatory sperm cells. The use of fresh testicular sperm cells resulted in better embryonic quality and pregnancies in all cases.[22] Contrary to Weissman findings, our study found similar laboratory outcomes when utilizing both ejaculated or testicular sperm and ICSI.

Normal chromosomal composition is a primary driver of embryonic competence and reproductive success in patients undergoing ART. It is well established that the most important factor to achieve a euploid embryo is the oocyte's age.[23] However, the paternal genome also plays a crucial role.[24] Because of this, there has been growing concerns regarding the possible chromosomal anomalies in offspring of men with severe male infertility.[24] Particularly in embryos derived from testicular versus ejaculate sperm in spite of a young female partner.[25] Even if the infertile male is chromosomally normal in his peripheral lymphocytes, a meiotic disruption may generate high rates of sperm chromosome abnormalities. Because of this, many researchers suggest evaluating the chromosomal complement of the spermatozoa in patients with severe male factor infertility and normal karyotypes.[26] Multiple studies utilized fluorescence in situ hybridization (FISH) to assess the genetic composition of ejaculate sperm in comparison with surgically retrieved spermatozoa. One of the earliest reports studied chromosomes X, Y, 18 in the spermatozoa of 34 men with severe male factor infertility. The authors claimed that testicular spermatozoa presented higher rates of chromosomal aneuploidy in comparison with ejaculate sperm (19.6% vs 13%).[27] In light of this, Cheung et al. compared sperm aneuploidy rates in ejaculated and testicular spermatozoa in the same individuals using FISH and NGS. After evaluating 9 chromosomes, the study reported that the total aneuploidy of surgically retrieved spermatozoa are comparable to that of ejaculated spermatozoa, corroborating that the use of testicular sperm is safe and does not increase aneuploidy rates.[28] Our study findings are similar as we found no association with surgical extracted sperm and lower odds of embryo euploidy (OR 0.69, CI95% 0.11-4.3, p=0.69).

Our study distinguishes itself as it was performed at a single, high-volume academic center with a team of embryologists all uniformly trained, thereby reducing the inherent variability that may arise from multicenter studies. Patients with recognizable risk factors for poor embryonic development, such as parental chromosomal rearrangements, were excluded from the analysis, thus making our findings more generalizable. Aside from a large cohort, we use clinically validated PGT-A techniques to assess the rates of embryonic ploidy for all embryos analyzed, ensuring uniformity within the embryonic genetic results.

Notwithstanding our best efforts to avoid biases, some shortcomings and limitations exist in the analysis. The most notable limitation is its retrospective design, which increases the chance of selection bias. Furthermore, the number of patients that underwent a TESE is limited, However, the retrieval of testicular sperm mandates a surgical intervention and embedded risks. In light of the lack of data about the preferable source of sperm cells for ICSI in patients with cryptozoospermia, it would be unethical to design such a prospective research rather than to first use ejaculated sperm.

CONCLUSION

To our knowledge, this study is the first to evaluate the rate of embryonic euploidy in blastocysts derived from testicular versus ejaculated sperm in cryptozoospermic patients. Our analysis shows that there is no genomic advantage to surgical sperm retrieval in cryptozoospermic patients. Furthermore, we demonstrated that the use of testicular or ejaculate spermatozoa for ICSI can compensate for the reproductive disadvantage associated with the semen parameters of patients with cryptozoospermia. These data can be used to counsel patients about the chromosomal composition of embryos and ART outcomes derived when cryptozoospermia is encountered and to reassure them that the method of sperm collection prior to insemination via ICSI will not influence their IVF clinical success. Further randomized prospective studies should be performed in order to generate personalized and evidence-based recommendations for couples facing cryptozoospermia.

FUNDING

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CONFLICT OF INTEREST

The authors declare they have no conflict of interest.

REFERENCES

[1]. World Health Organization. WHO Laboratory Manual for the Examination and Processing of Human Semen. 5th ed.Geneva: World Health Organization; 2010.

[2]. MacLeod J. Human male infertility. Obstet Gynecol Surv. 1971; 26:335–51.

[3]. Bessonnat J, Brouillet S, Sintzel S, Gillois P, Bergues U, Boutte-Busquet C, et al. In cryptozoospermia or severe oligozoospermia is sperm freezing useful? Basic Clin Androl. 2014; 24:15.

[4]. Tournaye H, Camus M, Goossens A, et al. Recent concepts in the management of infertility because of non-obstructive azoospermia. Hum Reprod. 1995; 10:115–9.

[5]. 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.

[6]. Hauser R, Bibi G, Yogev L, Carmon A, Azem F, Botchan A, et al. Virtual azoospermia and cryptozoospermia—fresh/frozen testicular or ejaculate sperm for better IVF outcome? J Androl. 2011; 32:484–90.

[7]. 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.

[8]. Palermo GD, Neri QV, Schlegel PN, Rosenwaks Z. Intracytoplasmic sperm injection (ICSI) in extreme cases of male infertility. PLoS One 2014:9.

[9]. 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.

[10]. Suganuma R, Yanagimachi R & Meistrich ML. Decline in fertility of mouse sperm with abnormal chromatin during epididymal passage as revealed by ICSI. Human Reprod. 2005; 20:3101– 3108.

[11]. Agarwal A, Ikemoto I, Loughlin KR. Effect of sperm washing on levels of reactive oxygen species in semen. Arch Androl. 1994; 33: 157-162.

[12]. Aitken RJ, Krausz C. Oxidative stress, DNA damage and the Y chromosome. Reproduction. 2001; 122:497-506.

[13]. Moskovtsev SI, Willis J, White J, Mullen JBM. Sperm DNA damage: correlation to severity of semen abnormalities. Urology. 2009; 74: 789-793.

[14]. Benchaib M. Sperm DNA fragmentation decreases the pregnancy rate in an assisted reproductive technique. Hum Reprod. 2003. 18; 1023-1028.

[15]. Everaert K, de Croo I, Kerckhaert W, Dekuype P, Dhont M, van der Elst J, et al. Long-term effects of micro-surgical testicular sperm extraction on androgen status in patients with non-obstructive azoospermia. BMC Urol. 2006:6: 9.

[16]. Schlegel PN, Su LM. Physiological consequences of testicular sperm extraction. Hum Reprod. 1997; 12:1688-1692.

[17]. 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 Jun;53(2):202-5.

[18]. Hernandez-Nieto C, Lee JA, Slifkin R, Sandler B, Copperman AB, Flisser E. What is the reproductive potential of day 7 euploid embryos? Hum Reprod. 2019 Sep 29;34(9):1697-1706.

[19]. Veeck L, Bodine R, Clarke RN, Berrios R, Libraro J, Moschini R, Zaninovic N, Rosenwaks Z. High pregnancy rates can be achieved after freezing and thawing human blastocysts. Fertil Steril. 2004; 82:1418-1427.

[20]. O'Connell M, McClure N, Lewis SE. Mitochondrial DNA deletions and nuclear DNA fragmentation in testicular and epididymal human sperm. Hum Reprod. 2002; 17:1565-1570.

[21]. Cui X, Ding P, Gao G, et al. Comparison of the clinical outcomes of intracytoplasmic sperm injection between spermatozoa retrieved from testicular biopsy and from ejaculate in cryptozoospermia patients. Urology 2017; 102:106–10.

[22]. 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;17:605–609.

[23]. Friedler S, Raziel A, Strassburger D, Soffer Y, Komarovsky D, Ron-El R.Testicular sperm retrieval by percutaneous fine needle sperm aspiration compared with testicular sperm extraction by open biopsy in men wit non-obstructive azoospermia. Hum Reprod 1997;12:1488–93.

[24]. Silber S, Escudero T, Lenahan K, Abdelhadi I, Kilani Z, Munne S. Chromosomal abnormalities in embryos derived from testicular sperm extraction. Fertil Steril. 2003; 79:30–8.

[25]. Schlegel PN. Testicular sperm extraction: microdissection improves sperm yield with minimal tissue excision. Hum Reprod. 1999; 14:131–5.

[26]. Franasiak JM, Forman EJ, Hong KH, et al. The nature of aneuploidy with increasing age of the female partner: a review of 15,169 consecutive trophectoderm biopsies evaluated with comprehensive chromosomal screening. Fertil Steril. 2014; 101: 656-663.

[27]. Vozdova M, Heracek J, Sobotka V, Rubes J. Testicular sperm aneuploidy in non-obstructive azoospermic patients. Hum Reprod. 2012; 27(7):2233–9.

[28]. Cheung S, Schlegel PN, Rosenwaks Z, Palermo GD. Revisiting aneuploidy profile of surgically retrieved spermatozoa by whole exome sequencing molecular karyotype. PLoS ONE. 2019; 14(1): e0210079.