Fertility outcomes after extended searches for ejaculated spermatozoa in men with virtual azoospermia

Netanella Miller, M.D.,[a] Tal Biron-Shental, M.D.,[a,b] Yael Pasternak, M.D.,[a] Michael Belenky, M.Sc.,[d] Shai Shefi, M.D.,[c,e] Pavel Itsykson, Ph.D.,[c] and Arie Berkovitz, M.D.[a,b,c]

[a] Department of Obstetrics and Gynecology, Meir Medical Center, Kfar-Saba; [b] Sakler School of Medicine, Tel Aviv University, Tel Aviv; [c] Assuta Medical Center, Tel Aviv; [d] Male Fertility Center, Rishon LeZion; and [e] Advanced Andrology Clinic, Tel Aviv, Israel

Objective: To assess the fertility outcomes of extended searches for ejaculated spermatozoa in men with virtual azoospermia.
Design: A retrospective cohort of 242 couples whose male partner suffered from nonobstructive azoospermia and who were treated with the use of intracytoplasmic sperm injection (ICSI).
Setting: Not applicable.
Patient(s): One hundred forty patients were referred to an extended search in the ejaculate and 102 patients were referred to micro-surgical testicular sperm extraction (microTESE).
Intervention(s): None.
Main Outcome Measure(s): Rates of sperm retrieval, fertilization, and pregnancy, take-home baby rate, and missed abortion rate were analyzed and compared.
Result(s): In the ejaculated spermatozoa group, motile spermatozoa were retrieved in 91 cases (65%) and on oocyte pick-up day in 71 cases (78%), compared with 70 cases (68%) in the microTESE group, with a similar incidence of sperm retrieval between groups. No significant difference was found between groups regarding mean number of embryo transfer and fertilization and pregnancy rates. There was no significant difference between groups regarding take-home baby rate. A significantly higher first-trimester missed abortion rate was found in the ejaculated sperm group (n = 14; 52%) compared with the microTESE group (n = 3; 8.6%).
Conclusion(s): Conducting an extended spermatozoa search in the ejaculate of men with virtual azoospermia can provide pregnancy rates similar to those obtained with the use of microTESE, with a higher rate of spontaneous abortions in the ejaculate group. (Fertil Steril® 2017;107:1305–11. ©2017 by American Society for Reproductive Medicine.)
Key Words: Virtual azoospermia, microTESE, ejaculated spermatozoa, intracytoplasmic sperm injection, ICSI, pregnancy rate, live birth rate

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Male factor contributes to 23%–25% of infertile couples (1, 2). Azoospermia, defined as the absence of sperm in the ejaculate, is identified in ~10%–15% of infertile men (3). Some men present with cryptozoospermia, when spermatozoa are absent from fresh preparations but are always observed in a centrifuged pellet in the semen sample (4). Semen is usually centrifuged at <3,000*g* for 15 minutes for spermatozoa detection, based on World Health Organization recommendations (4). A more extreme condition, virtual azoospermia, refers to the occasional presence of spermatozoa, after an extended search, in the ejaculate of men diagnosed with azoospermia (5). This condition is probably due to fluctuations in spermatogenesis(6). Men with virtual azoospermia are usually referred to intracytoplasmic sperm injection (ICSI) treatment for paternity, in which the presence of one spermatozoon per oocyte can achieve pregnancy (7). A great debate exists regarding the efficacy of ejaculated versus testicular sperm to achieve the best outcomes after ICSI in men with virtual azoospermia. Retrieving spermatozoa from ejaculated sperm is a noninvasive procedure, yet it requires a prolonged searching time and thorough examination (8). It is also speculated that the retrieved spermatozoa might suffer from oxidative stress (9, 10) and nuclear DNA damage (11) due to its transit through the male genital tract. An additional concern is related to the

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Reprint requests: Netanella Miller, M.D., 59 Tsharnichovski, Kfar Saba, Israel (E-mail: millerne@me.com).

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lack of certainty for the detection of sperm cells on the oocyte pick-up (OPU) day. This situation may lead to the necessity of oocyte cryopreservation, a procedure that is no longer considered to be experimental (12). Recent data also show that oocyte vitrification provides results similar to those achieved with fresh oocytes (13, 14).

Microsurgical testicular sperm extraction (microTESE) is another option for sperm retrieval for men with virtual azoospermia. It involves microsurgical exploration of the testicular parenchyma under an operating microscope to search for dilated seminiferous tubules (15). This method can minimize the amount of testicular tissue required and the vascular injury created compared with TESE (16). Yet, this procedure is still invasive and entails several risks, including damage to the testes as well as anesthesia and surgical complications (15). Few studies, based on relatively small cohorts, have attempted to evaluate the efficacy and fertility outcomes of ejaculated and testicular procedures in men with virtual azoospermia or cryptozoospermia, and they have reached controversial results (6,16–19). Consequently, a recent meta-analysis concluded that there is no specific recommendation for men with cryptozoospermia to use testicular sperm in preference over ejaculated sperm for ICSI (20). Of note, that meta-analysis did not address the time required to search for spermatozoa, which is a crucial parameter in sperm retrieval. Moreover, it did not separate the analysis of the results for men with virtual azoospermia, which is considered to be a more severe sperm pathology, and it included men with cryptozoospermia.

The primary aim of the present study was to evaluate the utility of an extended search carried out in ejaculated specimens compared with microTESE in men with nonobstructive azoospermia with no spermatozoa observed in a centrifuged pellet. A further aim was to assess whether conducting an extended search in ejaculated spermatozoa on OPU day can be a suitable option to offer for this population. Sperm retrieval, fertilization, pregnancy, live birth rate, and missed abortion rate were compared.

MATERIALS AND METHODS

Study Design

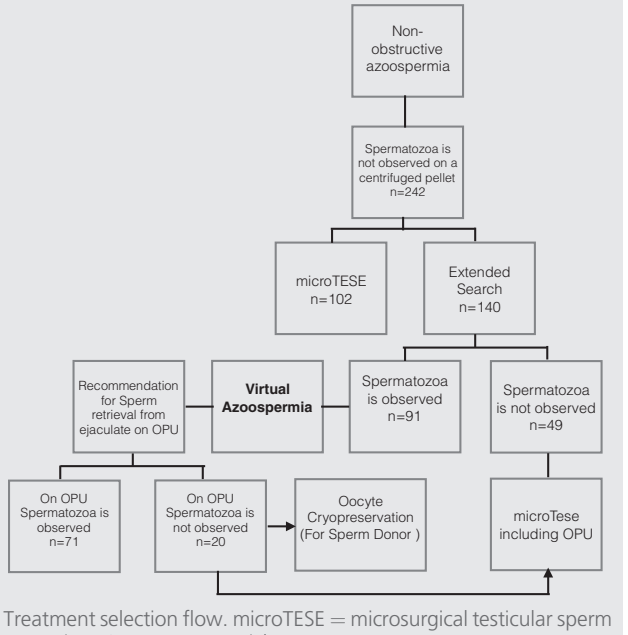
We studied a retrospective cohort of ICSI cycles achieved after extended search of ejaculated spermatozoa or microTESE for men with virtual azoospermia during the years 2006–2016. Data were collected from a single outpatient fertility IVF clinic's records (Assuta Medical Center, Rishon Letzion, Israel).

Patients

All couples whose male partner was diagnosed with nonobstructive azoospermia in which no spermatozoa were observed in centrifuged semen samples were included. Patients for whom spermatozoa were found in the centrifuged pellet, which is defined as cryptozoospermia, were excluded from the analysis. The men diagnosed with nonobstructive azoospermia were then categorized according to the procedure of sperm retrieval: ejaculated spermatozoa or microTESE. The mode of treatment that was offered to the patient, i.e., ejaculate or microTESE, was based on the IVF physician's personal experience with no clear policy of the IVF unit.

Men who were referred to the ejaculate treatment had their ejaculated semen examined with an extended search for 120–240 minutes during the initial evaluation of the male fertility (regardless of OPU date). If spermatozoa were absent in this extended search, these men were referred to microTESE and were excluded from the analysis. If spermatozoa were observed in the extended search, these men were diagnosed with virtual azoospermia, a condition defined as spermatozoa absent from fresh preparation and not observed in a centrifuged pellet, but visible after this extended search of the entire ejaculate. These men were then offered to continue for ejaculated sperm retrieval on OPU day, which was conducted an average of 3 hours before the OPU procedure. The second extended search on OPU day was usually conducted an average of 1–2 months after the initial extended search. If no spermatozoa were observed after an extended search on OPU day, oocyte cryopreservation (which was available from 2009) was conducted, and the men were referred to microTESE. As mentioned above, some of the patients with nonobstructive azoospermia were referred to microTESE with no extended search before the procedure. In the microTESE group, men in whom we did not find sperm in the procedure were referred to use donor sperm. Figure 1 shows the treatment selection flow. It is important to mention that all men in our cohort went through one procedure only, either extended search or microTESE. In addition, the vitrified oocytes were used only for sperm donors, if no spermatozoa were found in the extended search, and later on in the microTESE procedure.

FIGURE 1



Treatment selection flow. microTESE = microsurgical testicular sperm extraction; OPU = oocyte pick-up.

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All of the couples in our cohort received genetic counseling before treatment initiation, according to the Israeli Ministry of Health recommendations. In addition, all of the patients in both groups (extended search and microTESE) went through full evaluation of hormonal profile, karyotype, and testicular Doppler sonography before treatment.

Men with abnormal karyotype (47,XXY or balanced translocation) were excluded, because we think that this group might have different clinical outcomes than those with nonobstructive azoospermia of unknown etiology.

Ethical Approval

The study was approved by the Institutional Ethical Review Board. The authors declare that there was no conflict of interest regarding the publication of this article.

Spermatozoa Collection

Sperm samples were obtained by means of masturbation or with the use of a spermicide-free polyurethane condom after 7–10 days of abstinence. The duration of abstinence was based on Ron-El et al.'s method for extended sperm search preparation (21). This spermatozoa sperm collection method has been used in our laboratory for several years.

Spermatozoa Preparation

After being allowed to liquefy in room temperature for 20 minutes, the sperm samples were loaded on Pureception Sperm Separation Media (Sage In-Vitro Fertilization) gradient of 1 mL 40% v/v (upper phase) and 0.5 mL 80% v/v (lower phase) consisting of a sterile colloidal suspension of silane-coated silica particles in HEPES-buffered human tubal fluid (HTF) containing 10 mg/L gentamicin and centrifuged for 20 minutes at 300 rpm at room temperature. The upper liquid was then removed and the pellet was resuspended in Quinn Sperm Washing Medium (Sage In-Vitro Fertilization) and centrifuged again for 10 minutes at 600 rpm at room temperature. After this procedure was performed twice, the pellet was resuspended in the 100-200 μL sperm washing medium. The entire resulting suspension was distributed into 5–10-μL droplets on a 90-mm sterile plastic Petri dish (De-Groot). The droplets were then flattened by gently tapping the plate on the work surface to minimize droplet depth and debris clustering. A 4-μL droplet of polyvinyl pyrrolidone (PVP) 10% solution (Sage In-Vitro Fertilization) for filling the microcapillary and several collection droplets of 0.6 μL Quinn Sperm Washing Medium were placed on the plate and covered with paraffin oil (Sage In-Vitro Fertilization).

The droplets containing the pellet were thoroughly searched for spermatozoa under $\times$200 magnification with the use of a Nikon Eclipse Ti inverted phase-contrast microscope with an Invenio 3SII camera and Deltapix software. Any spermatozoa found were transferred to a collection droplet and, if present, a sperm exhibiting progressive motility was given priority. This procedure was performed with the use of the Transferman NK2 micromanipulation system with Celltram Oil pump (Eppendorf), equipped with a sterilized nonangulated glass microcapillary with a 12-μm diameter tip (TPC).

MicroTESE

MicroTESE procedures were all performed by a single surgeon (S.S.) in an operating room sharing a window with the embryologic laboratory. After usual placing and patient preparation and induction of general anesthesia, a skin cut was performed on the scrotal median raphe. Testicular envelopes of the larger/firmer testicle were first sharply cut. The tunica vaginalis was clamped on each side, and the testicle was brought outside of it and balanced with the use of gauze pads. A stay suture (Vicryl 5/0) was placed at the far side of an imaginary line of the equatorial plane of the testicle. A thin sharp blade was used for a delicate and long equatorial cut of the tunica albuginea, starting near the stay suture and encompassing two-thirds to three-fourths of the equatorial diameter.

The edges of the tunica cut surface were grasped with the use of fine Jacobson clamps, which both stop subtunical bleeding and help to retract the testicular upper and lower poles apart. The operating microscope was brought into the operative field to perform careful and meticulous screening of testicular seminiferous tubules at both parts of the testicle. Magnification at $\times$20 was used to find thicker or more opaque individual tubules or groups of tubules, in which there was a presumed higher chance to find sperm. Testicular tissue obtained by directed or systematic microsamples during microTESE was first cut in the operating room with the use of microscissors, and then these tiny tissue fragments were transferred to the embryologic laboratory for further mechanical and enzymatic disintegration followed by simultaneous meticulous sperm search by at least two embryologists using advanced micromanipulators. In cases when no thick or opaque tubules were found, systematic multiple microbiopsies were taken from the open testicle, encompassing both upper and lower cut surfaces, including superficial and deep areas. Dissection was stopped if at least ten sperm cells were reported by the embryologic laboratory. Further mechanical dissection of the tissue was performed at the embryologic laboratory under stereomicroscope ($\times$20) with the use of the edges of 24-gauge needles. Primary search for spermatozoa was performed at $\times$20 magnification with the use of an inverted microscope. The dissected testicular tissue was further washed and centrifuged at 1,000*g* for 10 minutes, and the pellet was resuspended and distributed into 5–10-μL drops and covered with oil. Secondary sperm search was performed on processed samples under $\times$20 magnification with the use of the inverted microscope. Motile and immotile spermatozoa were collected with the use of injection pipette and Integra1 micromanipulator for further injection into oocytes or cryopreservation.

After careful bipolar diathermic hemostasis, the operating microscope was taken out of the operative field and the tunica albuginea edges were closed by means of running Vicryl 5/0 from the stay suture. Tunica vaginalis was closed with the use of running Vicryl 4/0 suture, and the testicle was placed back inside its hemiscrotum. Testicular layers were approximated with the use of interrupted Vicryl 4/0 suture. In cases

in which sperm had not yet been found in the embryologic laboratory, the same procedure was performed on the contralateral testicle. Finally, 0.25% bupivacaine solution was injected at the surgical cut, and the skin edges were approximated with the use of interrupted Vicryl Rapide 4/0 suture. Cord block was performed for the operated side(s) with the use of 10 mL 0.25% Bupivacaine solution.

Stimulation Protocols

Two protocols were used for egg stimulation in both ejaculated spermatozoa and microTESE groups: GnRH agonist triptorelin (Decapeptyl) long protocol or GnRH antagonist protocol. Oocytes were retrieved by means of vaginal ultrasound–guided follicular puncture. Embryo transfer was performed on day 2 or 3 after oocyte retrieval.

Statistical Analysis

Statistical analysis was performed with the use of the SPSS 20.0 package for Windows. Categoric variables were analyzed by means of chi-square test or Fisher exact test. Continuous variables were analyzed by means of *t* test. A *P* value of $<.05$ was considered to be statistically significant. All statistical tests were two tailed.

Based on the assumption that the we accepted a difference of 25% in pregnancy rate between groups, we calculated that enrollment of 55 participants in each group would provide a power of 80% to show a treatment effect at a two-sided alpha level of 5%.

RESULTS

The cohort included a total of 242 cases. Out of these, nine cases were ongoing pregnancies beyond 24 gestational weeks. Out of the 242 available cases for analysis, 140 patients were referred to an extended spermatozoa search in the ejaculate (58%) and 102 to microTESE (42%).

In the ejaculate group, the total duration of time for retrieval of spermatozoa was 120–240 minutes. After conducting this extended sperm search in the ejaculated spermatozoa group, we retrieved motile spermatozoa in 91 cases (65%), thus constituting the virtual azoospermia group. On OPU day, after a second extended search, spermatozoa were found in 71 patients out of the 91, which represents 78% success of achieving spermatozoa in the virtual azoospermia group on OPU day and 22% retrieval failure. Table 1 presents the characteristics of the retrieved sperm on OPU day in this group. We found 1–80 spermatozoa in all of the samples (mean 28 ± 21.4). An average of 25 motile spermatozoa were found, which represents an 89% chance for finding a motile sperm out of all spermatozoa found.

In the microTESE group, we retrieved sperm in 70 patients (out of 102) on OPU day (68%), with a 32% failure rate. There was no significant difference between the virtual azoospermia and microTESE groups regarding spermatozoa retrieval rate on OPU day ($P=.142$).

Table 2 presents the baseline characteristics of the study groups including only the cycles for sperm retrieval. There was no difference between the mean female age in the ejaculated spermatozoa cycles compared with the microTESE cycles (31.1 ± 5.2 y vs. 30.8 ± 5.0 y, respectively; $P=.739$) nor in the mean male age (33.3 ± 6.0 y vs. 32.3 ± 5.5 y, respectively; $P=.299$). We found no significant difference regarding FSH, LH, and total T between groups ($P=.083$, $P=.081$, and $P=.059$, respectively). The usage of long (agonist) protocol was higher in the ejaculated spermatozoa group compared with the microTESE group (73% vs. 30%; $P=.0001$). Ovarian response as measured by maximum E_2 levels was similar between groups ($P=.953$), and the mean number of retrieved oocytes per cycle did not differ between groups ($P=.583$).

A comparison between the ICSI fertility outcome of ejaculated spermatozoa versus microTESE cycles is presented in Table 3. Fertilization rate was calculated as number of fertilized oocytes out of mature oocytes. Take-home baby rate was calculated as number of deliveries (including ongoing pregnancies beyond 24 gestational weeks) out of number of pregnancies. No significant differences were found between groups regarding mean number of embryo transfers and fertilization rate ($P=.645$ and $P=.873$, respectively). The pregnancy rate between groups was similar: 64% for the ejaculated spermatozoa group and 50% for the microTESE group ($P=.128$). Although there was a higher take-home baby rate (including pregnancies beyond 24 gestational weeks) in the microTESE group compared with the ejaculated spermatozoa group (82% vs. 58%), this difference was not significant ($P=.335$). A significantly higher first trimester missed abortion rate was found in the ejaculated sperm group (n = 14; 52%) compared with the microTESE group (n = 3; 8.6%; $P=.002$).

TABLE 1

Retrieved sperm characteristics in the ejaculated spermatozoa group (n = 71).

Basic characteristic	Mean ± SD	Min.	Max.
Total no. of sperm observed	28 ± 21.4	1	80
Nonmotile sperm, n	2.4 ± 7.4	0	52
Motile sperm, n	25 ± 21.1	1	80

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TABLE 2

Comparison of basic characteristics between the ejaculated spermatozoa and microTESE groups.

Basic characteristic	Ejaculated	MicroTESE	*P* value
Cycles, n	71	70	
Female age, y	31.1 ± 5.2	30.8 ± 5.0	.739
Male age, y	33.3 ± 6.0	32.3 ± 5.5	.299
FSH, IU/L	14 ± 7.26	24.7 ± 18.11	.083
LH, IU/L	7 ± 2.8	11.6 ± 7.8	.081
Total T, ng/dL	15.5 ± 6.7	11 ± 5.4	.058
Treatment type, % long/%short	73/27	30/70	.0001
E_2, pg/mL	8,069.9 ± 3,896.3	8,034 ± 3,514.7	.953
Eggs retrieved, n	10.9 ± 5	11.4 ± 5	.583

Note: Data presented as mean ± SD, unless otherwise noted. ICSI = intracytoplasmic sperm injection; microTESE = microdissection testicular sperm extraction.

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TABLE 3

Comparison of fertility outcomes after ICSI between the ejaculated spermatozoa and microTESE groups.

Fertility outcome	Ejaculated	MicroTESE	*P* value
Cycles, n	71	70	
Embryos transferred, n	2.13 ± 0.9	2.36 ± 0.8	.645
Fertilization rate, %	56	54	.873
Pregnancy, n (% of cycles)	46 (64)	35 (50)	.128
Take-home baby + ongoing pregnancy, n (% of pregnancies)	27 (58)	29 (82)	.335
Miscarriage in first trimester, n (% per pregnancy)	14 (52)	3 (8.6)	.002

Note: Data presented as mean ± SD or n (%) unless otherwise noted. ICSI = intracytoplasmic sperm injection; microTESE = microdissection testicular sperm extraction.

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In addition, we found a significantly positive correlation between number of embryo transfers (Pearson = 0.3; P=.006) and fertilization rate (Pearson = 0.24; P=.034). There was a negative correlation between male age and number of retrieved motile sperm (Pearson = −0.312; P=.008).

DISCUSSION

To the best of our knowledge, our study is the first to include a large cohort of men (91 patients) with specifically virtual azoospermia for addressing the issue of fertility outcomes. We investigated the ejaculate of men with virtual azoospermia and not cryptozoospermia, because we think that this group belongs to a different category with more severe pathology and therefore needs to be investigated separately.

To date, there is still no consensus regarding the best clinical approach regarding ejaculated versus testicular sperm for ICSI in men who are with virtual azoospermia. Because testicular sperm involves an invasive procedure with possible irreversible damage to the testis, many physicians as well as patients prefer to avoid it if possible.

To achieve the best results in sperm retrieval, we used an inverted microscope to perform an extended search of 120–240 minutes. With this search, we were able to find spermatozoa on OPU day in 71 cases, which reflected a 22% failure of spermatozoa retrieval in men with virtual azoospermia. Of those 71 cases, we detected motile sperm in 89%. The majority of earlier studies had not stated the specific duration needed to perform meticulous microscopic search to retrieve sperm in men with virtual azoospermia or cryptozoospermia (16, 17, 19). One report by Palermo et al., who conducted a meticulous extended search, stated a duration similar to ours, 30–225 minutes, for spermatozoa in ejaculated or surgically retrieved specimens in men with virtual azoospermia or cryptozoospermia (18). We assume, therefore, that our method was probably sufficient to detect spermatozoa if present.

In our study, we found a similar retrieval rate in the microTESE group (68% vs. 78%; P=.142). Alrabeeah et al. found a similar rate of 77% sperm retrieval in men with cryptozoospermia after microTESE (22). This high rate is probably due to the detection of larger and more opaque tubules containing a large number of germ cells. In addition, it is well known that microTESE is associated with a higher sperm retrieval rate than conventional TESE (23, 24).

There was a higher incidence of long protocol use in the ejaculated spermatozoa group (P=.0001), because the long protocol allows for better timing of both fertility experts and the availability of laboratory staff for an extended search procedure.

We found no significant differences in fertilization rate, pregnancy rate, and take-home baby rate between groups (Table 3), similarly to Hauser et al., who performed a comparison between 34 ejaculated cycles and 59 TESE fresh or frozen cycles in 13 couples (five with virtual azoospermia and eight with cryptozoospermia) and found similar pregnancy and take-home baby rates between ejaculated and TESE groups. However, in their study the fertilization rate and the quality of embryos were higher in the ejaculated sperm group (16). Bendikson et al. compared the outcome of ICSI cycles with the use of either ejaculated or testicular sperm, in 16 men diagnosed with virtual azoospermia or cryptozoospermia and also found no difference between fertilization rate, although fresh testicular sperm yielded better results in terms of clinical pregnancies and deliveries (6). In addition, Ben Ami et al., who compared the outcome of ICSI cycles using either fresh ejaculated or testicular sperm in 13 men diagnosed with cryptozoospermia, found higher implantation, pregnancy, and take-home baby rates for the TESE group and no difference regarding fertilization rate (17). However, they excluded the virtual azoospermia group. Palermo et al. found that TESE provided more consistent fertilization and pregnancy outcomes than retrieval from ejaculate and that pregnancy rate decreased as search time increased in the TESE group (18). Yet a meta-analysis that included some of these studies mentioned above, did not find differences between ejaculated sperm and TESE/microTESE regarding fertility and pregnancy rates (20).

We found take-home baby rates (which included number of take home babies and pregnancies beyond 24 gestational weeks) of 82% in the microTESE group and 58% in the ejaculated spermatozoa group. Earlier studies found lower live birth rates ranging from 42% to 47% after testicular sperm retrieval (6, 17) and from 14% to 20% for ejaculated spermatozoa (6, 16). It is possible that our comparatively higher rate is due to the extended search for sperm retrieval, which led to higher numbers of motile sperm and normal-morphology spermatozoa available for fertilization. In addition, we included ongoing pregnancies beyond 24 gestational weeks, which may also have contributed to this increased rate.

We found a significantly higher first trimester missed abortion rate in the ejaculated sperm group compared to the microTESE group (P=.002). In contrast, Ben Ami et al. found higher missed abortion rate in the TESE group compared to the ejaculate spermatozoa group (17). Whereas Palermo et al. found that pregnancy losses, including biochemical, blighted ova, and miscarriage, were similar between the ejaculated and TESE groups (18). One possible explanation is that ejaculated sperm retrieved from men with virtual azoospermia is prone to chromosomal aberrations and thus causes higher missed abortion rates.

We found a negative correlation between male age and number of retrieved motile sperm (Pearson = −0.312;

$P=.008$). Earlier studies have also shown that the percentage of normal sperm in older men is lower than in younger men (25) and that there is an inverse relationship between male age and semen volume and sperm quality (26). Our study supports those conclusions.

One of the difficulties for men with virtual azoospermia relates to the inconsistent presence of sperm in the ejaculate and thus the possibility of wasted oocyte retrieval. This difficulty can be resolved with the use of oocyte cryopreservation techniques. Recent studies have shown that treatment outcomes with the use of autologous oocyte vitrification and warming are as good as with the use of fresh oocytes (13, 14).

According to our data there was a 22% chance of failure of sperm retrieval on OPU day in the ejaculated spermatozoa group, compared with 32% in the microTESE group. Although this difference was not staistically significant, it may have a clinical value.

These findings suggest an appropriate solution when there is no immediate availability of sperm, such as in cases of virtual azoospermia. From this analysis, it appears that the use of extended spermatozoa search in the ejaculate of men with virtual azoospermia can provide pregnancy rates similar to those obtained with the use of microTESE. It is important to explain to couples that there is a higher chance of spontaneous abortion when extended spermatozoa search is conducted.

The two major strengths of our study are the relatively large sample size and the separate analysis of men with virtual azoospermia without including cryptozoospermia. Only one earlier study compared ejaculated sperm versus microTESE and not TESE. Although that study found similar fertilization rates and embryo quality between groups, it did not address missed abortion rate (19).

The limitations of the present study concern its retrospective design, although we think that presenting our gained experience provides sufficient epidemiologic support. In addition, our policy is to add hormonal treatment for men with low levels of testosterone. Yet, in this study we did not have the available data regarding which of the men received prior hormonal treatment to evaluate the effect of hormonal treatment on the sperm retrieval rate. Moreover, an additional analysis of males with abnormal karyotype (47,XXY or balanced translocation) might reveal interesting results.

In conclusion, our relatively large retrospective study suggests that conducting an extended spermatozoa search in the ejaculate of men with virtual azoospermia, can provide pregnancy rates similar to those obtained with the use of microTESE, with a higher rate of spontaneous abortions in the ejaculate group.

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