

ICSI Cycles Using Motile Sperm from Fresh Ejaculate in Cryptozoospermic Patients and the Extremely Severe Oligospermia Patients Yield Similar Reproductive Outcome

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What's known on the subject? and What does the study add?

Literature is insufficient about the clinical and laboratory in vitro fertilization (IVF) outcome with intracytoplasmic sperm injection (ICSI) for men with cryptozoospermia compared to men with extremely severe oligospermia (sperm count <1 mil/mL). Reproductive IVF-ICSI outcomes for men with cryptozoospermia are comparable to those with extremely severe oligospermia provided that motile sperm are used in men with cryptozoospermia.

Abstract

Objective: In this retrospective study, we analyzed the in vitro fertilization (IVF) clinical and laboratory outcome of cryptozoospermia cases compared to the extremely severe oligospermia cases in a single IVF center.

Materials and Methods: All the IVF laboratory and clinical outcomes of cryptozoospermia and extremely severe oligospermia cases were analyzed and compared between January 2014 and December 2019 in Istanbul Florence Nightingale IVF Center. The same reproductive group treated all couples. Virtual azoospermia or cryptozoospermia were diagnosed once the mature sperm cells could be recognized after centrifugation (group 1). Patients without motile sperm were excluded. Extremely severe oligospermia was defined as a sperm count was less than <1 mil/mL (group 2). The study consisted of 33 virtual azoospermic patients with 40 cycles, whereas there were 40 severely oligospermic patients with 45 cycles. All patients underwent the intracytoplasmic sperm injection (ICSI) procedure and all the embryos were let to grow until the blastocyst stage on day 5. Groups were compared for clinical and laboratory reproductive outcome.

Results: Both the median maternal and paternal ages were similar. All outcomes including fertilization rates, blastulation rates, clinical pregnancy and delivery rates were comparable. The miscarriage rates did not also show any statistical difference.

Conclusion: Reproductive outcomes in cryptozoospermic IVF patients are comparable to those of extremely severe oligospermic patients provided that the ICSI is performed using motile spermatozoa. Our results favor using sperm from fresh ejaculate rather than surgical sperm retrieval when motile sperm is available in cryptozoospermic IVF patients.

Keywords: Cryptozoospermia, male infertility, oligospermia, IVF

Introduction

With recent technological advances in reproductive medicine, the success rates are steadily increasing in in vitro fertilization (IVF) practice. Despite these developments, there are still some controversial issues that have yet to be clarified. One of them is how to approach the cryptozoospermia patients.

According to World Health Organization (WHO) "cryptozoospermia" or "virtual azoospermia" refers to the situation when spermatozoa cannot be observed in a fresh sample, instead it could be found after an extended centrifugation followed by meticulous microscopic search (1). Since the number is so few, it is extremely difficult to find, and

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in most laboratories, it is very easy to miss as well. That is why all the patients diagnosed to have azoospermia should be referred to a tertiary andrology center where a detailed semen analysis should be performed and used if any spermatozoa are recovered. This is extremely important to avoid unnecessary surgical interventions for the male partner, such as testicular sperm extraction (TESE) and to save the patients from unnecessary surgical expenses.

These oligospermia are usually classified into 3 classes; mild (10-15 mil/mL), moderate (5-10 mil/mL) and severe (<5 mil/mL) (2). Additionally, here in this study we referred to the oligospermia patients having less than <1 mil/mL on semen analysis as extremely severe oligospermia.

The aim of this retrospective study was to compare the outcome of the intracytoplasmic sperm injection (ICSI) cycles using motile spermatozoa recovered from fresh ejaculate of the cryptozoospermia patients to that of ICSI cycles with extremely severe oligospermia cases in the same IVF program.

Materials and Methods

In this retrospective study, we analyzed all the infertile couples with male factor IVF cycles managed at the Istanbul Florence Nightingale Hospital IVF Unit (including the andrology work-up) between January 2014 and December 2019. Couples were managed and ovum pickups were performed by a single IVF clinician (M.A.A.) and all andrology work-up was accomplished by the same andrology technician. Ethics approval was obtained from Demiroglu Bilim University Ethics Committee (ethics approval no: 2021-12-02).

According to the semen analysis on the day of oocyte pick up, we grouped the cycles into two: in the group 1 we included the cryptozoospermia cases, whereas in group 2 there were cycles in which sperm analysis showed less than 1 million/mL sperm (extremely severe oligospermia group). We excluded the cycles in which no motile sperm was detected and the ones where the ICSI was performed through the frozen sperm. None of the patients with the presence of motile spermatozoa on the day of oocyte pick up underwent surgery for sperm recovery. All sperm analyzes were performed at least twice.

Additionally, to decrease the possibility of female factor as the confounding factor as much as possible, the cycles where maternal age was more than 40 years and less than 5 mature oocytes (MII oocytes) aspirated were excluded from the study in which fertilization failure may have resulted from the female factor.

Briefly stimulation was done as follows: In all controlled ovarian hyperstimulation cycles the patients were stimulated with the use of the same gonadotropin, namely u-hMG (Merional® IBSA,

Switzerland). The initial dose was 75-225 units daily s.c. which was adjusted based on the response to previous cycles and/or body mass index. When the leading follicle reached 14 mm in diameter one ampoule of GnRH antagonist Cetrorelix 0.25 mg daily s.c. (Cetrotide®, Merck, Germany) was added daily until the day of trigger. Once the follicles reached 18-20 mm in diameter, r-hcg 250 mcg sc (group 1) (Ovitrelle®, Merc Serono, Italy) was administered. Thirty-five hours later, oocytes were retrieved under general anesthesia. ICSI was used for all cycles and all the embryos were let to grow until the blastocyst stage on day 5. On the day of oocyte pick up, semen analysis was performed according to the WHO criteria (1).

Cryptozoospermia was only diagnosed when the mature sperm cells were reported after centrifugation at 1800 x g for at least five minutes in an azoospermic man at semen analysis.

The embryo transfer (ET) was performed by the same clinician with a full bladder under transabdominal guidance. Twelve days post ET, all the patients gave blood pregnancy test and test-positive ones were called for transvaginal ultrasonography 15 days later. We defined clinical pregnancy once we saw the gestational sac with fetal cardiac activity inside.

Power analysis was performed based on the study of Ben-Ami et al. (3). They found 15% (8/68) pregnancy rate for ejaculated sperm per cycle compared with 42% (17/48) in couples who underwent surgical sperm retrieval. Considering into account these results, with a power of 80% and alpha value 0.05 and beta value 0.2, the sample size calculated is 84 cycles (42+42) (<https://clincalc.com/stats/samplesize.aspx>).

Statistical Analysis

For data analysis, the Statistical Package for the Social Sciences (SPSS), version 21.0 (SPSS Inc., Chicago, IL) statistical computing software was used. Variables were given as medians (interquartile range). The quantitative data of the groups were compared with Mann-Whitney U test. Categorical data and relationships between the groups were analyzed using the chi-squared test. P<0.05 was considered statistically significant.

Results

In the group 1, there were 33 patients with 40 cycles and in group 2, there were 40 patients with 45 cycles (Table 1). Group 1 and group 2 did not differ in the mean age, the amount of hMG consumed, endometrial thickness on the day of hCG trigger, total and mature oocytes retrieved, fertilization rates and the number of embryos transferred. Additionally, the obstetric outcomes regarding clinical pregnancy, miscarriage and delivery rates did not reach statistically significant differences among the groups (Table 1).

Table 1. Patient and cycle characteristics with pregnancy outcome

Variable	Group 1 (Cryptozoo spermia) (n=40 cycles)	Group 2 (<1 mil/mL) (n=45 cycles)	
Maternal age (year)*	31.50	28	NS
Range	(21-39)	(21-39)	
Consumed hMG medication (units) [‡]	2697.50±865.78	2419.44±773.77	NS
The end. thickness on hCG day [‡]	9.85±1.24	9.74±1.30	NS
Total oocytes retrieved*	9	12	NS
Range	(5-23)	(5-27)	
Number of M2 oocytes retrieved*	7	9	NS
Range	(5-17)	(5-20)	
Fertilization rate (%)*	80	80	NS
Range	(40-100)	(33.33-100)	
Number of transferred embryos*	2	2	NS
Clinical pregnancy rate (%)	67.50	64.44	
Miscarriage rate (%)	7.50	13.33	NS
Delivery rate (%)	60	51.11	NS

*values are in median, ‡: values are in mean ± standard deviation

Discussion

In this study, we found that the primary reproductive outcomes, including clinical pregnancy, miscarriage and delivery rates, were similar among the groups. Additionally, secondary outcomes, including the number of gonadotropins consumed, endometrial thickness on the day of hCG trigger, total and mature oocytes retrieved, fertilization rates and the number of embryos transferred did not differ between groups.

The cryptozoospermia is really a big challenge in IVF practice. If the ejaculate does not contain a suitable sperm sample due to inadequate motility or abnormal morphology, etc. the clinical decision is straightforward to proceed with performing surgical operation to recover the sperm. However, the ideal approach to cryptozoospermia in the presence of motile spermatozoa following centrifugation is still not clear. Some advocate the direct use of motile spermatozoon for ICSI from fresh ejaculate and others favor surgery to recover sperm (4-8). Hauser et al. (4) found that fresh TESE should be considered a treatment of choice in cryptozoospermia cycles showing superior fertility potential compared to use of motile sperm recovered after centrifugation. In another study, Miller et al. (5) compared the micro-TESE surgery with fresh ejaculate and found that they gave similar pregnancy rates. But the miscarriage rates were higher in the ejaculate group and they suggest doing fresh TESE in cryptozoospermic IVF patients. However, in our study, although the pregnancy and delivery rates were higher and miscarriage rate was lower in the group 1 compared to group 2, this difference did not reach statistical significance.

Even, several published meta-analyses showed some contradictory results. In their meta-analysis, Abhyankar et al. (6) concluded that the existing literature does not support a recommendation for men with cryptospermia to use TESE in preference over ejaculated sperm. Contrary to this, in another meta-analysis Ku et al. (7) found that the take home baby rates are higher in the TESE group and concluded that TESE has more advantages for ICSI patients with cryptozoospermia, especially in younger couples. Similarly, in another meta-analysis Kang et al. (8) found that TESE yields better embryo quality, higher pregnancy and implantation rates and recommend that TESE should be the treatment of choice in cryptozoospermic IVF patients.

In our current practice, we do not proceed to surgery if fresh ejaculate yields motile spermatozoa in the cryptozoospermic IVF patients. We think the presence of motile spermatozoa on the day of oocyte pick up is the most essential factor in proceeding with surgery or not. It is a very critical decision to perform surgery in these patients with very limited numbers of spermatozoa. It is well known that spermatogenesis is a focal and periodic process. This means one can miss the sperm producing region during surgery and deceptively present the case as complete azoospermia. Additionally, one can find the focus and may harm that solely sperm producing region and unfortunately, if the IVF treatment fails, these patients may never father a child. Therefore, considering these potential disadvantages of surgery and the absence of solid-state evidence of superiority, we think balance tips toward non-surgical approach. Therefore, considering our results, since performing ICSI with motile

spermatozoa derived from fresh ejaculates in cryptozoospermia cases yields comparable results to those cases with extremely severely low sperm counts (<1 mil/mL), we can comment that the use of motile spermatozoa derived from fresh ejaculates is at least as good as (maybe even better) surgical sperm recovery in cryptozoospermia cases and should be the method of choice.

Study Limitations

To note, this study was limited by its retrospective nature. However, we do not think that this limitation decreases its scientific quality to much extent because our criteria in defining the groups were strict such as the female age (<40) and at least more than 5 oocytes were picked up at OPU procedure. The single and same clinical and laboratory technician may also be considered among the study strengths.

Conclusion

In conclusion, we found that the outcome of using spermatozoa derived from fresh ejaculate in cryptozoospermic IVF patients is comparable to that of extremely severe oligospermic patients provided that the ICSI is performed using motile spermatozoa. Thus, we think that the preferred approach should be to use motile spermatozoa obtained from fresh ejaculate for ICSI and avoid surgical operation in cryptozoospermia.

Ethics

Ethics Committee Approval: Ethics approval was obtained from Demiroglu Bilim University Ethics Committee (ethics approval no: 2021-12-02).

Informed Consent: Retrospective study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: M.A., M.A.A., Concept: M.A., M.A.A., Design: M.A., M.A.A., Data Collection or Processing:

M.A., M.A.A., Analysis or Interpretation: M.A., M.A.A., Literature Search: M.A., M.A.A., Writing: M.A., M.A.A.

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