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Role of Repeating Testicular Sperm Extraction on Sperm Recovery by Using Combination Enzymatic and Mechanical Methods in Non-obstructive Azoospemic Men.

Amina Al-Tameemi [1], Ali Ibrahim Rahim [2], Ula Al-Kawaz [1]

[1] High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al Nahrain University, Baghdad, Iraq.

[2] College of Medicine, University of Kufa, Najaf, Iraq.

aminaakram77@gmail.com

ABSTRACT

Azoospermic patients have the option of undergoing in vitro fertilization procedures, specifically intracytoplasmic sperm injection (ICSI), for which sperm is retrieved from the patient's own testicles and then injected directly into the oocyte. The aim of the study: evaluate the role of repeated sperm extraction by enzymatic and mechanical mincing techniques in azoospemic patients. A total of 32 males with non-obstructive azoospermia participated in the research. Testicular sperm extraction (TESE) was performed under spinal or general anesthesia after a comprehensive history, physical examination, and investigations. After that, the biopsies underwent through a series of mincing processes, both mechanical and enzymatic. Patients gave information including age, body mass index and previous biopsy attempts. In both enzymatic and mechanical methods there was no significant higher sperm recovery in patients with history of previous testicular biopsies (66.7% vs. 38.5%; p=0.209) and (66.7% vs. 30.8%; p=0.102) respectively. Patients who had undergone prior testicular biopsies had marginally higher sperm recovery rates using both enzymatic and mechanical techniques

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KEYWORD

Sperm Extraction, TESE, Mechanical, Enzymatic

1. Introduction

Male fertility has been on the decline, and this trend may have multiple causes. Smoking, alcohol consumption, drug abuse, obesity, high levels of stressful conditions, advanced paternal age, an unhealthy diet, and drinking too much coffee are just some of the lifestyle factors linked to male infertility. Testicular heat stress, strenuous cycling training, sleep deprivation, and mobile phone radiation are also contributors (Durairajanayagam D., 2018 [1]; Dissanayake et al., 2019 [2]).

A non-rare condition, azoospermia, which is defined as the lack of sperm cells in the ejaculate after evaluation of centrifuged semen on at least two occasions, can be diagnosed in 1 percent of the male population and in up to 15 percent of the male subjects who are infertile (Caroppo et al., 2017 [3]). Azoospermic patients have the option of undergoing in vitro fertilization procedures, specifically intracytoplasmic sperm injection (ICSI), in which sperm is retrieved from the patient's own testicles and then injected directly into the oocyte. It has been demonstrated that the reproductive ability of azoospermic patients entering an ICSI cycle is roughly comparable to that of oligozoospermic subjects (Oldereid et al., 2014 [4]).

Azoospermia can be further classified into two types: non-obstructive azoospermia (NOA), which results from failure of spermatogenesis, and obstructive azoospermia (OA), which is caused by an obstruction in the ejaculatory route (Yuen et al., 2021 [5]).

Patients with nonobstructive azoospermia often have flat epididymis and testes with volumes less than 15 cm3. Through a thorough investigation of clinical diagnostic characteristics, obstructive azoospermia can be clearly separated from nonobstructive azoospermia in

the great majority of patients. FSH values of 7.6 mIU/ml or less or a testicular long axis longer than 4.6 cm were present in 96% of males with obstructive azoospermia. In contrast, FSH levels higher than 7.6 mIU/ml or a testicular long axis of 4.6 cm or less were present in 89% of males with nonobstructive azoospermia (Schoor et al., 2002 [6]).

Intracytoplasmic sperm injection (ICSI) is the only treatment option for men with severe oligozoospermia and azoospermia who do not have an obstructive form of the condition. Spermatozoa extraction via testicular biopsy is the standard treatment for men with non-obstructive azoospermia (NOA). Spermatozoa can be collected from the testes and used for ICSI in about 50%-60% of men with NOA and 100% of men with OA (Jungwirth et al., 2012 [7]) .

Several studies have shown that the best chance of discovering the few remaining foci of spermatozoa within the functionally impaired gonads requires an open biopsy for testicular sperm extraction (TESE). Mechanically prepare testicular tissue by mincing and shredding whole tissue to yield injectable sperm in TESE (Modarresi et al., 2008 [8]).

2. Patients and Methods

Between 2022 and 2023, this clinical trial study was carried out at the "High Institute for Infertility Diagnosis and Assisted Reproductive Technologies/Al-Nahrian University." The local medical ethical committee approved the trial, and patients gave their verbal consent to participate.

A total of 32 males with non-obstructive azoospermia participated in the research. Testicular sperm extraction (TESE) was performed under spinal or general anesthesia after a comprehensive history, physical examination, and investigations. After that, the biopsies went through mechanical mincing and then

enzymatic digestion. Patients gave information, including age, body mass index, and previous biopsy attempts.

The TESE procedure was used to obtain testicular tissue from patients under spinal anesthesia or general anesthesia. The testicular tissue underwent mincing between two microscopic slides, placed in a buffered medium, and sperm released. Inverted microscope examination determines positive or negative results and sperm count in positive biopsies. All biopsies were then processed using GM501 collagenase media for enzymatic digestion. Collagenase, derived from Clostridium histolyticum culture filtrates, has 1000 CDU/ml activity and World Health approval (WHO, 2021 [9]).

3. Results

1- **Comparison of Demographic Features between Patients with Positive and Negative Sperm Recovery** in Enzymatic and Mechanical Methods

In the enzymatic method, there were no significant differences between patients with positive and negative sperm recovery regarding the mean ages (35.29 ± 5.84 vs. 31.33 ± 5.51 & p=0.059) and body mass indices (27.89 ± 3.39 vs.28.70 ± 3.20; p=0.504).

In addition, there was also no significant difference in mean patients' ages (34.67 ± 6.11 vs. 32.10 ± 5.72; p=0.240) and body mass indices (28.17 ± 3.59 vs. 28.42 ± 3.11; p=0.837) in mechanical methods between patients with positive and negative sperm recovery, as illustrated in (Table 1).

2- The relation of previous testicular biopsies with the efficacy of both methods in positive sperm recovery. In both Mechanical mincing and combining mechanical with enzymatic methods, there was no significant higher sperm recovery in patients with a history of previous testicular biopsies (66.7% vs. 38.5%; p=0.209) and (66.7% vs. 30.8%; p=0.102) respectively, as demonstrated in (Table 2).

(Table 1): Comparison of Demographic Features between Patients with Positive and Negative Sperm Recovery in Combining both Methods and Mechanical Methods

Combining Methods			
Parameters	Patients with positive sperm recovery No.=14	Patients with negative sperm recovery No.=18	*p*-value
Age (years) (Mean ± SD)	35.29 ± 5.84	31.33 ± 5.51	0.059 Ŧ NS
BMI (kg/m²) (Mean ± SD)	27.89 ± 3.39	28.70 ± 3.20	0.504 Ŧ NS
Mechanical Methods			
Parameters	Patients with positive sperm recovery No.=12	Patients with negative sperm recovery No.=20	*p*-value
Age (years) (Mean ± SD)	34.67 ± 6.11	32.10 ± 5.72	0.240 Ŧ NS
BMI (kg/m²) (Mean ± SD)	28.17 ± 3.59	28.42 ± 3.11	0.837 Ŧ NS

NS: Not significant (p > 0.05); Ŧ: Independent sample t-test

(Table 2): The relation of previous testicular biopsies with the efficacy of both methods in positive sperm recovery

Positive sperm Recovery	**History of previous testicular biopsies No.=6**	**No history of testicular biopsies N.=26**	***p*-value**
Combining method	4 (66.7 %)	10 (38.5 %)	0.209 NS
Mechanical method	4 (66.7 %)	8 (30.8 %)	0.102 NS

NS: Not significant (p > 0.05)

4. Discussion

In the present study, both enzymatic and mechanical methods had no significant higher sperm recovery in patients with a history of previous testicular biopsies (p = 0.209, p = 0.102, respectively). Although no previous studies focused on this comparison, many studies discussed repeated biopsies in non-obstructive azoospermic men. Similar to the present study results, a study by (Dabaja AA. And Schlegel PN., 2013 [10]) mentioned that most men with NOA needed more than one biopsy to retrieve sperm, and further testicular evaluation may be required. Vernaeve and his colleagues (Vernaeve et al., 2006 [11]) also found that repeated conventional TESE has been shown to improve outcomes for patients with Sertoli cell-only syndrome and maturation arrest, according to some retrospective studies.

(Kalsi et al., 2012 [12]) Proposed that testicular biopsy is insufficient to predict TESE success, as evidenced by the fact that spermatozoa can be retrieved in a significant proportion of patients whose testicular biopsy revealed Sertoli cell-only syndrome.

In contrast, the study by (Herwig et al., 2004 [13]) suggested that there is a chance that repeated random biopsies may cause intratesticular scars, lowering androgen levels and preventing future sperm production. However, (Tsujimura et al., 2006 [14]) reported the possibility of retrieving spermatozoa using a salvage micro-TESE if a standard TESE has failed, with a success rate of 45%.

The reason behind the increase in success rates of repeated testicular biopsies may be due to intensive management given to the patient after the failure of sperm retrieval or to avoiding the previous biopsied sites which may not have spermatogenesis.

5- Conclusion

Patients who had undergone prior testicular biopsies had marginally

higher sperm recovery rates using both enzymatic and mechanical techniques. In order to find the rare spermatozoa, present in the testes of men with low or no sperm yields in a previous biopsy, a repeated testicular biopsy is needed.

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Author Contribution

Amina Al-Tameemi performed the study, and Ali Ibrahim Rahim Ula Al-Kawaz supervised the work .

Conflict of Interest

The authors declare no conflict of interest .

Ethical Clearance

The study was approved by the Ethical Approval Committee.

Financial Disclosure

There is no financial disclosure.

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