Middle East Journal of Science

https://dergipark.org.tr/mejs **MEJS**

e-ISSN:2618-6136

Research Article

INVESTIGATION OF GLOBOZOOSPERMIA'S MORPHOLOGICAL STRUCTURE AND DNA FRAGMENTATION IN OLIGOZOOSPERMIA CASES IN INFERTILE MALES

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Abstract: *Total globozoospermia is diagnosed by the presence of 100% round-headed spermatozoa without acrosomes. It is still unclear whether patients whose ejaculate contains both normal and globozoospermic cells (partial globozoospermia) suffer from a variation of the same syndrome. Affected men may experience decreased fertility and even infertility. In some cases, an increased number of cells with DNA fragmentation has also been observed in patients with globozoospermia..In this study, standard semen analysis methods in accordance with WHO criteria were applied to infertile male patient groups consisting of 20 normozoospermic and 20 oligozoospermic individuals who were admitted to our clinic. Age and sperm parameters (volume, vitality, concentration, total motility, and morphology) were determined and statistically analyzed in normozoospermic and oligozoospermic infertile men. Sperms were stained with the Eosin-Nigrosin method and were visualized under an immersion lens light microscope and evaluated for vitality. The slides were stained using sperm staining solutions with the Spermac technique and the sperms were evaluated morphologically. Sperm DNA fragmentation damage was evaluated by the acridine orange staining method. Our results revealed that sperm morphological features (Kruger test) and sperm DNA fragmentation, obtained with various staining techniques, are important in the clinical approach to male infertility and ART methods, and should be used together.*

Keywords: *Infertility, Oligozoospermia, Roundhead, Globozoospermia, DNA fragmentation*

Received: December 23, 2021 *Accepted: May 10, 2022*

1. Introduction

World Health Organization (WHO) defines infertility as the inability to achieve pregnancy despite unprotected and regular sexual intercourse for 12 months or longer [1]. Despite the chance of conceiving in a healthy couple is 85-90% in twelve months and 75% in six months, this rate decreases to 20-25% in one month [2-6]. In women over 35 years of age, the inability to achieve pregnancy despite regular sexual intercourse for 6 months without protection is an indication to start the process of diagnosis and treatment [2, 6, 7]. The promising results of Assisted Reproductive Therapy (ART) and the increase in the number of couples using these methods have caused an increase in treatment applications and paved the way for developments in this field [8]. Evaluation of the female factor is an important criterion in diagnosis and treatment in infertile couples who admit for treatment [9]. It has been observed that the advanced age of the patient causes a decrease in the oocyte reserve and the quality and the number of

embryos, the frequency of aneuploidy, implantation rates, and an increase in the fragmentation rate in the embryo [10, 11].

1.1. DiagnosticTests in Male Infertility

While semen analysis is at the beginning of the application to help the evaluation and diagnosis of men in terms of infertility, it is very important to evaluate non-sperm cells and determine the steps for diagnosis and treatment with functional tests.

Semen Analysis: During semen analysis; fresh semen obtained after at least 48 hours of sexual avoidance is evaluated. The number, motility, and morphological structure of sperm are determined by these analyzes [12].

- **Macroscopic Examination:** The priority in semen analysis is to start inspection with the unaided eye. In order to minimize the effect of environmental conditions on semen quality; analysis should be performed within the first 30-60 minutes after ejaculation [13]. During these examinations, color, liquefaction, viscosity, volüme, and pH are evaluated.
- **Microscopic Examination:** For the examination of liquefied fresh semen preparations, the use of phase-contrast microscopy is recommended. During these examinations, concentration, vitality, motility, and morphology are evaluated [14,15].

Sperm DNA Damage (Sperm DNA Fragmentation = SDF)

Although there is not enough information about the causes and mechanisms of sperm DNA damage today, three fundamental mechanisms are mentioned. These are; anomalies in sperm chromatin packaging, apoptosis, and oxidative stress [16].

Total globozoospermia is diagnosed by the presence of 100% round-headed spermatozoa without acrosomes. In some cases, an increased number of cells with DNA fragmentation has also been observed. This study aims to determine the semen parameters, globozoospermia, and DNA fragmentation rates in normozoospermic and oligozoospermic cases and to investigate their relationship with male infertility.

2. Materials and Methods

Working Groups: Normozoospermic and oligozoospermic semen samples were obtained from patients aged 21-44 years who applied to Diyarbakır Dicle University Hospital Urology policlinic. All patients were informed about patient data confidentiality and data sharing, and a written consent form was obtained from all the patients.

Semen Supply and Analysis: Semen samples were taken from patients who came to the urology outpatient clinic and abstained from sexual abstinence for 2-7 days. Parameters were evaluated such as duration of sexual abstinence, volume, color, viscosity, and liquefaction time.

Vitality Assessment by Eosin-Nigrosin Administration: One drop (1µl) of semen was mixed with two drops (2µl) of Eosin-Y in an experiment tube. After 30 seconds, 3 drops of Nigrosin were added and mixed gently. Thus, dead cells were stained red, and living cells were not.

Preparation of Sperm with Swim-Up Technique: The prepared tube was incubated at 37°C, at an angle of 45°, for 30-60 minutes. The supernatant was aspirated and discarded.

Evaluation of Sperm Concentration and Motility: It was evaluated according to WHO classification: Fast-forward motile sperm (+4), Slow-forward motile sperm (+3), Insitu-motile sperm (+2), Immotile sperm (+1) [14].

Sperm Staining and Morphology Evaluation: The preparations were stained with the spermac method.

Evaluation of Sperm DNA Fragmentation: Sperm samples fixed with Carnoy fixative for one hour were stained with AO dye for 5 minutes in the dark and examined under a fluorescence microscope.

Statistical Analysis

The normality control of the data was tested with the Kolmogorov Smirnov Test. The student's T-test was used for statistical analysis. The level of significance was accepted as a p=0.05 limit.

3. Results

Forty voluntary infertile male individuals were included in this prospective study. Semen analyzes were performed on a total of 40 patients, 20 of them were normozoospermic patients and the rest of them were oligozoospermic study group patients, who met the study criteria. Sperm samples were evaluated morphologically and DNA fragmentation indexes were investigated.

3.1. Vitality in Normozoospermic and Oligozoospermic Cases

Sperms stained with the Eosin-Nigrosin method were evaluated for vitality. Live and dead sperms were visualized under the x100 immersion objective light microscope (Figure 1).

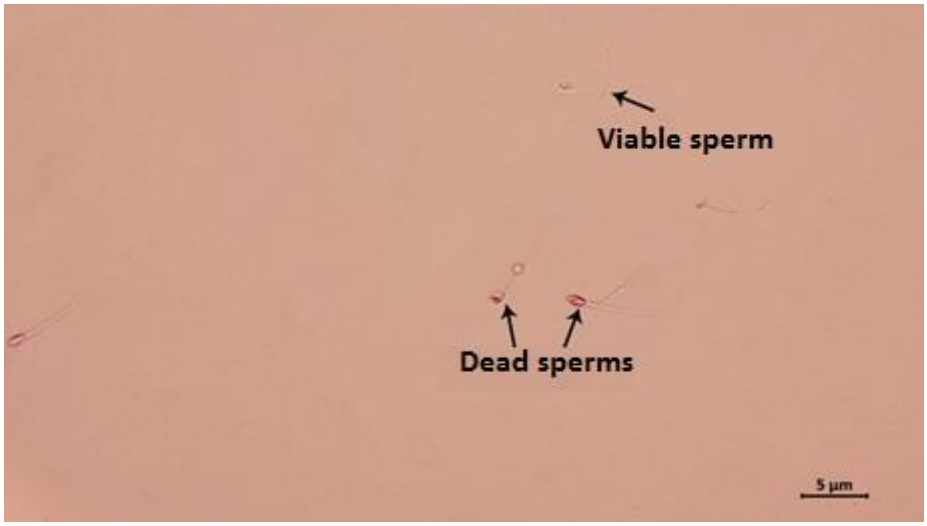

Figure 1. Live and dead sperms

3.2. Morphology in Normozoospermic and Oligozoospermic Cases

Sperms stained with the spermac method were evaluated morphologically. Normal (Figure 2) and abnormal morphological sperms were visualized under an x100 immersion objective light microscope (Figure 3).

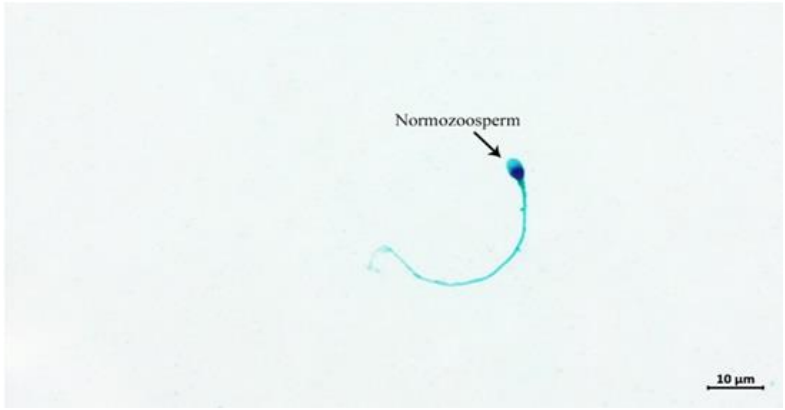

Figure 2. Normal morphological sperm

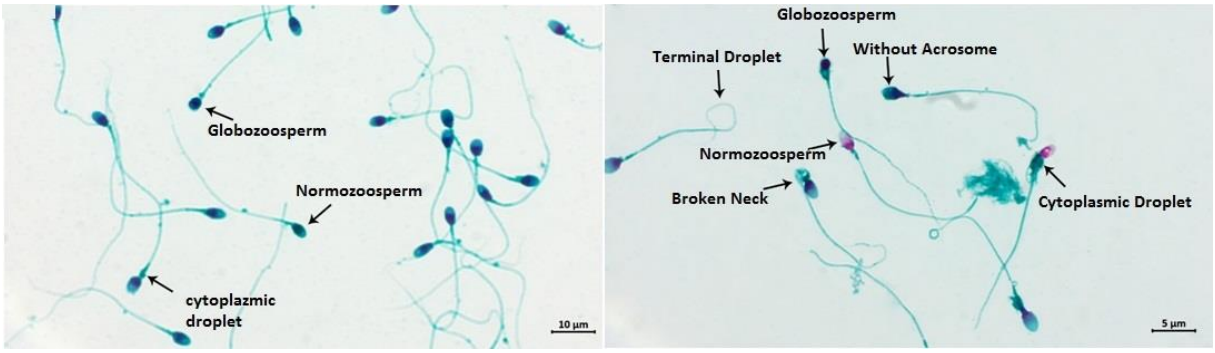

Figure 3. Abnormal morphological sperms

3.3. DNA Fragmentation in Normozoospermic and Oligozoospermic Cases

Sperm stained with acridine orange method were evaluated for DNA fragmentation. By looking at one hundred sperm, the percentages of damaged and healthy sperm were calculated. It was visualized by fluorescence microscopy with an x100 immersion objective (Figure 4).

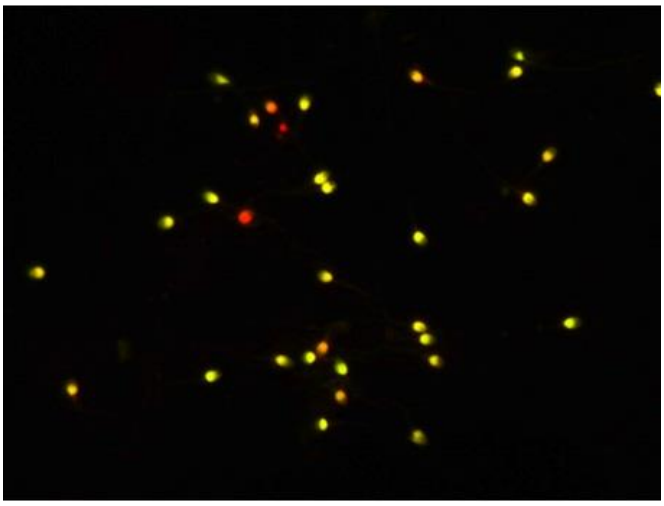

Figure 4. Sperm stained with acridine orange method

3.4. Statistical Findings

The statistical analysis results of the mean values of age, sperm parameters, globozoospermia incidence, and DNA fragmentation in normozoospermic and oligozoospermic infertile men are presented in Table 1.

The mean ages of normozoospermic and oligozoospermic infertile men were found to be 28.80 ± 5.473 years and 29.05 ± 3.348 years, respectively, and there was no statistically significant difference between the groups (p = 0.86) (Table 1).

While the sperm volume was 1,700 ±.9921 ml in normozoospermic infertile men, it was found as 1,700 ±.8176 ml in oligozoospermic men, and no significant difference was found (p =1.00) (Table 1). The mean sperm concentration of normozoospermic and oligozoospermic infertile men was 83,945 ± 45.4547 106/ml and 10,910 ± 3.6092 106/ml, respectively, with a statistically significant difference (p = 0.000) (Table 1).

Total motility was found to be significantly higher in normozoospermic men (62.80 ± 19.691) compared to oligozoospermic individuals (44.35% ± 6.368) (p=0.001). Sperm morphology rates (Kruger) were found to be significantly higher in normozoospermic males (11.40 ± 6.870%) than in

oligozoospermics (5.15 ± 4.246%) (p=0.001). The mean vitality percentages of normozoospermic and oligozoospermic infertile men were found to be 71.80 ±13,387% and 65.30±15.941%, respectively, and no significant difference was detected (p = 0.17).

In addition, the mean rate of globozoospermia in normozoospermic and oligozoospermic male groups was 2.3500 ± 2.27746% and 19.7778 ± 20.09597%, respectively, and it was determined that there was a significant difference between the groups (p = 0.001) (Table 1). Sperm DNA fragmentation rates were 16.55 ± 6.304% and 22.75% ± 10.627% in normozoospermic and oligozoospermic male groups, respectively, and were significantly higher in oligozoospermic males compared to normozoospermic males (p = 0.03). (Table 1).

Table 1. Statistical analysis results of age, sperm parameters, the incidence of globozoospermia and mean values of DNA fragmentation in normozoospermic and oligozoospermic infertile men.

	Groups	n	mean	Standard deviation	Student's T
Age	N	20	28,80	5,473	t=-0,174 p=0,86, NS
	O	20	29,05	3,348	
Volume (ml)	N	20	1,700	,9921	t=00 p=1,00, NS
	O	20	1,700	,8176	
sperm concentration (Million/ ml)	N	20	83,945	45,4547	t=7,163 p=0,000, S
	O	20	10,910	3,6092	
Total motility (%)	N	20	62,80	19,691	t=3,987 p=0,001, S
	O	20	44,35	6,368	
Morphology (Kruger) (%)	N	20	11,40	6,870	t=3,461 p=0,002, S
	O	20	5,15	4,246	
Vitality (%)	N	20	71,80	13,387	t=1,317 p=0,17, NS
	O	20	65,30	15,941	
Globozoospermia rate (%)	N	20	2,3500	2,27746	t=-3,854 P=0,001, S
	O	20	19,7778	20,09597	
DNA fragmentation index (%)	N	20	16,55	6,304	t=-2,244 p=0,03, S
	O	20	22,75	10,627	

t: Student T-test; p: Mann Whitney p değeri; NS: nonsignificant, S: significant; n: number of subjects; N: normozoospermic group; O: oligozoospermic group.

4. Discussion

Globozoospermia is a rare but serious condition that can cause male infertility and is characterized by sperm morphology disorder. Globozoospermia was first defined as 'Rundkopfspermatozoen', which means round-headed spermatozoa in German, in the light of light microscopic analyzes performed by Myhöfer in 1965 [17]. In the case reports published in the following years, the morphological and etiological aspects of globozoospermia were emphasized [18]. Despite all this time, the genes responsible for globozospermia or the patterns of heredity remain unclear [19].

It has been reported that there are many differences between normozoospermic and oligozoospermic infertile men in terms of various semen parameters such as volume, motility, and vitality [20]. In these studies, it was reported that semen volume was different between the groups, but this difference was not statistically significant. In our study, we found that sperm volume differed between the groups, but this difference was not statistically significant.

Schirren et al. reported the first case of globozoospermia after examination of 2200 patients undergoing routine andrological screening, indicating an incidence of less than 0.05%. Later, the same study group reported the incidence of globozoospermia in andrological patients by correcting it to 0.1% [21]. In subsequent studies, the incidence of this irregularity among infertile men has been suggested to be between <0.05% and 0.1% in different studies [22, 23]. However, Holstein et al. reported patients with only 20-60% round-headed spermatozoa in their ejaculate [24]. In our study, 100% of globozoospermia cases were not found in both normozoospermic and oligozoospermic infertile cases. Globozoospermia was observed at a rate ranging from 1% to 7% in 7 cases out of 20 normozoospermic cases and between 4% and 96% in all 20 oligozoospermic cases. The results of our study are in agreement with the information reported by Siddique et al in the literature that "morphological anomalies are generally more common in oligozoospermia when compared to normozoospermia" [25]. Male infertility can be caused by low sperm count, low sperm quality, or both [25]. Similar to the studies in the literature, or results revealed that sperm motility and concentration decrease and the rate of globozoospermia increases in infertile men [26].

In our study; sperm samples of the patients were examined based on Kruger criteria and evaluated morphologically. However, it is known that this conventional semen analysis does not reflect the fertility status of a man alone [22, 27]. Sperm with normal morphology and motility may have approximately 8% abnormal chromatin/ DNA [22]. It has been reported that infertile men with normal sperm parameters may also have high levels of DNA damage [28]. In addition to these conventional routine analyzes, information on the fragmentation rates of DNA is obtained by using the AO method in semen samples obtained from oligozoospermic and normozoospermic infertile groups. In our study, DNA fragmentation was investigated using the AO method in normozoospermic and oligozoospermic infertile male groups, and DNA fragmentation was significantly higher in the oligozoospermic group, in line with the literature.

Fragmentation rates of DNA are important for appropriate sperm selection in ART, because the use of sperm with abnormal DNA during the IVF program leads to a significant decrease in the success rate [22, 27]. As seen in our study results, globozoospermia is probably associated with DNA damage, like other sperm morphological abnormalities.

5. Conclusion

Based on the results we obtained, in addition to conventional approaches, sperm should be stained with various staining methods such as spermac staining to examine their morphological features and analyze sperm DNA fragmentation. As a result of these analyzes, the relationship between the incidence of globozoospermia and DNA fragmentation can be demonstrated. But it is necessary to study in larger series in order to clearly demonstrate this relationship.

Ethical statement

This work was approved by Dicle University Research Ethics Committee. Approval number and date: 2020/74; 06.02.2020

Financial support

This study was financially supported by Dicle University Scientific Research Projects Commission Headquarters (DUBAP, Project No: TIP.20.028).

Conflict of interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Z.Ç: Writing - Original draft preparation
M.A: Methodology
E.Y: Formal analysis, Writing
D.A: Conceptualization, Methodology
F.A: Investigation
O.D: Resources
M.AF: Investigation
All authors read and approved the final manuscript.

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