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The Sperm Vitality Test with Eosin-Nigrosin can be used as an Indicator of Globospermia Type III

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ABSTRACT

Introduction: *Teratozoospermia is characterized by the presence of spermatozoa with abnormal morphology in sperm. Globozoospermia is a rare (incidence 0.1%) and severe form of teratozoospermia characterized by the presence in the ejaculate of a large majority of round spermatozoa without acrosome. Globozoospermia is most commonly caused by mutations in the DPY19L2 gene. Globozoospermic spermatozoa are thus unable to adhere and penetrate the zona pellucida, causing primary infertility.* ***Results:*** *Total teratozoospermia 100% (globozoospermia 89% and other forms of abnormal morphology 11%), necrozoospermia 69%, asthenozoospermia 99%.* ***Conclusion:*** *The case presented is the first to be published as a pathology of infertility in the Republic of Kosovo. Teratozoospermia in the form of globozoospermia was 89%, and we called it globozoospermia severe or Globospermia type III, based on the results of 100% atypical morphology (89% globozoospermia +11% other atypical forms) and necrozoospermia 69% as well as asthenozoospermia 99%.*

Keywords: Spermatozoa, Teratozoospermia, Globozoospermia, Necrosospermia, Asthenozoospermia

INTRODUCTION

Infertility is defined as the inability of couples to have a baby after one year of regular unprotected intercourse, affecting 10-15 percent of couples [1-3]. Large-scale studies have shown that about half of all cases of infertility occur due to female factors, 20 to 30 percent male factors, and 20 to 30 percent due to common causes of both gender [4-6]. Recent meta-analysis studies by researchers show that male factors are present in 20-70 percent of infertility cases [7]. Among the factors leading to male infertility, alterations of spermatogenesis are the major cause including quantitative defects (azoo-or oligozoospermia) or qualitative defects (teratozoospermia or asthenozoospermia) [8,9]. Teratozoospermia is characterized by the presence of spermatozoa with abnormal morphology in sperm. The literature is limited to three forms of teratozoospermia. The first group consists of "polymorphic teratozoospermia", where a majority of spermatozoa display more than one type of abnormality. The second group, named "globozoospermia", is characterized by round spermatic heads, absence of acrosome, and disorganization of mid-piece and tail. The third group consists of "enlarged head teratozoospermia", where almost all spermatozoa have an enlarged head, multiple tails, and abnormal acrosome [10]. Globozoospermia is a rare (incidence 0.1%) and severe form of teratozoospermia characterized by the presence in the ejaculate of a large majority of round spermatozoa without acrosome [11]. Analysis of the incidence of cases with globozoospermia demonstrated history of consanguinity and a familial occurrence. Familial cases of globozoospermia suggest that this pathology has genetic origins, but the mode of inheritance remains unexplained, probably showing X-linked, sex-restricted dominant, or autosomal recessive modes of inheritance [12]. Globozoospermia is most commonly caused by mutations in the *DPY19L2* gene [13]. Mutations in other genes likely also cause globozoospermia [13-15]. Globozoospermic sperm are thus unable to adhere and penetrate the zona

pellucida, causing primary infertility. It is important to differentiate total globozoospermia referring to patients with a homogeneous phenotype with ~100% round-headed sperm and partial globozoospermia with a variable percentage of spermatozoa of typical shape. A minimum threshold of 20%-50% of round-headed spermatozoa is commonly used in the literature to confirm the diagnosis of globozoospermia [11,16,17].

CASE PRESENTATION

History of Illness

22/06/2017, a male patient born in 1984 was reported to the Bioloab-Zafi-F Medical Laboratory from the city of Peja, to perform a spermiogram analysis, referred by a specialist urologist with an indication of primary infertility after 6.5 years of infertility. A diagnosis was made from a urologist's report based on previous analyzes conducted in various clinics and laboratories in Kosovo; Asthenospermia and normal morphology.

RESULTS

The urologist did not find anything significant after the physical and genital examination of the patient. Testicular ultrasound parameters were normal (right testicular volume 32 ml, left testicular volume 25 ml).

Hormone biochemical analyses were normal values (Table 1).

Table 1 Hormone biochemical analysis

	Results	Normal value
FSH (mIU/mL)	3.62	0.60-12.00
LH (mIU/mL)	3.22	0.60-12.00
Testosterone (ng/mL)	6.77	2.40-8.70

From the gynecologist's medical reports owned by the patient's wife, it was seen that his wife had done ultrasonography several times, and in the last 6 months, hysterosalpingography and hormone analysis (FSH.LH, EII, P4, PROL, TSH) were normal. The patient delivered ejaculate on the third day of abstinence, and the ejaculate analysis was performed 2x based on the criteria for analysis of ejaculate by the World Health Organization [18].

The main characteristics of sperm were as follows; volume=4.8 ml, pH 7.41, concentration=26.4 × 10^6 l, and total mobility=1% (grade c, mobility in place). However, sperm morphology revealed a diagnosis of 89% partial globozoospermia after staining by the May Grunewald-Giemsa method (MGG). An Eosin-Necrosis test, the viability in normal sperm samples, was also performed, and the result was 69% [18-20]. After detailed consultations with the couple, the man was found to be infertile for 6.5 years, and the ejaculate-spermiogram analysis was repeated two more times to confirm that it was a rare diagnosis of globosperm and first detected as pathology in an infertile man in the Republic of Kosovo (Figures 1-6).

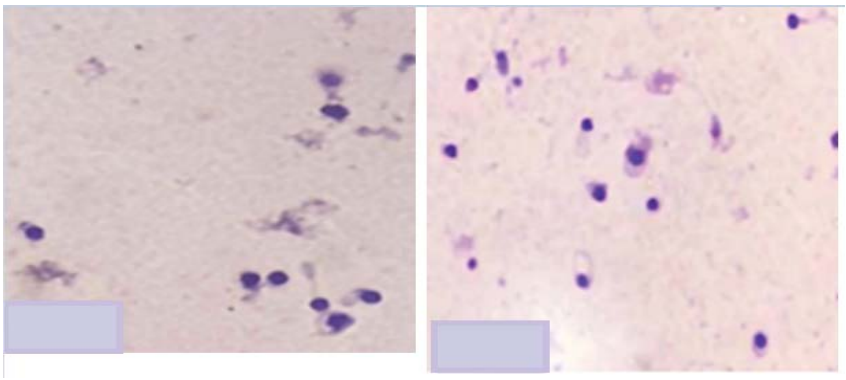

Figure 1 Optical microscopic observations of sperm from a patient with partial globozoospermia; total lack of acrosome at the top of the sperm head

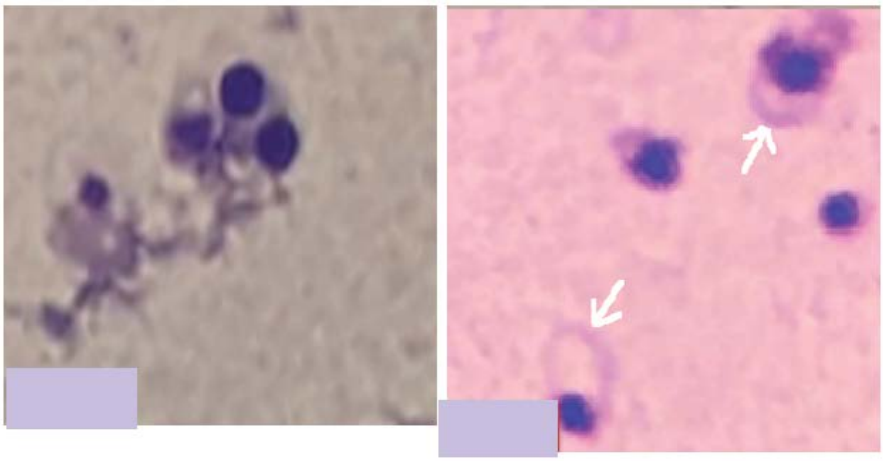

Figure 2 Zoom on a spermatozoon's head of typical s-round shape, the shape of the head follows the shape of the nucleus; staining by the May Grunwald-Giemsa method (MGG)

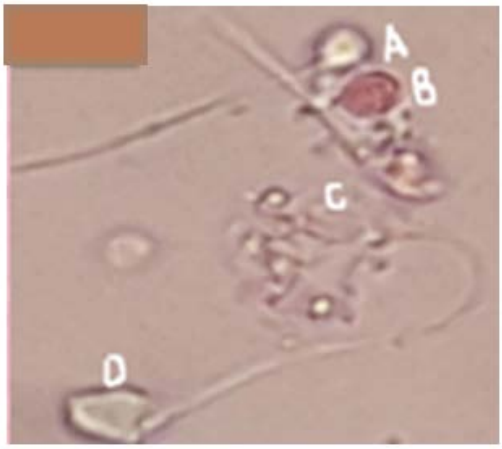

Figure 3 Sperm A live globospermic sperm; C, D, living spermatozoa of atypical morphology, but not globospermic, B dead globospermic spermatozoa

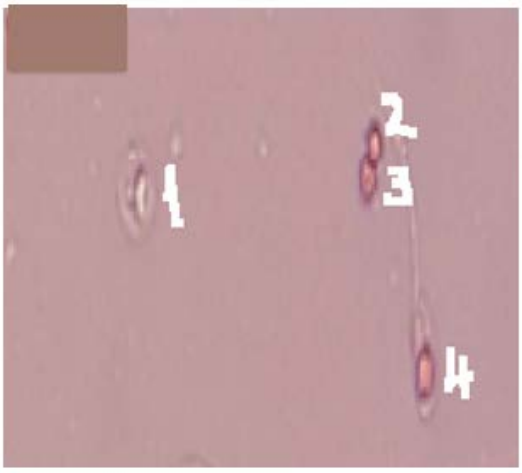

Figure 4 Sperm1 lives globospermically with a characteristic tail surrounding the head; sperm 2, 3, 4 globospermic dead sperm

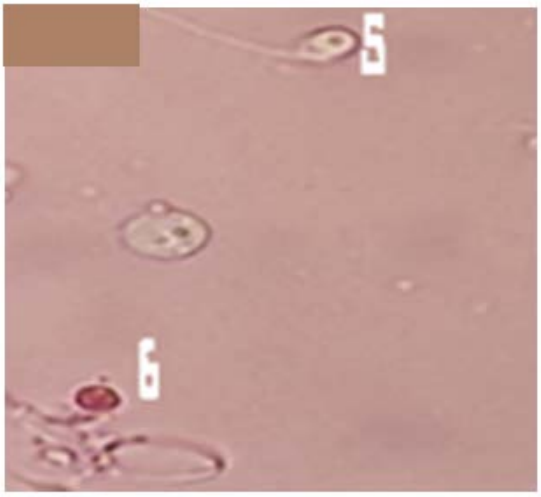

Figure 5 Sperm 5 live globospermic sperm and 6 dead globospermic sperm

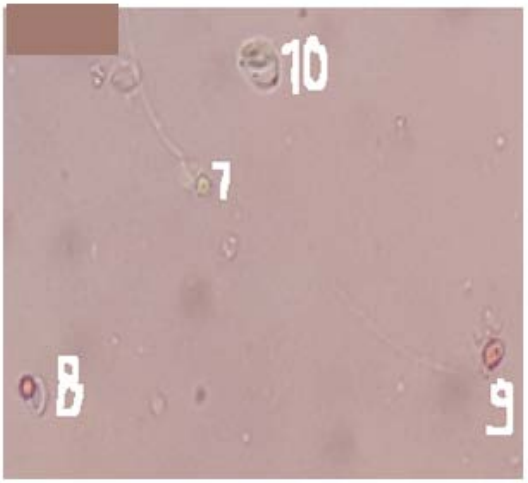

Figure 6 Globospermic live spermatozoa 7 and 10, and spermatozoa number 8 and 9 dead globospermic spermatozoa

The results are:

-total teratozoospermia 100% (globozoospermia 89% and other forms of abnormal morphology 11%)

-necrozoospermia 69%

-asthenozoospermia 99%

Figures 3-6 show the eosin-nigrosin staining technique for human sperm vitality assessment. Optical microscopic observations of sperm from a patient with partial globozoospermia

In our patient, the Eosin-Nigrosine vitality test was 31% live (unpainted heads of sperm) and 69% dead spermatozoa (red-head spermatozoa).

Normal range >58% vitality in normal sperm samples [18].

The presence of 89% globozoospermia is not teratozoospermia of the same percentage (globozoospermia 89% *vs.* necrozoospermia 69%). It is important to note that in the group of live sperm (31%) after the Eosin-Nigrosine test, 20% were globospermic and 11% live sperm with an atypical morphology of another non-globospermia (Figures 3-5).

DISCUSSION

Globozoospermia is a rare incidence <0.1%, a genetic defect that causes male infertility [16]. It is characterized by multiple alterations such as spermatozoa with round heads, absence of acrosomes, and anomalies in chromatin condensation [11,21]. In this case presentation, we report our findings to spermatozoa with total teratozoospermia dominated by globozoospermic sperm as the first case detected in an infertile man in the Republic of Kosovo with total teratozoospermia and where globospermia dominates. In our case, the patient was with primary infertility and 89% are dominated by globozoospermic sperm which are characterized by the presence of round-headed sperm and total lack of acrosome (Figure 1 and Figure 2). The classification of globozoospermia based on morphological characteristics and the percentage of round-headed spermatozoa is not consensual. Singh classified globozoospermia into type I and type II [22]. Accordingly, in type I globozoospermia, also known as total or classic globozoospermia, or true round-head only syndrome, 100% of the spermatozoa have a small, round, and acrosome-free head. Since round-headed spermatozoa are unable to penetrate the zona pellucida because of having no acrosome, this kind of disorder causes primary male infertility [22]. Contrary, men with type II globozoospermia have both normal and round-headed sperm cells with large which impair motility. In this type of globozoospermia, 20%-90% of spermatozoa have no acrosome; therefore, it is also known as partial globozoospermia [8,9,15,16].

We agree with the above-mentioned authors about this division of globozoospermia into two types but based on the case findings we present we have the dilemma that there are arguments that there must be type III globozoospermia, or severe globozoospermia as is the case of our patient. Our hypothesis that we can divide globosperm into three types is based on the percentage mentioned by the authors that type II globosperm counts from 20%-90% sperm with globosperm [8,9,16]. We thought it could not be concluded that it is globospermia type II result with 20% globospermic sperm. For example, the case presented by our group where globospermic sperm are 89% (Figure 1 and Figure 2) cannot be called globosperm type II. We did not find any positive encouraging results in achieving pregnancy in vivo when we had 20% or 50% of globospermic sperm. Therefore, we could not even think that there could be *in vivo* fertilization in cases with >50% or in our case 89% with atypical sperm, i.e. globozoospermia.

Dam concluded that partial globozoospermia is characterized by oval sperm cells with pronounced malformations on the sperm head matrix compared with normozoospermia, but normal sperm cells are also found [9]. We agree that type II partial globospermia has these characteristics; dominance of a higher percentage of globular spermatozoa and the presence of some spermatozoa of normal morphology. In our case, we have total teratozoospermia (100%), globospermic sperm 89%, and 11% non-glospermic spermatozoa, but with atypical morphology, as opposed to 69% necrosospermia. Necrosospermia was confirmed by the Eosin-Nigrosin test (Figures 3-6). The results show 31% of unstained head sperm i.e. live sperm. Within this percentage, 20% are globospermic live spermatozoa and 11% live morphologically atypical spermatozoa of other morphology than normal and not globospermic forms.

Based on our results we have hypothesized that we can talk about severe globozoospermia in the case of our patient and not with partial globozoospermia because we have more than 80% of sperm (89%) with globozoospermia associated with total teratozoospermia (100%) and the Eosin-Nigrosine vitality test has provided us with additional and reliable (easily verifiable) knowledge that we are dealing with another form of globozoospermia which we call "severe globozoospermia" when we have a high percentage of globozoospermia followed by 100% teratozoospermia. In cases with total globozoospermia as well as cases with severe globozoospermia as our case when we also have 99% asthenozoospermia, the sperm vitality test Eosin-Nigrosine gives us enough data that we can have live spermatozoa to use for the ICSI procedure after we have worked the HOS test.

The question is being raised, about in vitro conditions (IVF/ICSI). Now our hypothesis is "proud". We agree with many authors that the only solution for cases with globozoospermia whether type I (total) or type II (partial) is the medically assisted fertilization procedure, the ICSI method.

For the ICSI method, the sperm with the best morphology is selected, the greatest mobility if there is movement and when it does not move (mobility 1%, movement in place) or 100% immobile and globospermic in percentage 89% as our case as we know that a globospermic sperm is also immobile, so which sperm will we use for ICSI. We have worked on the Eosin-Nigrosine test (Figures 3-6) to conclude that; our results have shown that globospermic and immobile sperm can be dead (heads are stained red) and alive (sperm heads are not stained). Such a finding could greatly contribute to IVF procedures to select live sperm to fertilize the oocyte with ICSI procedures. It is known

that sperm cannot be used for fertilization with the ICSI method after the Eosin-Nigrosine test but it helps us a lot to trust the next Hypo-Osmotic Swelling (HOS) test-the test results from all the numerous articles have shown that the results of the HOS test and Eosin-Nigrosine are in fairly large consistency. It is known that sperm is immobile but with a positive HOS-test and can be used for oocyte fertilization with the ICSI method. We did not use Hypo-Osmotic Swelling (HOS) test or DNA fragmentation of sperm (for objective reasons), but we believe and expect that the author of other studies will confirm our hypothesis and come to a valid conclusion in cases with severe globozoospermia (80%-99%) type III must be checked Eosin-Nigrosine by a test in which percentage are globozoospermic sperm are necrozoospermic, to then do a HOS test on the day we need sperm for the ICSI procedure.

CONCLUSION

The case presented is the first to be published as a pathology of infertility in the Republic of Kosovo. Teratozosperm in the form of Globospermis was 89%, and we called it Globospermia severe or Globospermia type III, based on the results of 100% atypical morphology (89% globospermia+11% other atypical forms) and Necrosospermis 69% as well as asthenozoospermia 99%.

DECLARATIONS

Conflicts of Interest

The authors declared no potential conflicts of interest concerning the research, authorship, and/or publication of this article.

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