

Ultrastructural analysis of a globozoospermia case

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Özet

Globozoospermia olgusunun ultrastrüktürel analizi

Globozoospermi, infertiliteye sebep olabilen ve nadir görülen bir sperm bozukluğudur. Globozoospermiye ikincil uzun süreli infertilite sorunu olan bir çift değerlendirildi. Globozoosperminin ICSI yöntemiyle başarılı şekilde tedavisi sonrası Transmisyon Elektron Mikroskopik (TEM) inceleme yapıldı. İncelenen çiftin ilk ICSI denemesinde %50 fertilizasyon oranı elde edildi. İlk denemede, başarılı embriyo transferi sonrasında bir gebelik oluştu ancak düşükle sonlandı. İkinci denemede, ICSI işlemi uygulanan 7 oositin 3'ü fertilize oldu ve normal yarıklanma elde edildi. Spontan oosit aktivasyonu sonrası elde edilen bu embriyoların transferi sonrasında dişi fötüse sahip tekli gebelik oluştu ve gestasyonun 36.haftasında doğum gerçekleşti. Spermatozoanın geriye dönük incelenmesinde orta parça ve kuyruk defekti olmaksızın globozoosperminin morfolojik bulguları saptandı. Mevcut ICSI işlemleri ile globozoospermiye ikincil gelişen infertilite sorununun çözülebileceği sonucuna varıldı ve bu tip vakalarda globozoosperminin ultrastrüktürel düzeyde analizinin, sonuçların önceden tahmininde faydalı bir rehber olabileceği düşünüldü.

Anahtar kelimeler: Globozoospermi, ICSI, ultrastrüktürel analiz

Abstract

Globozoospermia is an uncommon sperm disorder which may lead to infertility. A couple with long term infertility secondary to globozoospermia was evaluated. The successful outcome of ICSI treatment associated with globozoospermia was evaluated with Transmission Electron Microscopy (TEM). The couple experienced 50% fertilization rate after ICSI at their first attempt. On the first cycle, successful embryo transfer and a pregnancy was achieved but resulted with abortion. On the second cycle, 3 of the 7 injected oocytes were fertilized after ICSI and normal cleavage was achieved. Transfer of these embryos obtained by spontaneous oocyte activation with globozoospermia resulted a singleton pregnancy with a female fetus and delivery occurred after 36 weeks of gestation. Retrospective analysis of spermatozoa reflected the morphologic abnormalities of globozoospermia without mid-piece and tail defects. It is concluded that current ICSI procedures may overcome the infertility secondary to globozoospermia and ultrastructural analysis of globozoospermia seems to be a useful guide in order to predict the outcomes in such cases.

Key words: Globozoospermia, ICSI, ultrastructural analysis

Introduction

Round-headed, acrosomeless human spermatozoa are termed as globozoospermia which is first described in 1976 [1]. The absence of the acrosome renders globozoospermic spermatozoa unable to bind to the zona pellucida or fuse with the oocyte oolemma [2-4]. Therefore, globozoospermic men were considered infertile until the advent of intracytoplasmic sperm injection (ICSI) in 1992 [5].

Assisted reproductive technology has undergone significant advances and ICSI has become the first choice assisted reproductive technique for globozoospermic patients for which no other treatment is available.

There have been several case reports involving patients with globozoospermia and ICSI [6-8]. Although the first live birth from these cases was reported in 1995 [7], subsequent studies suggested lower fertilization and pregnancy rates than average [6-9] and also there were significant number of patients for whom ICSI completely fails [9-10].

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In this report, we examined the results of two ICSI cycles of a long term infertile couple with globozoospermia as the round headed, acrosomeless spermatozoa injections were able to induce oocyte activation and embryonic development, and analyzed the ultrastructural aspects of spermatozoa whether a meaningful information could be provided to predict ICSI success.

Case Report

A 34-year-old woman and her 35-year-old male partner presented with infertility of 12 years' duration. The female partner had an unilateral right tubal adhesion demonstrated by hysterosalpingography and globozoospermia had been detected in the male partner with 100% of spermatozoa exhibiting round-headed morphology.

Ovarian stimulation was accomplished by exogenous gonadotropin administration following a desensitization protocol with long-acting GnRH analogues. Only mature oocytes in second meiotic metaphase were selected for ICSI.

On the day of oocyte retrieval, the semen was collected and placed in a 37°C incubator for 20-30 minutes to liquefy. After liquefaction, conventional semen parameters, including volume (ml), concentration ($\times 10^6/\text{ml}$), motility (percentage), and forward progression (1-4) were evaluated according to WHO (1992) criteria [11]. Morphology (percent normal) was assessed using Tyberg strict criteria. Liquefied semen was washed by centrifugation two times in Earle's Balanced Salt Solution (EBSS) (Sigma-Aldrich Chemie GmbH, Sweden). The seminal plasma removed and the final suspension was maintained in a 5% CO₂ incubator until injection. The remaining semen was pipetted into vials and analyzed by transmission electron microscopy (TEM). Assessment of fertilization was performed 18 hours after injection and the presence of two pronuclei was recorded as a sign of fertilization. The embryos that cleaved without fragments or with minimal fragmentation scored as Grade A were transferred and a positive quantitative β -human chorionic gonadotropin (β -HCG) was included as a positive pregnancy. Ultrasound detection for fetal sac was used to confirm a positive clinical pregnancy.

Transmission Electron Microscopy (TEM)

Semen sample was prepared for TEM by diluting the semen with 2 times the volume of EBSS and centrifuging for 5 minutes at 300 \times g. The pellet was fixed at room temperature in 2% paraformaldehyde

/ 2,5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.25). The pellet was rinsed with the same buffer, postfixed in 1% osmium tetroxide for 2 hours, dehydrated in a graded series of ethanol, and embedded in Araldite. Polymerized resin blocks were sectioned at 120 nm on an LKB IV ultramicrotome with a diamond knife. Sections were placed on 200-mesh grids, stained with lead citrate and uranyl acetate, then examined and photographed at 60 Kv on a JEM-100 CXII transmission electron microscope. In initial sperm analysis, globozoospermia had been recognized reliably with SperMAC stain (Ferti Pro Inc. Beernam, Belgium) by light microscopy (Figure 1). On the day of ICSI, semen parameters included sperm counts of $55 \times 10^6/\text{ml}$, 20% motility, and $60 \times 10^6/\text{ml}$, 30% motility respectively for the two consecutive ICSI cycles.

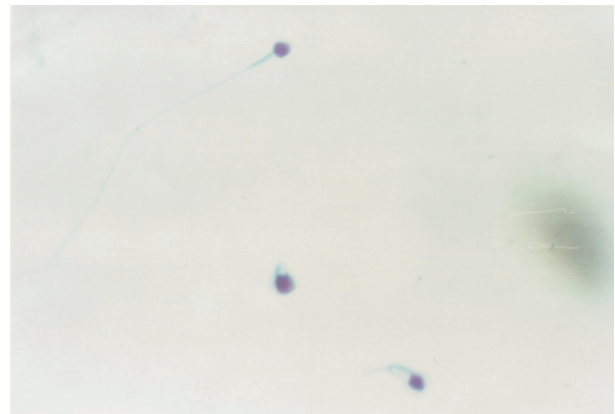


Figure 1. Light micrograph demonstrating apparently the round-headed morphology of the spermatozoa with SperMAC stain. Original magnification= $\times 1000$.

The TEM revealed that nearly 100% of the spermatozoa had more compact, round head shape than normal oval head shape and exhibited large vacuolated regions within the nucleus and spermatozoa lack nuclear envelope, acrosome, and postacrosomal sheath (Figure 2). Therefore spermatozoa had normal, dense midpiece with normal axoneme, outer dense fibres and mitochondrial sheath structure and the basic 9+2 pattern of microtubules were seen as in normal structure which forms the core of the human sperm tail (Figure 3).

In the first cycle, 4 of 8 oocytes (50%) injected fertilized and four embryos (two 2-cell embryos and two 4-cell embryos with no fragmentation) was transferred 48 h later. Pregnancy resulted after transfer but ended with abortion on the sixth week. In the second ICSI cycle, 6 months later, 7 oocytes

were retrieved. Three of the injected oocytes (43%) exhibited signs of normal fertilization. A total of three embryos (one 4-cell, one 2-cell embryos without fragmentation and one 3-cell embryo with minimal fragmentation) were transferred 48 h after microinjection. The last attempt resulted with pregnancy and delivery occurred after 36 weeks of gestation.

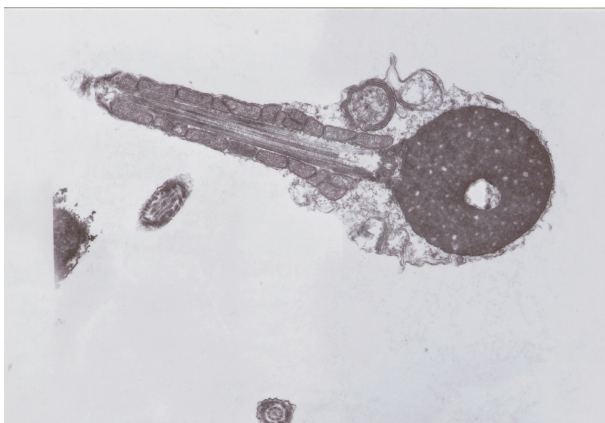


Figure 2. Transmission electron micrograph (TEM) of a spermatozoon demonstrating compact, round head shape and large vacuolated regions within the nucleus. Spermatozoon lack nuclear envelope, acrosome, and postacrosomal sheath, therefore normal mid-piece structure is visible. Original magnification= $\times 10,000$.

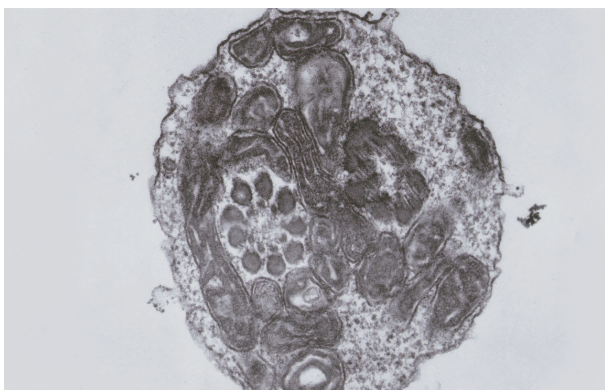


Figure 3. Transmission electron micrograph (TEM) of a round-headed spermatozoon showing the basic 9+2 pattern of microtubules as in normal structure which forms the core of the spermatozoon tail. Original magnification= $\times 35,970$.

Discussion

ICSI may be the most effective and safe technique for the treatment of globozoospermia. Hence this procedure allows us to bypass natural barriers such as cells of the corona radiata, the zona pellucida and the cytoplasmic membrane without spermatozoon and oocyte interaction. The couple with long term infertility resulting from globozoospermia proceeded to ICSI, and ICSI treatment has led to fertilization, embryonic development, and pregnancy in two

consecutive cycles with delivery of one healthy female infant in the second cycle.

Therefore, beyond identifying patient as globozoospermic, conventional semen analysis is ambiguous in predicting the fertility of globozoospermic patients. Although the oocyte activating capacity of the round-headed spermatozoa can not be predicted from the morphology, the spermatozoa used in these cycles demonstrated by the transmission electron microscope (TEM) as it is the principal technique used for diagnostic purposes. TEM examination of thin sections allows an experienced morphologist to identify numerous structural defects of spermatozoon which exhibited characteristic of globozoospermia by acrosomeless round-head shape with large vacuolated regions within the nucleus. In contrast, there were no other ultrastructural defects within the midpiece and tail regions of the spermatozoa that would directly effect fertilization capacity of these round-headed spermatozoa. In a previous study, it has been reported that no abnormalities were noted for hypoosmotic swelling changes and morphologic evaluation of the tail with globozoospermia [12]. We have demonstrated that acrosomeless round headed sperm morphology is not always associated with midpiece, and tail abnormalities.

In order to fertilize an oocyte successfully, ICSI requires only a genetically functional paternal genom, a functional microtubule-organizing center (MTOC), the paternally –inherited sperm centrosome, and an oocyte activating factor. It is clear that the spermatozoa of some patients with globozoospermia will fail with ICSI because of their lack of factors needed for oocyte activation [10,13,14].

It has been demonstrated in an experimental model using mouse oocytes that sperm-associated oocyte-activating factor may be present to a variable degree in these spermatozoa [14]. With this evidence, reported success rates of ICSI in cases of globozoospermia are rather variable ranging from total failure of fertilization to almost normal fertilization rates with the establishment of pregnancy [7,10,15]. Variable fertilization, and pregnancy rates in globozoospermia render the outcomes unpredictable.

Although the relevance of the Mouse oocyte activation test for predicting the outcome of ICSI in globozoospermic men has been demonstrated, there is variation in human oocyte activation rates among different cohort of oocytes [13,14].

There were a few successes including our couple patient which resulted delivery after spontaneous oocyte activation in globozoospermia [8,15]. In our case, we assume that adequate cytogenetic factors were likely to have contributed to the spontaneous oocyte activation followed by successful fertilization, cleavage rates, and embryonic development evidenced by full-term pregnancy resulted in successful delivery of a healthy infant.

In conclusion, further research is needed for understanding the mechanisms which are responsible for the embryonic development and full-term pregnancy in globozoospermia cases. Also, TEM analysis of globozoospermia seems to have predictive value in order to assess the outcomes in such cases since conventional semen parameters, including strict morphologic evaluation, did not provide predictive information for ICSI success. Therefore as additional various cases are explored, appropriate treatment strategies may be developed to handle the problem.

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