CASE REPORT

RJME Romanian Journal of Morphology & Embryology http://www.rjme.ro/

Healthy birth in a case of total globozoospermia after intracytoplasmic sperm injection and assisted oocyte activation

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Abstract

Globozoospermia is a rare (incidence <0.1%) and very severe disorder, with major implications in male fertility. Total globozoospermia is represented by the presence of spermatozoa with 100% rounded heads and a lack of acrosomes. These specific morphological modifications seem to be connected to defects occurring in the last stage of spermatogenesis, spermiogenesis, and will result in anomalies of the acrosomal reaction and a defective adherence of the spermatozoa to the oocytes *zona pellucida*. This will result in a failure of natural fertilization. This article aims to present the case of a couple diagnosed and successfully treated for primary male infertility. The 26-year-old male partner underwent two semen analyses that revealed the presence of fully rounded spermatozoa heads (morphological abnormality) and consequently was proposed for *in vitro* fertilization treatment. Semen preparation and the use of assisted reproductive techniques, intracytoplasmic injection of sperm cells into the assisted oocyte activation, have resulted in the conceivement of a healthy child. The particularities of this case lie in the early recognition of the total abnormal globozoospermia morphology. This is the first case reported in Romania where specific assisted reproductive techniques and treatments have resulted in a successful pregnancy for a couple with male total globozoospermia.

Keywords: male infertility, globozoospermia, assisted oocyte activation.

Introduction

Infertility is defined as a couple's incapacity to obtain a pregnancy after one year of frequent and unprotected sexual intercourse [1]. The male factor is represented by a modification in one or more seminal parameters. Globally, male infertility represents 40–50% of the total cases of infertility and affects approximately 7% of males [2]. On a world scale, over 186 million people are suffering of infertility [3]. One of the rarer types is represented by globozoospermia (incidence <0.1%), a morphological modification of the spermatozoa heads. It is a severe disorder that always leads to male infertility [4] and is characterized by round headed and acrosomeless sperm cells [5]. The spermatozoa have a very low or inexistent level of phospholipase C-zeta (PLCζ), an important physiological agent that is necessary to induce the activation of the oocytes [6]. The primary morphologic defect is characterized by an absent or gravely malformed acrosome. The apparition of this defect takes place during spermiogenesis, and it most probably has its origins in a faulty fusion of the acrosomal vesicles and in flaws of the cytoskeleton, but the exact mechanisms are still unclear [4]. In literature, there have been two types of globozoospermia described: type I and type II, although it is still up for debate if they are variations of the same syndrome or if they are the result of different deficiencies [7].

Type I globozoospermia is also known as total or classical globozoospermia or as the rounded head and acrosomeless syndrome. Because the spermatozoa with a rounded head are not capable to fertilize the oocyte through the penetration of the *zona pellucida* due to the inexistence of the acrosome, this type of disorder leads to primary male infertility [8].

The deoxyribonucleic acid (DNA) structure from the heads of the spermatozoa with globozoospermia is not well known. Some studies have identified an increased rate of aneuploidy in sperm cells, a phenomenon studied with the help of fluorescence *in situ* hybridization (FISH) [9].

Men suffering from type II globozoospermia present in the ejaculate, besides round-headed acrosomeless sperm cells (>25%), normal spermatozoa, therefore making this an uneven pathology [10].

Before the discovery of intracytoplasmic sperm injection (ICSI), patients with total globozoospermia were considered sterile. Yet now, with the use of the ICSI, men with this disorder can overcome the problem of infertility in a couple, although, in general they still experience lower rates of fertilization, pregnancy and birth, compared to other groups of infertile males [11].

This article presents a successful case of pregnancy and healthy birth after utilizing the ICSI, using sperm cells from a man with globozoospermia and assisted oocyte activation.

Aim

The aim of this paper was to present an extremely rare case of a Romanian patient with total globozoospermia and the conceiving and ulterior birth of a healthy child and to include a review of the literature, where very few cases such as this have been reported.

Case presentation

On March 2021, an infertile couple, with one year of previous pregnancy attempts, was admitted for *in vitro* fertilization (IVF) procedure: a 19-year-old female and a 26-year-old male. The woman already had one healthy child from a previous relation. The medical history of both partners did not reveal any major problems. The body mass index was also normal. Laboratory analyses of the female patient showed a hormonal profile in normal ranges: the anti-Müllerian hormone (AMH) was 6.8 ng/mL. The medical and physical history of the male partner, which included a urogenital examination, was normal, with one exception: a varicocele surgery on the left testicle in the year 2020. Hormonal tests [follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin, testosterone, inhibin B] were in normal ranges. Sperm analysis revealed globular headed acrosomeless spermatozoa, indicating a total globozoospermia. Seminal parameters were evaluated according to *World Health Organization* (WHO) 2010 criteria [12]. Both partners had normal serological analyses for hepatitis B, C, syphilis, and human immunodeficiency virus (HIV).

Following the evaluation of the sperm parameters, the couple was proposed for the IVF procedure and ICSI. In addition, two other techniques are proposed: ZyMōt (the selection of spermatozoa processed through this technique will lead to a greater sample integrity and a lower degradation of their DNA), compared to conventional methods of semen preparation [13], and the artificial activation of the oocytes with Calcium Ionophore, which pumps extracellular Ca^{2+} into the oocyte, against the concentration gradient, increasing the Ca^{2+} concentration in the oocyte plasma and finally activating the oocyte [14].

The male patient had collected three samples of fresh sperm and their preparation took place using the ZyMōt (ZyMōt Fertility, USA) technique to select only the mobile spermatozoa with a minimum degree of DNA fragmentation. The prepared samples were suspended in washing solution, Sperm Medium (Cook Medical Ltd., Ireland), and kept at 37°C and 6% carbon dioxide (CO_2), in an incubator (Cook Medical Ltd., Ireland).

Controlled ovarian hyperstimulation using short gonadotropin-releasing hormone (GnRH) antagonist protocol was performed, during the IVF, and several oocytes were obtained (18 oocytes in total) from 26 follicles. The 18 oocytes were then washed in Gamete Buffer (Cook Medical Ltd., Ireland) solution and incubated in fertilization medium (Cook Medical Ltd., Ireland) at 37°C and 6% CO_2 and brought to an equilibrium under humidified conditions.

Just 14 oocytes were mature in metaphase II (MII) and for every mature recovered oocyte, a single sperm was selected and injected into the oocyte's cytoplasm, using an ICSI micro-injection pipette (5 μm inner diameter, Gynemed, Germany). The oocytes were injected with the partner's sperm and immediately after injection were activated for 15 minutes in the Calcium Ionophore (Gynemed, Germany) solution.

Nineteen hours later, the fertilization assessment was made and showed two fertilized oocytes with the presence of the two polar bodies and two pronuclei (PNs), signs of sperm–oocyte interaction. The development of embryos until day 5 was observed in sequential culture media (Cleavage and Blastocyst Medium, Cook Medical Ltd., Ireland), in low oxygen (O_2) environment: a 6% CO_2, 5% O_2 and 89% nitrogen (N_2) Cook MINC incubator (Cook Medical Ltd., Ireland), at 37°C. On day 5, the assessment of the embryos was made according to Gardner's grading system and the criteria of the consensus [15].

As a result of the procedure, two blastocytes were obtained. They were vitrified, thus avoiding the risk of ovarian hyperstimulation for the patient. The vitrification was done using Cryotop (Kitazato, Japan) type open system straws and vitrification solutions (Kitazato, Japan) following the manufacturer's protocol.

Patient preparation for the transfer meant an ultrasound (US) and hormonal analysis during a natural cycle immediately after controlled ovarian stimulation. Warming an embryo was done using thawing media (Kitazato, Japan) following the manufacturer's protocol. The transfer of the embryo in the uterus was performed under US-guided control with a Guardia Access Nano (Cook Medical Ltd., Ireland) catheter.

The analyzed samples of sperm showed a concentration of spermatozoa between 1×10^6 to 10×10^6/mL, with a progressive mobility comprised between 5% and 20% (Table 1). All spermatozoa presented, from a morphological point of view, a rounded head, and a lack of acrosome (Figure 1, A–C). In comparison, it can be observed that a morphologically normal sperm cell displays an oval shaped head, with a well-defined acrosome, intermediary piece, and tail (Figure 2).

Table 1 – ***Sperm parameters of the patient and the normal WHO 2010 parameters***

Variable	First sample	Second sample	Third sample	Normal parameters (*WHO*^{2010})
Volume [mL]	2	1	0.8	1.5
Concentration [10^6/mL]	1	7	1	15
Progressive motility [%]	20	20	5	32
Non-progressive motility [%]	10	10	5	–
Normal sperm morphology [%]	0	0	0	4

WHO: *World Health Organization*.

Eighteen oocytes were extracted, and 14 oocytes were in MII. These 14 were injected with the partner's spermatozoa (Figure 3) and immediately after injection, they were activated in the Calcium Ionophore solution. After 19 hours, the fertilization was evaluated and it was ascertained that from the 14 oocytes, two were fertilized and presented two PNs and two polar bodies (Figure 4, A and B). After 72 hours from the ICSI procedure, the evolution of two "A quality" embryos can be observed, with eight blastomeres (Figure 5, A and B).

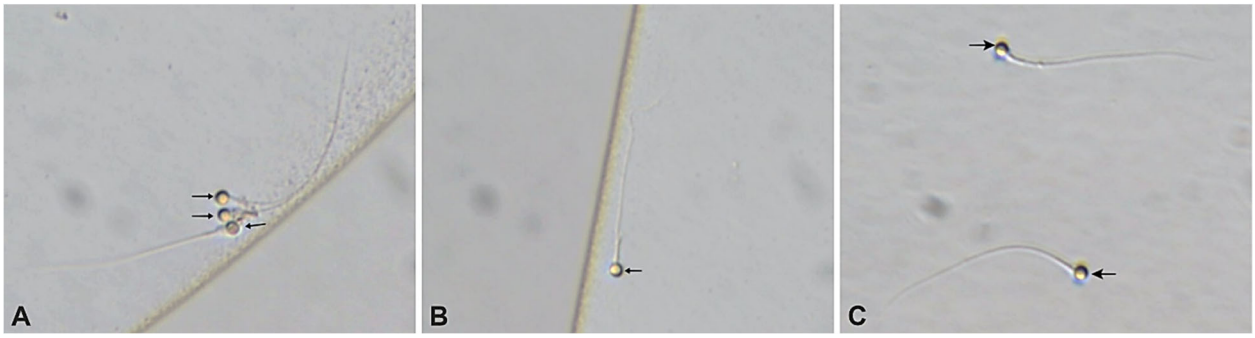


Figure 1 – ***(A–C) Globozoospermia. Microscopic evaluation of the spermatozoa: rounded head and lack of acrosome (black arrow) (100×).***

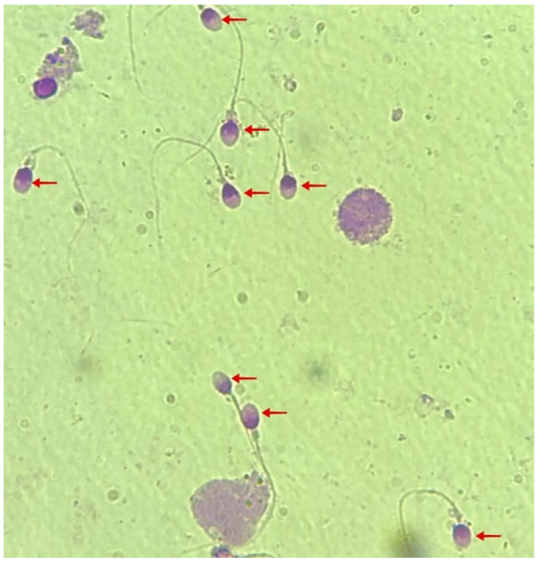


Figure 2 – ***Morphologically normal sperm cells (red arrows). May-Grünwald–Giemsa staining (200×).***

In day 5 of culture, two quality embryos could be observed, marked 2AB (Figure 6, A and B), which were vitrified. According to Gardner's criteria, a blastocyst 2AB is characterized as having a moderate degree of expansion and presents a compact inner cell mass (ICM), with tightly packed numerous cells which, during embryonal development, will form the fetus and the trophectoderm – multiple cells organized in a lax epithelium –, which will form the placenta.

After warming and transferring one of the blastocysts (Figure 7), the result of the beta-human chorionic gonadotropin (β-HCG) at 10 days post-transfer was of 95 mIU/mL, tripling after 48 hours. After the gestational period, the patient delivered, through Caesarean section (C-section), a perfectly healthy baby girl.

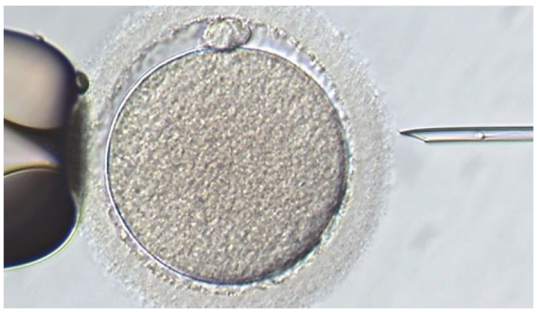


Figure 3 – ***Images of ICSI procedure on mature MII oocyte; extruded polar body at 12 o'clock position (200×); using an ICSI pipette, a single sperm is injected through the oolemma directly into the oocyte's cytoplasm. ICSI: Intracytoplasmic sperm injection; MII: Metaphase II.***

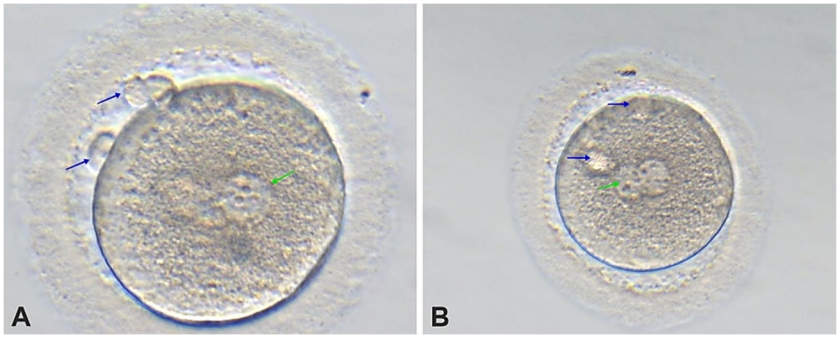
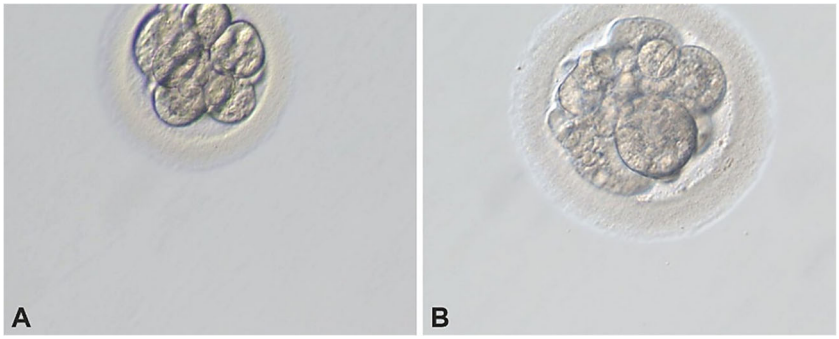


Figure 4 – ***(A and B) The presence of the pronuclei (green arrow) and the two polar bodies (blue arrow) in the fertilized oocytes (19 hours evaluation) (200×).***

Figure 5 – ***(A and B) Embryo aspect at 72 hours after fertilization, presenting eight "A quality" cells (200×).***



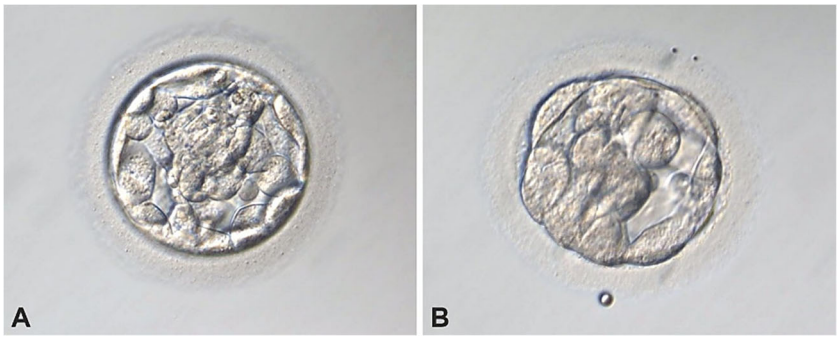


Figure 6 – ***(A and B) The aspect of the day 5 cryopreserved blastocysts (200×).***

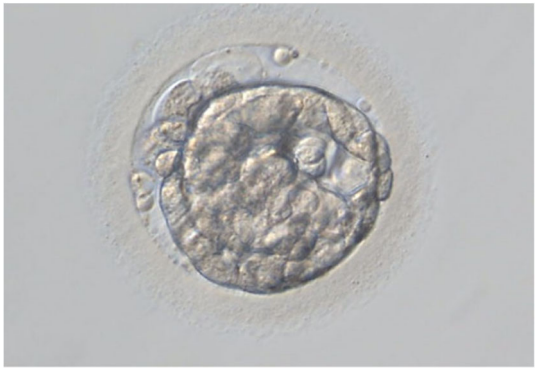


Figure 7 – ***The aspect of the embryo two hours after warming (200×).***

Discussions

Teratozoospermia is a spermatozoa anomaly represented by shape modifications. Polymorphic teratozoospermia comes into discussion when we encounter sperm cells with multiple and diverse morphologic anomalies, while monomorphic teratozoospermia is seen when all spermatozoa present a unique anomaly. Globozoospermia is part of the latter category and is also known as the rounded head sperm cell syndrome [16].

In the case of globozoospermia, the only way in which a couple can conceive their own biological child is by using the ICSI methods [17]. Spontaneous fertilization in such cases has not been previously reported in literature. The absence of spontaneous fertilization is because acrosomeless sperm cells are not capable to bind to the *zona pellucida* and to further fuse with the oocyte's membrane [16]. In IVF, according to Rybouchkin *et al*. (1996) [18], the process was improved when Calcium Ionophore was added immediately after the injection of the oocytes. The study suggested that the association between a normal spermatozoon and an oocyte activates the factor that normally determines the flux of Ca^{2+} needed for fertilization to take place. In this case, the activation of the oocytes is a spatiotemporal process, caused and adjusted by a series of intracellular Ca^{2+} oscillations from the moment that the sperm cell enters the ooplasma. This process is triggered by the PLCζ [19], which has been marked, through immunofluorescence analysis, to be predominantly present in the equatorial segment of the spermatozoon, but also in the acrosome, post-acrosome, tail or sometimes a combination of these segments [20]. Spermatozoa, which contain PLCζ, encourage the release of Ca^{2+} from the intracellular deposits of the oocyte through the hydrolysis of the membrane lipids phosphatidylinositol 4,5-bisphosphate (PIP2) and, in consequence, through the stimulation of the inositol 1,4,5-trisphosphate (InsP3) receptor's Ca^{2+} signaling pathway. This leads to the activation of the oocyte and early embryogenesis [21]. For patients with globozoospermia, anomalies of the spermatozoa and the lack of PLCζ will not allow for fertilization to take place. Insufficient calcium can lead to a failure in fertilization and splitting anomalies. Therefore, through the artificial activation of the oocyte after injection (ICSI), failures in fertilization can be avoided. According to studies by Tejera *et al*. (2006) [22] and Kamiyama *et al*. (2012) [23], previous instances of successful deliveries of live babies have been reported, because of oocyte treatment with Calcium Ionophore after ICSI injection with rounded head sperm cells. On the other hand, previous attempts of injecting the same type of spermatozoa without prior activation of the oocytes has led to failures in fertilization [24].

Conclusions

After evaluating the available literature on the subject, the case reported in this paper represents the first instance, in Romania, of a healthy baby resulted from assisted reproduction techniques using spermatozoa from a patient with total globozoospermia and aided by the Calcium Ionophore oocyte activation method.

Conflict of interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be considered as a potential conflict of interests.

Ethics Statement

We obtained the approval of the Ethics Committee of Gynera Fertility Clinic, Bucharest, Romania, for the publication of this manuscript. The authors obtained agreement for publication of any accompanying images.

Consent

Written informed consent was obtained from patient for the publication of any potentially identifiable images or data included in this article.

References

[1] Babakhanzadeh E, Nazari M, Ghasemifar S, Khodadadian A. Some of the factors involved in male infertility: a prospective review. Int J Gen Med, 2020, 13:29–41. https://doi.org/10.2147/IJGM.S241099 PMID: 32104049 PMCID: PMC7008178

[2] Hanson BM, Kaser DJ, Franasiak JM. Male infertility and the future of *in vitro* fertilization. Urol Clin North Am, 2020, 47(2): 257–270. https://doi.org/10.1016/j.ucl.2019.12.012 PMID: 32272997

[3] Akalewold M, Yohannes GW, Abdo ZA, Hailu Y, Negesse A. Magnitude of infertility and associated factors among women attending selected public hospitals in Addis Ababa, Ethiopia: a cross-sectional study. BMC Womens Health, 2022, 22(1):11. https://doi.org/10.1186/s12905-022-01601-8 PMID: 35012515 PMCID: PMC8751301

[4] Dam AHDM, Feenstra I, Westphal JR, Ramos L, van Golde RJT, Kremer JAM. Globozoospermia revisited. Hum Reprod Update, 2007, 13(1):63–75. https://doi.org/10.1093/humupd/dml047 PMID: 17008355

[5] Canepa P, Casciano I, De Leo C, Massarotti C, Anserini P, Remorgida V, Scaruffi P. A successful healthy childbirth and an ongoing evolutive pregnancy in a case of partial globozoospermia by hyaluronic acid sperm selection. Andrologia, 2019, 51(2): e13178. https://doi.org/10.1111/and.13178 PMID: 30324675

[6] Niu X, Ruan Q, Witz CA, Wang W. Comparison of human oocyte activation between round-headed sperm injection followed by calcium ionophore treatment and normal sperm injection in a patient with globozoospermia. Front Endocrinol (Lausanne), 2020, 11:183. https://doi.org/10.3389/fendo.2020.00183 PMID: 32318024 PMCID: PMC7154056

[7] Sermondade N, Hafhouf E, Dupont C, Bechoua S, Palacios C, Eustache F, Poncelet C, Benzacken B, Lévy R, Sifer C. Successful childbirth after intracytoplasmic morphologically selected sperm injection without assisted oocyte activation in a patient with globozoospermia. Hum Reprod, 2011, 26(11): 2944–2949. https://doi.org/10.1093/humrep/der258 PMID: 21857011

[8] Fesahat F, Henkel R, Agarwal A. Globozoospermia syndrome: an update. Andrologia, 2020, 52(2):e13459. https://doi.org/10.1111/and.13459 PMID: 31724759

[9] Vicari E, Perdichizzi A, De Palma A, Burrello N, D'Agata R, Calogero AE. Globozoospermia is associated with chromatin structure abnormalities: case report. Hum Reprod, 2002, 17(8): 2128–2133. https://doi.org/10.1093/humrep/17.8.2128 PMID: 12151448

[10] Dam AH, Ramos L, Dijkman HB, Woestenenk R, Robben H, van den Hoven L, Kremer JA. Morphology of partial globozoospermia. J Androl, 2011, 32(2):199–206. https://doi.org/10.2164/jandrol.109.009530 PMID: 20864651

[11] Chansel-Debordeaux L, Dandieu S, Bechoua S, Jimenez C. Reproductive outcome in globozoospermic men: update and prospects. Andrology, 2015, 3(6):1022–1034. http://doi.org/10.1111/andr.12081 PMID: 26445006

[12] Murray KS, James A, McGeady JB, Reed ML, Kuang WW, Nangia AK. The effect of the new 2010 World Health Organization criteria for semen analyses on male infertility. Fertil Steril, 2012, 98(6):1428–1431. https://doi.org/10.1016/j.fertnstert.2012.07.1130 PMID: 22921910

[13] Quinn MM, Jalalian L, Ribeiro S, Ona K, Demirci U, Cedars MI, Rosen MP. Microfluidic sorting selects sperm for clinical use with reduced DNA damage compared to density gradient centrifugation with swim-up in split semen samples. Hum Reprod, 2018, 33(8):1388–1393. https://doi.org/10.1093/humrep/dey239 PMID: 30007319

[14] Xu Z, Yao G, Niu W, Fan H, Ma X, Shi S, Jin H, Song W, Sun Y. Calcium Ionophore (A23187) rescues the activation of unfertilized oocytes after intracytoplasmic sperm injection and chromosome analysis of blastocyst after activation. Front Endocrinol (Lausanne), 2021, 12:692082. https://doi.org/10.3389/fendo.2021.692082 PMID: 34335469 PMCID: PMC8320372

[15] Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology. The Istanbul Consensus Workshop on embryo assessment: proceedings of an expert meeting. Hum Reprod, 2011, 26(6):1270–1283. https://doi.org/10.1093/humrep/der037 PMID: 21502182

[16] De Braekeleer M, Nguyen MH, Morel F, Perrin A. Genetic aspects of monomorphic teratozoospermia: a review. J Assist Reprod Genet, 2015, 32(4):615–623. https://doi.org/10.1007/s10815-015-0433-2 PMID: 25711835 PMCID: PMC4380889

[17] Kyono K, Nakajo Y, Nishinaka C, Hattori H, Kyoya T, Ishikawa T, Abe H, Araki Y. A birth from the transfer of a single vitrified-warmed blastocyst using intracytoplasmic sperm injection with Calcium Ionophore oocyte activation in a globozoospermic patient. Fertil Steril, 2009, 91(3):931.e7–931.e11. https://doi.org/10.1016/j.fertnstert.2008.10.002 PMID: 19046584

[18] Rybouchkin A, Dozortsev D, Pelinck MJ, De Sutter P, Dhont M. Analysis of the oocyte activating capacity and chromosomal complement of round-headed human spermatozoa by their injection into mouse oocytes. Hum Reprod, 1996, 11(10):2170–2175. https://doi.org/10.1093/oxfordjournals.humrep.a019071 PMID: 8943524

[19] Shan Y, Zhao H, Zhao D, Wang J, Cui Y, Bao H. Assisted oocyte activation with Calcium Ionophore improves pregnancy outcomes and offspring safety in infertile patients: a systematic review and meta-analysis. Front Physiol, 2022, 12:751905. https://doi.org/10.3389/fphys.2021.751905 PMID: 35140624 PMCID: PMC8819094

[20] Jones C, Meng X, Coward K. Sperm factors and egg activation: Phospholipase C zeta (PLCZ1) and the clinical diagnosis of oocyte activation deficiency. Reproduction, 2022, 164(1):F53–F66. https://doi.org/10.1530/REP-21-0458 PMID: 35312629 PMCID: PMC9175550

[21] Nomikos M, Swann K, Lai FA. Starting a new life: sperm PLC-zeta mobilizes the Ca^{2+} signal that induces egg activation and embryo development: an essential phospholipase C with implications for male infertility. Bioessays, 2012, 34(2):126–134. https://doi.org/10.1002/bies.201100127 PMID: 22086556

[22] Tejera A, Mollá M, Muriel L, Remohí J, Pellicer A, De Pablo JL. Successful pregnancy and childbirth after intracytoplasmic sperm injection with Calcium Ionophore oocyte activation in a globozoospermic patient. Fertil Steril, 2008, 90(4):1202.e1–1202.e5. https://doi.org/10.1016/j.fertnstert.2007.11.056 PMID: 18359025

[23] Kamiyama H, Shimizu T, Oki T, Asada T, Araki Y, Araki Y. Successful delivery following intracytoplasmic sperm injection with Calcium Ionophore A23187 oocyte activation in a partially globozoospermic patient. Reprod Med Biol, 2012, 11(3): 159–164. https://doi.org/10.1007/s12522-012-0123-z PMID: 29699121 PMCID: PMC5907117

[24] Battaglia DE, Koehler JK, Klein NA, Tucker MJ. Failure of oocyte activation after intracytoplasmic sperm injection using round-headed sperm. Fertil Steril, 1997, 68(1):118–122. https://doi.org/10.1016/s0015-0282(97)81486-0 PMID: 9207595

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Received: June 15, 2022

Accepted: November 10, 2022