Evaluation of PLCζ and PAWP Expression in Globozoospermic Individuals

Majid Kamali-Dolat Abadi, M.Sc.1,2, Marziyeh Tavalaee, M.Sc.1, Abdolhossein Shahverdi, Ph.D.3, Mohammad Hossein Nasr-Esfahani, Ph.D.1, 4*

1. Department of Reproductive Biotechnology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
2. Department of Molecular and Cellular Biology, Faculty of Basic Sciences and Advanced Technologies in Biology, University of Science and Culture, Tehran, Iran
3. Department of Embryology, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
4. Isfahan Fertility and Infertility Center, Isfahan, Iran

*Corresponding Address: P.O.Box: 8165131378, Department of Reproductive Biotechnology, Reproductive Biomedicine Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
Email: mh.nasr-esfahani @ royaninstitute.org

Received: 5/Aug/2015, Accepted: 17/Jan/2016

Abstract

Objective: Globozoospermia is a rare type of teratozoospermia with incidence of 0.1% among infertile individuals. Phospholipase C zeta (PLCζ) and postacrosomal sheath WW domain binding protein (PAWP) are the main candidates in sperm taking responsibility for oocyte activation during fertilization. Therefore, we aimed to evaluate the expression of these two genes at RNA and protein levels in globozoospermic individuals and compare the results with fertile individuals.

Materials and Methods: In this experimental study, semen samples of 21 infertile men with globozoospermia and 25 fertile men were collected. Expression of PLCζ and PAWP at RNA and protein levels were assessed and compared between two groups by quantitative real time polymerase chain reaction (qPCR) and Western blot, respectively.

Results: Expression of both PLCζ and PAWP were significantly reduced at RNA and protein levels in infertile men with globozoospermia compared to fertile men.

Conclusion: This is the first study that simultaneously assessing the respective factors in a large population of globozoospermia, suggested that intra-cytoplasmic sperm injection (ICSI) along with artificial oocyte activation may rescue failed fertilization in routine ICSI.

Keywords: Globozoospermia, PLCζ, PAWP

Citation: Kamali-Dolat Abadi M, Tavalaee M, Shahverdi AH, Nasr-Esfahani MH. Evaluation of PLCζ and PAWP expression in globozoospermic individuals. Cell J. 2016; 18(3): 438-445.

Introduction

With regards to high fertilization rate (around 70-80%) obtained by intra-cytoplasmic sperm injection (ICSI), this procedure remains at the forefront of assisted reproductive techniques for treating male infertility (1). Despite data highlighting remarkable application of ICSI, this technique remains futile in treatment of 3-5% of applicants due to totally failed or low number of fertilization (2). This phenomenon is determined in men with globozoospermia.

Two kinds of globozoospermia are recognized in infertile men; i. Total or homologous globozoospermia, composed of round shape without acrosome in all sperms and ii. Partial or heterologous globozoospermia, consisting of some sperms with intact acrosome. Globozoospermia has been principally ascribed to individuals with sperm inability to activate oocyte (3). Therefore, to overcome this dearth, artificial oocyte activation (AOA) in conjunction with ICSI has been implemented, providing an opportunity for such couples to have
children (4, 5). Failure of sperm to induce oocyte activation has been mainly attributed to absence of sperm-borne oocyte-activating factor(s) (SOAFs) which is believed to be present in the post-acrosomal sheath of sperm perinuclear theca (PAS-PT) (6, 7).

So far, several factors have been proposed as the potential candidates for SOAFs, including phospholipase C zeta (PLCζ), postacrosomal sheath WW domain binding protein (PAWP), truncated form of KIT(tr-Kit) and citrate synthase (8-11). Among these factors, PLCζ has been well studied and gained the highest rank as the potential candidate. Previous study demonstrated that injection of PLCζ and PAWP recombinant proteins into the oocytes led to induce Ca\(^{2+}\) oscillations. Therefore, reduction or absence of these factors in the sperm could cause fertilization failure (12). Each of these proteins have been studied by two different research groups (12, 13) and each group has provided evidences that the other factor may not be the potential candidate (14, 15). Considering this ambiguity, we aimed in present study to evaluate the expression of these two genes at RNA and protein levels in globozoospermic individuals and compare the results with fertile individuals.

**Materials and Methods**

**Sperm sample preparation**

This experimental study received the approval of Institutional Review Board of Isfahan Fertility and Infertility Center (IFIC) and Royan Institute (Iran). Semen samples were collected from men who had been referred to the IFIC. This study was performed on 21 infertile men with total globozoospermia and 25 fertile men recruited from couples participating in the embryo donation program, during the time period of February 2012 to April 2015. All individuals gave informed consent prior to participation in the study. All semen samples were collected by masturbation in to the sterile containers, following on 3-4 days sexual abstinence, and delivered to the laboratory within 45 minutes after ejaculation. Immediately, one portion of the semen was used for evaluation of sperm concentration and motility according to World Health Organization (WHO) guidelines (16).

The remaining portion of the semen samples were washed twice in phosphate buffer saline (PBS, pH=7.4) and used for assessment of relative expression of PLCζ and PAWP at mRNA and protein levels by quantitative real time polymerase chain reaction (qPCR) and Western blot, respectively.

Briefly, sperm concentration was defined using sperm counting chamber (Sperm Processor, India), sperm motility was assessed by computer assisted semen analysis (CASA) software and sperm morphology was carried out using Papanicolaou staining according to the WHO-2010 instruction.

**Preparation of samples for quantitative real time polymerase chain reaction and Western blot techniques**

Briefly, for protein and RNA extractions, semen samples were washed with PBS and lysed with total RNA isolation (TRI) reagent (Sigma-Aldrich, USA) according to the manufacturer’s protocol. In order to eliminate possible contamination of genomic DNA, RNA-containing samples were treated with DNaseI (Fermentas, USA). First strand cDNA synthesis was carried out using 1 mg of total RNA with the RevertAid First Strand cDNA Synthesis kit (TaKaRa, Japan). Subsequently, the obtained cDNA was kept in -70°C freezer.

**Western blotting technique**

Briefly, approximately 35 µg of protein was run on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to a polyvinylidene fluoride membrane (PVDF, Biorad, USA). The membranes were blocked with skimmed milk (Merck, USA) and polyclonal anti-PLCζ antibody (1:32000, Covalab, France), polyclonal anti-PAWP antibody (1:5000, abcam, UK) and monoclonal anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH), clone 6C5 (1:5000, Millipore, USA), were used as specific primary antibodies. After three times washing, the secondary antibodies, used for PAWP and PLCζ, were horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG and for GAPDH was anti-rabbit IgG (all purchased from Dako, Japan). After three times washing, target proteins band were detected with an Amersham ECL Advance Western Blotting Detection Kit (GE Healthcare, Germany). The fire reader (Uvitec, UK) was used for recording chemiluminescence images. Densitometric
analysis of the images was performed by Quantity One Software v 4.6.9 (Bio-Rad, Germany). Results were expressed as mean relative intensity (mean intensity of the patient’s band/mean intensity of fertile bands) (17). Figure 1 showed Western blot of PLCζ and PAWP in infertile men with globozoospermia (n=4) and fertile men (n=5).

Fig.1: Western blot analysis of PAWP and PLCζ in 4 infertile men with globozoospermia and 5 fertile men.

Table 1: Primers used to assess PLCζ, GAPDH and PAWP mRNA levels in human sperm

| Gene symbol | Primer sequences (5´-3´)                           |
|-------------|--------------------------------------------------|
| GAPDH       | F: CCACCTCCTCCACCTTGACG                          |
|             | R: CCACCACCTGGTGCTGAG                            |
| PLCζ        | F: ATGCCGTTGTTGGAGATTG                           |
|             | R: AGTTTGCTTGGAGATTG                             |
| PAWP        | F: CAGATGCTTTGTCAGTTATCGTC                       |
|             | R: GCCTTCATTCCCTACCGGTG                          |

Statistical analysis

For descriptive results, the data were expressed as mean ± error of mean (SE). Independent samples t test with a threshold of 0.05 was used for comparison of mean values between fertile and globozoospermic individuals. Pearson analysis was used to assess the correlations between different parameters. All statistical analyses were carried out using Statistical Package for Social Sciences (version 11.5, SPSS, Chicago, IL, USA).

Results

The mean age of men were 40.06 ± 1.19 and 30.91 ± 1.68 years old in the fertile and globozoospermic groups, respectively. Table 2 shows the descriptive parameters including sperm concentration, percentage of motility and sperm abnormal morphology in fertile men (n=25) and individuals with globozoospermia (n=21).

Relative expression of PAWP and PLCζ at mRNA and protein levels were compared between fertile and individuals with globozoospermia and results are presented in the Figure 2. At mRNA level, the relative expression of both PAWP (1.41 ± 0.2 vs. 0.07 ± 0.03, P<0.05) and PLCζ (1.55 ± 0.23 vs. 0.12 ± 0.07, P<0.05) were significantly lower in individuals with globozoospermia, compared to fertile individuals. Similarly, relative expression of both PAWP (1.04 ± 0.13 vs. 0.51 ± 0.08, P<0.05) and PLCζ (1.26 ± 0.36 vs. 0.40 ± 0.1, P<0.05) at protein levels were significantly lower in individuals with globozoospermia in comparison with fertile individuals.

Pearson correlation analysis between PAWP and PLCζ revealed positive significant correlations between these genes at protein level (r=0.703, P=0.000) and also at mRNA (r=0.476, P=0.003).
However, no significant correlation was observed between the relative expression of mRNA with protein for both PAWP and PLCζ (Fig.3).

In this study, we also assessed the correlation between semen parameters with PAWP and PLCζ at both the protein and RNA levels (Table 3). Only a significant correlation was observed between sperm morphology with the relative expression of both PAWP ($r=-0.367$, $P=0.022$) and PLCζ proteins ($r=-0.375$, $P=0.045$). At mRNA level, only PLCζ showed significant correlations with all the three semen parameters.

**Table 2:** Comparison of sperm concentration, percentage of motility and sperm abnormal morphology between individuals with globozoospermia and fertile men

| Parameters                        | Fertile Mean ± SE (n=25) | Globozoospermia Mean ± SE (n=21) | P value |
|-----------------------------------|---------------------------|----------------------------------|---------|
| Sperm concentration ($10^6$/ml)   | 68.82 ± 5.0               | 41.57 ± 6.71                     | 0.002   |
| Sperm motility (%)                | 58.86 ± 1.54              | 38.44 ± 5.27                     | 0.002   |
| Abnormal sperm morphology (%)     | 94.70 ± 0.49              | 100 ± 00                         | 0.000   |

![Fig.2: Comparison of relative expression of PLCζ and PAWP at both protein and mRNA levels between infertile individuals with globozoospermia and fertile men. *: Significant difference: $P<0.05$.](image)
Fig. 3: Pearson’s correlation coefficient analysis between PAWP and PLCζ at protein (r=0.703, P=0.00) and at mRNA (r=0.476, P=0.003) levels in 21 infertile men with globozoospermia and 25 fertile individuals.

| Parameters                        | Protein      | RNA          |
|-----------------------------------|--------------|--------------|
|                                   | PLCζ | PAWP | PLCζ | PAWP |
| Sperm concentration (10^6/ml)     | 0.194 | 0.316 | 0.378* | 0.017 |
| Sperm motility (%)                | 0.092 | 0.194 | 0.371* | 0.310 |
| Abnormal sperm morphology (%)     | -0.375* | -0.367* | -0.387* | -0.349 |

*; Significant difference: P<0.05.

Discussion

Before the advent of ICSI, failure of fertilization in globozoospermia was mainly attributed to lack of acrosome and inability of such sperm to penetrate into the oocyte. However, with implementation of ICSI, it became evident that in a considerable portion of globozoospermic cases, sperm do not have the potential of inducing oocyte activation with exception of few reports achieving fertilization and pregnancy following ICSI in globozoospermic individuals (19-21).

Further research and advances in the field of reproductive medicine and animal biotechnology revealed that an essential step in the process of fertilization is the ability of sperm to induce oocyte activation. In this context, several factors have been considered as the potential candidates for SOAF, including PLCζ, PAWP, tr-kit, and citrate synthase (8-11). Among these candidates PLCζ and PAWP have gained the most attentions of two different groups, scientifically arguing which factor is more likely to be the real candidate for SAOAF (12, 13).
showed that in globozoospermia, acrosome
at RNA and protein but at low levels. 
ected both PLCζ and PAWP in globozoospermia
the former possibility is more likely, since we de-
results revealed significant reduction at both RNA and
protein levels for these two factors in globozoospermic
patients compared to fertile individuals, further reiterating on our previous report implicating
reduced level of PLCζ mRNA in these patients
. But, to our knowledge this is the first study
resulting in the degree of expression of PAWP at
both RNA and protein levels and comparing the
results with fertile individuals. These results are
also consistent with literature report indicating that
increased expression levels of PLCζ and PAWP in
human sperm strongly correlated with high fertil-
ization rate following ICSI, and improved embry-
one quality (25, 26).

Two possibilities may account for reduced levels
of PLCζ and PAWP in globozoospermia: i. Anom-
aliies in the process of acrosome biogenesis and ii.
Genetic defects underlying globozoospermia (27-
29). Acrosome biogenesis has been shown to be
dependent on the presence and functionality of the
perinuclear theca that shields the nucleus during
sperm development and contributes to signaling
molecules which may be essential for oocyte ac-
tivation (30-32). Therefore, lack of acrosome may
account for general absence of molecules that are
present in the region of pronuclear theca and can-
didate of SOAFs may be among the factors which
are absent in such sperm (28).

Presence of high percentage of sperm with small
acrosome may lead to fertilization failure after
ICSI due to similar phenomenon. The latter con-
clusion is in line with previous literature (27). On
the other hand, reduced SOAFs may be related to
genetic defects, like gene deletions and mutations
associated with globozoospermia. Although it is
difficult to define which option is the main cause
of reduced PLCζ and PAWP in globozoospermia,
the former possibility is more likely, since we de-
tected both PLCζ and PAWP in globozoospermia
at RNA and protein but at low levels.

Electron microscopic study by Singh et al. (33)
showed that in globozoospermia, acrosome is pre-
sent in early spermatids and develops independent-
ly from the nucleus in the cytoplasm. The ques-
tions that remain to be defined are whether proper
attachment of acrosome with nucleus could have
role in localizing SOAFs in the pronuclear theca
and lack of this association lead to reduced expres-
sion of PLCζ and PAWP? In addition, transfer of
them into sertoli cell’s cytoplasm could lead to en-
gulbing the residual body (27, 29, 34, 35). Simi-
lar events have been reported for three acrosomal
markers, including acrosin, intra-acrosin inhibi-
tor and purified outer acrosomal membrane (36).
Another possibility is the malfunctioning of Golgi
apparatus and manchette, which may have role in
propelling cargo of protein during acrosome bi-
genesis. Thus, such anomaly may lead to reduced
deposition of PLCζ and PAWP in the perinuclear
theca (37). In this regard, Dam et al. (27) and Lon-
go et al. (38) stated that "calcin, a basic protein
that is almost exclusively located in the posterior
part or calyx of the sperm nuclear theca, appeared
to be absent in globozoospermic cells", thereby in-
dicating that impaired development of the sperm-
specific skeleton may affect protein localization in
globozoospermia (39, 40). Such observation has
been verified in mouse model of globozoospermia
with DPY19L2 deletion, the most common genetic
defect associated with human globozoospermia
(29). This may also describe the punctate pattern
of PLCζ staining within the sperm head in globo-
zoospermic individuals (20, 29).

It is notable that despite partial absence of PLCζ
in the sperm of some globozoospermic individu-
aliments, some oocytes became activated (41). Although
the oolema deformation and the subsequent rup-
ture of the membrane caused by ICSI procedure
leads to Ca2+ influx. This raise may not be suffi-
cient to induce oocyte activation (42, 43). Never-
theless, it is possible that in some cases, this Ca2+
influx together with the low traces of PLCζ asso-
ciated with acrosomal buds might be capable of
supporting oocyte activation. The importance of
acrosomal buds in oocyte activation was initially
suggested in a globozoospermic patient with no
mutations or deletions in SPATA16 and DPY19L2
genes (29, 44) and confirmed later in non-gen-
typed globozoospermic patients by Kashir et al,
showing that spemms exhibited an acrosomal bud
could present a punctate pattern of PLCζ staining
within the head (45).
Conclusion

Level of both SOAFs candidates, PLCζ and PAWP, are reduced at both RNA and protein levels in globozoospermia. Studies of literature mainly attribute this phenomenon to impaired development of the sperm-specific skeleton in these individuals.

Acknowledgments

This study was financially supported by Royan Institute and we would like to express our gratitude to the staff of Isfahan Fertility and Infertility Institute and we would like to express our gratitude.

References

1. Palermo GD, Neri OV, Takeuchi T, Rosenwaks Z. ICSI: where we have been and where we are going. Semin Reprod Med. 2009; 27(2): 191-201.
2. Esfandian N, Javed MH, Gofflief L, Casper RF. Complete failed fertilization after intracytoplasmic sperm injection—analysis of 10 years’ data. Int J Fertil Womens Med. 2005; 50(4): 187-192.
3. Rybouchkin A, Dozortsev D, Pelinck MJ, De Sutter P, Dhoff 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.
4. Heindryckx B, Van der Elst J, De Sutter P, Dhoff M. Treatment option for sperm–oocyte-related fertilization failure: assisted oocyte activation following diagnostic heterologous ICSI. Hum Reprod. 2005; 20(8): 2237-2241.
5. Borges E Jr, de Almeida Ferreira Braga DP, de Sousa Bonetti TC, Iaconelli A Jr, Franco GJ Jr. Artificial oocyte activation with calcium ionophore A23187 in intracytoplasmic sperm injection cycles using surgically retrieved spermatzoa. Fertil Steril. 2009; 92(1): 131-136.
6. Sutovsky P, Manandhar G, Wu A, Oko R. Interactions of sperm perinuclear theca with the oocyte: Implications for oocyte activation, anti-polyspermy defense, and assisted reproduction. Microsc Res Tech. 2003; 61(4): 362-378.
7. Amdani SN, Yeste M, Jones C, Coward K. Sperm factors and oocyte activation: current controversies and considerations. Biol Reprod. 2015; 93(2): 50.
8. Swann K, Saunders CM, Rogers NT, Lai FA. PLCζ (zeta): a sperm protein that triggers Ca2+ oscillations and egg activation in mouse oocytes. Dev Biol. 2007; 306(2): 797-808.
9. Aarabi M, Sutovsky P, Oko R. Re: is PAWP the ‘real’ sperm factor? Asian J Androl. 2015; 17(3): 446-449.
10. Nomikos M, Swann K, Lai FA. Is PAWP the “real” sperm factor? Asian J Androl. 2015; 17(3): 444-448.
11. Nomikos M, Sanders JR, Theodoridou M, Kashir J, Matthews E, Nounessi G, et al. Sperm-specific post-acrosomal WW-domain binding protein (PAWP) does not cause Ca2+ release in mouse oocytes. Mol Hum Reprod. 2014; 20(10): 938-947.
12. Aarabi M, Yu Y, Xu W, Tse MY, Pang SC, Yi YJ, et al. The testicular and epididymal expression profile of PLCζ in mouse and human does not support its role as a sperm-borne oocyte activating factor. PloS One. 2012; 7(3): e33496.
13. WHO. WHO laboratory manual for the examination and processing of human semen. 5th ed. Geneva, Switzerland: WHO Press; 2010.
14. Motei M, Tavalaee M, Rabiei F, Hajhosseini R, Nasr-Esfahani MH. Evaluation of HSPA2 in fertile and infertile individuals. Andrologia. 2013; 45(1): 66-72.
15. Aghajanpour S, Ghadie K, Salamian A, Deemeh MR, Tavalaee M, Mostaghanian J, et al. Quantitative expression of phospholipase C zeta, as an index to assess fertilization potential of a semen sample. Hum Reprod. 2011; 26(11): 2950-2956.
16. Kim ST, Cha YB, Park JM, Gye MC. Successful pregnancies and delivery of frozen-thawed embryos after intracytoplasmic sperm injection using round-headed spermatzoa and assisted oocyte activation in a globozoospermic patient with mosaic Down syndrome. Fertil Steril. 2001; 75(2): 445-447.
17. Taylor SL, Yoon SY, Morshedi MS, Lacey DR, Jellerette T, Fissore RA, et al. Complete globozoospermia associated with PLCζ deficiency treated with calcium ionophore and ICSI results in pregnancy. Reprod Biomed Online. 2010; 20(4): 559-564.
18. 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.
19. Swain JE, Pool TB. ART failure: oocyte contributions to unsuccessful fertilization. Hum Reprod Update. 2008; 14(5): 431-446.
20. Nasr-Esfahani MH, Deemeh MR, Tavalaee M. Artificial oocyte activation and intracytoplasmic sperm injection. Fertil Steril. 2010; 94(2): 520-526.
21. Nasr-Esfahani MH, Razavi S, Javad Z, Tavalaee M. Artificial oocyte activation in severe teratozoospermia undergoing intracytoplasmic sperm injection. Fertil Steril. 2008; 90(6): 2231-2237.
22. Aarabi M, Balakier H, Bashar S, Moskovtsev SI, Sutovsky P, Librach CL, et al. Sperm content of postacrosomal WW binding protein is related to fertilization outcomes in patients undergoing assisted reproductive technology. Fertil Steril. 2014; 102(2): 440-447.
23. Amdani SN, Jones C, Coward K, Phospholipase C zeta (PLCζ): oocyte activation and clinical links to male factor infertility. Adv Biol Regul. 2013; 53(3): 292-308.
24. Dam AH, Feenstra I, Westphal JR, Ramos L, van Golde LJ, et al. Successful pregnancy and childbirth after intracytoplasmic sperm injection with calcium ionophore A23187 in mouse oocytes. Mol Hum Reprod. 2007; 13(1): 63-75.
25. Perrin A, Coat C, Nguyen MH, Talagas M, Salamian A, Deemeh MR, Tavalaee M, Mostaghanian J, et al. Subcellular localization of phospholipase
Cζ in human sperm and its absence in DPY19L2-deficient sperm are consistent with its role in oocyte activation. Mol Hum Reprod. 2015; 21(2): 157-168.

30. Ito C, Akutsu H, Yao R, Kyono K, Suzuki-Toyota F, Toyama Y, et al. Oocyte activation ability correlates with head flatness and presence of perinuclear theca substance in human and mouse sperm. Hum Reprod. 2009; 24(10): 2588-2595.

31. Oko R, Sutovsky P. Biogenesis of sperm perinuclear theca and its role in sperm functional competence and fertilization. J Reprod Immunol. 2009; 83(1-2): 2-7.

32. Ito C, Yamatoya K, Yoshida K, Kyono K, Yao R, Noda T, et al. Appearance of an oocyte activation-related substance during spermatogenesis in mice and humans. Hum Reprod. 2010; 25(11): 2734-2744.

33. Singh G. Ultrastructural features of round-headed human spermatozoa. Int J Fertil. 1992; 37(2): 99-102.

34. Alvarez Sedó C, Rawe VY, Chemes HE. Acrosomal biogenesis in human globozoospermia: immunocytochemical, ultrastructural and proteomic studies. Hum Reprod. 2012; 27(7): 1912-1921.

35. Wu AT, Sutovsky P, Xu W, van der Spoel AC, Platt FM, Oko R. The postacrosomal assembly of sperm head protein, PAWP, is independent of acrosome formation and dependent on microtubular manchette transport. Dev Biol. 2007; 312(2): 471-483.

36. Förke-Gerloff S, Krause W, Töpfer-Petersen E, Tschetsche H, Müller-Esterl W, Engel W. On the teratogenesis of round-headed spermatozoa: investigations with antibodies against acrosin, an intraacrosomally located acrosin-inhibitor, and the outer acrosomal membrane. Andrologia. 1985; 17(2): 126-138.

37. Nistal M, Panigagua R. Morphogenesis of round-headed human spermatozoa lacking acrosomes in a case of severe teratozoospermia. Andrologia. 1978; 10(1): 49-51.

38. Longo FJ, Krohne G, Franke WW. Basic proteins of the perinuclear theca of mammalian spermatozoa and spermatids: a novel class of cytoskeletal elements. J Cell Biol. 1987; 105(3): 1105-1120.

39. Escalier D. Failure of differentiation of the nuclear-perinuclear skeletal complex in the round-headed human spermatozoon. Int J Dev Biol. 1990; 34(2): 287-297.

40. Courtot AM. Presence and localization of the 60 KD calcin in human spermatozoa presenting postacrosomal sheath defects: preliminary results. Mol Reprod Dev. 1991; 28(3): 272-279.

41. Dam AH, Ramos L, Dijkman HB, Woestenenk R, Robben H, van den Hoven L, et al. Morphology of partial globozoospermia. J Androl. 2011; 32(2): 199-206.

42. Sato MS, Yoshitomo M, Mohri T, Miyazaki S. Spatiotemporal analysis of [Ca2+]i rises in mouse eggs after intracytoplasmic sperm injection (ICSI). Cell Calcium. 1999; 26(1-2): 49-58.

43. Tesank J, Sousa M, Testart J. Human oocyte activation after intracytoplasmic sperm injection. Hum Reprod. 1994; 9(3): 511-518.

44. Sermondade N, Hafhouf E, Dupont C, Bechoua S, Palacios C, Eustache F, et al. Successful childbirth after intracytoplasmic morphologically selected sperm injection without assisted oocyte activation in a patient with globozoospermia. Hum Reprod. 2011; 26(11): 2944-2949.

45. Kashir J, Sermondade N, Sifer C, Oo SL, Jones C, Mounce G, et al. Motile sperm organelle morphology evaluation-selected globozoospermic human sperm with an acrosomal bud exhibits novel patterns and higher levels of phospholipase C zeta. Hum Reprod. 2012; 27(11): 3150-3160.