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REVIEW ARTICLE

Reproductive outcome in globozoospermic men: update and prospects

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SUMMARY

Infertility affects approximately 15% of couples in reproductive age. Male infertility is estimated to represent about 20% of the etiologies. Among them, a rare type of teratozoospermia known as globozoospermia leads to disappointing pregnancy outcomes. Morphological, physiological and genetic aspects of this severe disorder have been described. We undertook a complete review of the available data on the reproductive outcomes in globozoospermic patients. To this end, a literature review in both English and French, over a 20-year time period using PubMed/Medline, ScienceDirect, and Scopus was performed. A total of 45 publications describing 172 attempts of treatment with assisted reproduction techniques (ICSI or IMSI with or without oocyte activation) were identified. We reviewed 28 deliveries and 34 children. However, for these patients, the fertilization rate after ICSI remained low. The present review suggests that oocyte activation (in particular with calcium ionophore) could improve the pregnancy rate significantly when dealing with globozoospermia. Once the exact pathogenesis of human globozoospermia is clearly identified, it is likely that other treatments such as recombinant phospholipase C zeta (PLC zeta, PLCζ), which seems to be a promising biological tool, would be developed.

INTRODUCTION

Globozoospermia is a rare but severe cause of male infertility, which is defined by round-headed spermatozoa devoid of the acrosome. Spermatozoa are unable to fertilize oocytes naturally. Before the discovery of intracytoplasmic sperm injection (ICSI), these patients were considered infertile, even sterile. With ICSI, children were born. Nevertheless, the outcomes are still disappointing with a high risk of fertilization failure. The description of this pathology in siblings pointed to an underlying genetic origin (Kullander & Rausing, 1975; Nistal & Paniagua, 1978; Edirisinghe *et al.*, 1998; Carrell *et al.*, 1999, 2001; Viville *et al.*, 2000; Kilani *et al.*, 2004; Demir *et al.*, 2008; Dirican *et al.*, 2008; Bechoua *et al.*, 2009). However, only recently have different genetic anomalies been discovered (Dam *et al.*, 2007a; Liu *et al.*, 2010; Harbuz *et al.*, 2011; Koscinski *et al.*, 2011). The need to improve knowledge of this pathology has been underlined in many publications. Only an appropriate assessment of this condition will enable proper management of these patients in assisted reproductive medicine. Hence, in this review, we describe in a first part this rare pathology from a morphological and genetical point of view. Second, we undertook an inventory of all cases of ICSI (intracytoplasmic sperm injection) and IMSI (intracytoplasmic morphologically selected sperm injection) attempts and their outcomes in terms of fertilization, pregnancy, delivery, and birth rates.

METHODS

For the review, a complete study which consisted of an inventory of existing data on globozoospermia was undertaken. Therefore, literature search was conducted on three electronic databases including PubMed/Medline, ScienceDirect, and Scopus. The search was performed using a combination of the following terms: [globozoospermia OR (round AND headed AND sperm) OR acrosomeless]. The review of the reproductive outcome covers the time period from the beginning of December 1994 to the end of April 2015. We included abstracts and articles

written in both French and English which dealt with the features and pathogenesis of acrosomeless spermatozoa.

We focused on publications where patients were diagnosed with total globozoospermia (complete teratozoospermia observed on light microscopy with 70–100% round-headed sperm). Spermatozoa were either acrosomeless or presented a rudimentary acrosome.

A total of 45 publications were selected and included in our review of the reproductive outcome. The following were excluded from the review:

- Publications reporting pregnancies after assisted reproductive technology (ART) in partial globozoospermia (Carrell *et al.*, 2001; Dam *et al.*, 2012).
- Publication reporting pregnancies in non-homogeneous groups (partial and total globozoospermia mixed together) (Kuentz *et al.*, 2013).
- Intrauterine insemination, conventional *in vitro* fertilization and subzonal sperm microinjection (SUZI) attempts.

RESULTS

Globozoospermia

During spermiogenesis, many abnormalities can occur in the acrosome, the nucleus or the flagella. Lack of acrosome is associated with a rare but severe type of infertility defined as globozoospermia.

It is a genetic pathology which causes severe male infertility. The first optical microscopic observation of round-headed spermatozoa was performed by Meyhöfer (1965). Schirren *et al.* (1971) and Holstein *et al.* (1973a,b) clarified the ultrastructural characteristics of these spermatozoa by electron microscopy and identified the lack of acrosome leading to round-headed spermatozoa (Holstein *et al.*, 1973a). The name 'globozoospermia' was introduced by Wolff *et al.* (1976).

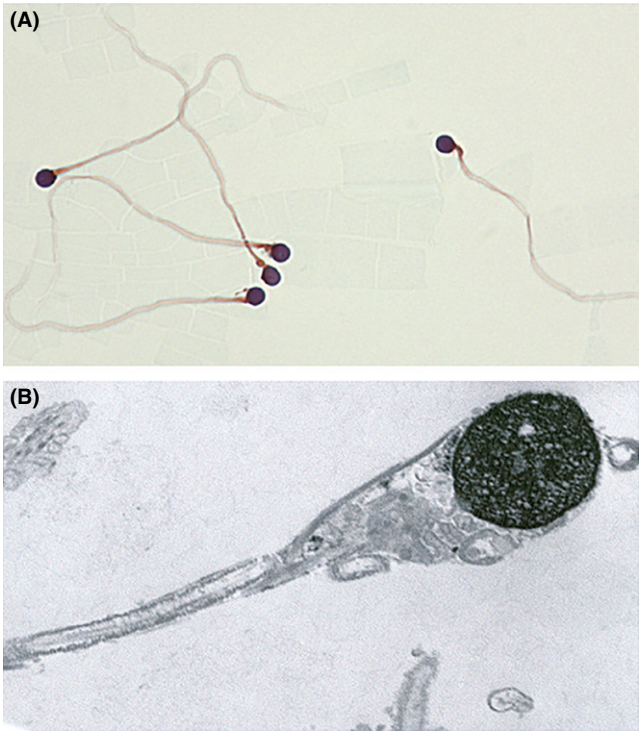
Since the first description, the incidence of globozoospermia has been stable, hovering around 0.1% of the infertile men (Holstein *et al.*, 1973a). Analysis of the spermatozoa of fertile males revealed a small proportion of round-headed spermatozoa (0.5%). However, in infertile patients, this percentage was found to be higher (2.3% ± 0.5; n = 114 infertile males vs. 0.5% ± 0.1; n = 60 fertile males; Kalahanis *et al.*, 2002). In case of total globozoospermia, patients displayed primary infertility. However, two spontaneous abortions were reported in a couple before 3 years of involuntary childlessness but no paternity test was performed (Arrighi *et al.*, 1980; review in Dam *et al.*, 2007b).

Overall, men with globozoospermia have normal physical and mental development, normal clinical features and a normal hormonal status (Pedersen & Rebbe, 1974; Kullander & Rausing, 1975; Anton-Lamprecht *et al.*, 1976; Weissenberg *et al.*, 1983; Lalonde *et al.*, 1988; Von Bernhardi *et al.*, 1990; Dale *et al.*, 1994; Bourne *et al.*, 1995). For some patients, varicocoeles, orchidectomy, epididymitis, with no link to the severity of the teratozoospermia, were reported. No recurrent chromosome abnormalities were stated in globozoospermia (Dam *et al.*, 2007b). In the literature, all the globozoospermic patients analyzed (n = 46) were normal except one who had a mosaic Down syndrome (Kim *et al.*, 2001).

The first electron microscopic observation indicated an absence of the acrosome and the presence of a rounded nucleus (Holstein *et al.*, 1973a,b; Fig. 1). These two criteria were considered as the main characteristics of globozoospermia. Thereafter, other abnormalities were described (Holstein *et al.*, 1973b; Pedersen & Rebbe, 1974; Anton-Lamprecht *et al.*, 1976; Baccetti *et al.*, 1977; Nistal & Paniagua, 1978; Syms *et al.*, 1984; Jeyendran *et al.*, 1985; Lalonde *et al.*, 1988; Aitken *et al.*, 1990; Escalier, 1990; Singh, 1992) such as:

- lack of post-acrosomal cap
- abnormalities in nuclear membrane
- presence of coiled flagella
- abnormalities in nuclear maturation and in chromatin condensation
- disorganization of mitochondria in the transitional piece
- abnormalities in the axoneme
- presence of cytoplasmic rest.

Figure 1 Representative view of globozoospermic spermatozoa. (A) Shorr-stained round-headed spermatozoa. Magnification with the oil immersion, ×100. (B) Transmission electron microscopy showing one acrosomeless spermatozoon.



The classification of globozoospermia based on morphological characteristics and the percentage of round-headed spermatozoa is not consensual. Two phenotypes have been established by Anton-Lamprecht *et al.* (1976). The first one utilized the classification of Schirren *et al.* (1971) which corresponds to 100% round-headed spermatozoa with spherical nucleus and a lack of acrosome. The second one described, with optical microscopy, 80% round-headed spermatozoa and with electron microscopy, the presence of cytoplasmic rest around the nucleus and the acrosome. Hence, two terms were suggested: globozoospermia type 1 for the first classification and globozoospermia type 2 for the second one (Anton-Lamprecht *et al.*, 1976).

Two forms of globozoospermia were also described by Singh (1992). Type 1 was the complete form described by Schirren *et al.* (1971) with 100% round-headed spermatozoa without acrosome, enzymes, and post-acrosomal cap. These

spermatozoa were unable to fertilize oocytes and patients were sterile. Type 2 gathered together spermatozoa with reduced acrosomal equipment and sometimes spermatozoa with other morphological abnormalities but capable of fertilizing an oocyte (Singh, 1992).

Despite these classifications, in some studies (Pedersen & Rebbe, 1974; Tyler *et al.*, 1985; Lanzendorf *et al.*, 1988; Coetzee *et al.*, 2001), optical microscopy did not show a round head for all spermatozoa, whereas consistent absence of acrosome was described with electron microscopy. In other studies (Syms *et al.*, 1984; Carrell *et al.*, 1999), only spermatozoa with round head were devoid of the acrosome. Finally, even if cells were not round, acrosome could be either absent or be really abnormal (Flörke-Gerloff *et al.*, 1984; Rybouchkin *et al.*, 1996; Carrell *et al.*, 2001; Larson *et al.*, 2001). These differences reflect an important phenotypic variability resulting from maturation abnormalities during spermiogenesis (Anton-Lamprecht *et al.*, 1976).

Apart from complete and homogeneous forms (100% of round-headed and acrosomeless spermatozoa), partial forms exist in which patients present 20–60% of round-headed spermatozoa (Holstein *et al.*, 1973b). Therefore, a simplified classification was suggested. Hence, partial globozoospermia was defined when less than 100% of spermatozoa were round-headed and acrosomeless. Partial globozoospermia is characterized by oval sperm cells with distinctive malformations to the sperm head matrix compared with normozoospermia, but normal sperm cells are also found (Dam *et al.*, 2011).

Many hypotheses to explain the absence of the acrosome and the abnormal form of sperm head in globozoospermia have been put forward. Holstein *et al.* (1973b) studied testicular biopsy and indicated that proacrosomal vesicles develop apart from the nucleus and stay in the cytoplasm before being integrated in Sertoli cells during sperm release from the germinal epithelium. In addition, acrosome production is disrupted but flagella construction is normal in most of the spermatids. Without the acrosome anchored on the nucleus, the sperm head remains spherical. Holstein *et al.* (1973b) did not describe any alteration in the nucleus suggesting that chromatin condensation was independent of acrosome malformation.

Similarly, Baccetti *et al.* (1977) observed an incomplete development of the acrosome with the acrosomal vesicle attached to the nuclear membrane which will degenerate subsequently in the late spermatid stage. Moreover, a hypoplasic aspect of the Golgi apparatus was found and was suggested as being a possible cause of malformation of the acrosome (Baccetti *et al.*, 1977). Indeed, acrosome biogenesis is impaired and leads to either an abnormal acrosome or to a totally absent acrosome. To evaluate the acrosome, biologists can focus on the spermocytogram but only electron microscopy allows a direct and precise assessment of the acrosome. In common practice, it is hard to implement such a technique. Thus, the integrity of the acrosome can be monitored more readily by the use of peanut agglutinin labeling: an intact acrosome exhibits a uniform fluorescence. Anti-CD46 labeling consists in visualizing the inner acrosomal membrane by a monoclonal antibody: fluorescence is detected after the acrosomal reaction.

Previous studies have described cytoskeletal abnormalities such as the absence of calicin, a cytoskeletal protein known to play a role in the interaction between the nucleus, acrosome, and the plasma membrane (Escalier, 1990; Courtot, 1991). The absence or deficiency of cytoskeletal proteins would be responsible for: (i) absence of nuclear elongation, (ii) absence of the post-acrosomal cap, and (iii) acrosomal abnormalities (Escalier, 1990).

We now focus on the other semen parameters in globozoospermic patients. An important heterogeneity in volume, concentration, and motility was observed in a study including 72 cases of globozoospermia (Dam *et al.*, 2007b). Aside from asthenozoospermia, the other semen parameters were within normal ranges (Dam *et al.*, 2007b). Sperm capacitation in globozoospermic patients was similar to that in fertile patients (Aitken *et al.*, 1990). Nevertheless, primary infertility found in most cases proves the incapacity of spermatozoa to fertilize spontaneously. Unlike the spermatozoa of fertile men, round-headed spermatozoa cannot bind to the plasma membrane and so are unable to fuse with the oocyte. The failure of SUZI has confirmed these observations (Lundin *et al.*, 1994; Trokoudes *et al.*, 1995). However, once injected in the hamster oocyte, a male pronucleus was formed and the decondensation of sperm nucleus was not impaired (Lanzendorf *et al.*, 1988). So, spermatozoa without acrosome can initiate fertilization once injected in the oocyte, which gives hope to treat patients with globozoospermia.

Morphological alterations could be associated with abnormalities of chromatin structure, DNA, and cytogenetic defects. Hence, chromatin condensation, DNA fragmentation, and aneuploidy were evaluated. Overall, it has been demonstrated that round-headed spermatozoa have more histones and less protamines than normal spermatozoa (Blanchard *et al.*, 1990; Yassine *et al.*, 2015). The percentage of spermatozoa with immature chromatin is higher (Vicari *et al.*, 2002; Gatimel *et al.*, 2013; Vozdova *et al.*, 2014) and DNA fragmentation increased. In 14 publications, 24 of 29 cases showed an increase in the fragmentation index compared with fertile patients (from a slight increase to a 100-fold increase compared with control values). The terminal uridine nick-end labeling assay was used in 10 studies and other techniques in the remaining four studies (Baccetti *et al.*, 1996; Larson *et al.*, 2001; Vicari *et al.*, 2002; Tejera *et al.*, 2008; Egashira *et al.*, 2009; Taylor *et al.*, 2010; Brahem *et al.*, 2011a,b; Perrin *et al.*, 2011, 2013; Sermondade *et al.*, 2011; Zhioua *et al.*, 2011; Gatimel *et al.*, 2013; Vozdova *et al.*, 2014; Yassine *et al.*, 2015). Concerning the aneuploidy rate, comparison between studies was difficult to perform. Controversial results were obtained with either no relationship between globozoospermia and aneuploidy (Viville *et al.*, 2000; Vicari *et al.*, 2002; Morel *et al.*, 2004; Schmiady *et al.*, 2005) or an increased frequency of aneuploidy in globozoospermia (Carrell *et al.*, 1999; Perrin *et al.*, 2011, 2013; Vozdova *et al.*, 2014). Using fluorescence *in situ* hybridization (FISH) analysis, aneuploidy was found increased in 60% of cases for at least one chromosome studied. The chromosomes concerned were mainly chromosomes 8, 13, 15, 16, 18, 21, X, and Y (Carrell *et al.*, 1999, 2001; Martin *et al.*, 2003; Morel *et al.*, 2004; Ditzel *et al.*, 2005; Moretti *et al.*, 2005; Strassburger *et al.*, 2007; Tejera *et al.*, 2008; Brahem *et al.*, 2011a,b; Perrin *et al.*, 2011, 2013; Vozdova *et al.*, 2014).

Globozoospermia, a genetic disease

In mice, genetic studies have demonstrated that mutation of at least 13 genes, including *Gopc* (Golgi-associated PDZ and coiled-coil motif containing protein) (Yao *et al.*, 2002), *Hrb* (HIV-1 rev

binding protein) (Kang-Decker *et al.*, 2001), *Csnk2a2* (Casein kinase 2, α prime polypeptide) (Xu *et al.*, 1999), and *Spaca1* (Sperm acrosome associated 1) (Fujihara *et al.*, 2012) results in a globozoospermia phenotype which suggests their potential role in this pathology (reviewed in Coutton *et al.*, 2015).

However, no mutation of these genes with a clear link to globozoospermia has been identified in humans (Chianese *et al.*, 2015; Christensen *et al.*, 2006; Pirrello *et al.*, 2005). Mutations of other genes (see below) segregating on an autosomal recessive mode were identified and described as being involved in globozoospermia in humans (De Braekeleer *et al.*, 2015).

DPY19L2

DPY19L2 [*dpy-19-like 2* (*C. elegans*)] seems to be implicated in acrosome formation. *DPY19L2* codes for a transmembrane protein expressed predominantly in spermatids, with specific localization limited to the internal nuclear membrane in front of the acrosomal vesicle. The protein is involved in anchoring the acrosome to the spermatozoa nucleus (Pierre *et al.*, 2012). In *DPY19L2*−/− knock-out mice, a blockage in sperm head elongation and acrosome formation has been reported. Indeed, acrosome in formation sets up normally but cannot resist to attractive strengths which induce its detachment. This abnormal acrosome is then eliminated during spermiation. Moreover, without the acrosome, microtubules involved in sperm head elongation cannot stand properly. This mechanism leads to the characteristic round-headed sperm. Patients without the *DPY19L2* gene have a normal or subnormal sperm concentration which indicates that *DPY19L2* plays a role in spermiogenesis but not in germ cell proliferation or meiosis (Tang *et al.*, 2010; Koscinski *et al.*, 2011; Pierre *et al.*, 2012). Koscinski *et al.* identified the homozygous deletion of *DPY19L2* located at 12q14.2 in patients. It is the most frequent genetic cause of globozoospermia. The frequency is estimated to be 1/85 and 1/290, respectively, for *DPY19L2* duplication and heterozygous deletion in the general population. Different genetics defects in the *DPY19L2* gene exist: intragenic deletions, deletion of the whole *DPY19L2* by non-allelic homologous recombination, splice-site mutations, nonsense mutations resulting in truncated proteins and missense mutations localized mainly in the central part of the DPY19L2 protein. A correlation exists between the severity of the phenotype and oocyte fertilization and the type of DPY19L2 mutations (Koscinski *et al.*, 2011; Chianese *et al.*, 2015; Coutton *et al.*, 2015; De Braekeleer *et al.*, 2015).

PICK1

In mouse, Pick1 (protein interacting with C kinase 1) is a cytosolic protein involved in protein trafficking. It is strongly expressed in brain, testis, and pancreas. In testis, *Pick 1* is expressed in proacrosomal vesicles in round spermatids and deletion of this gene leads to round-headed spermatozoa and oligozoospermia. In knock-out mice *Pick1*−/−, proacrosomal vesicles do not merge during the Golgi phase and the spermatids have a fragmented acrosome. Anomalies in nuclear elongation and in mitochondria organization are also present. Pick1 has been implicated in vesicular transport mechanism and to participate in acrosome biogenesis in cooperation with others proteins located nearby (for instance Gopc) (Xiao *et al.*, 2009). Liu *et al.* (2010) discovered a homozygous missense mutation (G198A) in exon 13 of the *Pick1* gene located in chromosome 22 in a Chinese family. The mutation modifies amino acid G393R in the C terminal domain. Patients had a primary infertility without any other associated symptoms. Asthenozoospermia was also described. This phenotype matches with the phenotype observed in the mutated mouse (Liu *et al.*, 2010).

SPATA 16

In mouse, SPATA 16 (Spermatogenesis associated 16) plays a role in spermatogenesis because of its localization in the Golgi apparatus and in proacrosomal vesicles. SPATA 16 is implicated in acrosome biogenesis during proacrosomal vesicle transport between the Golgi apparatus and the acroplaxome (Dam *et al.*, 2007a).

Dam *et al.* (2007a) identified in a consanguineous family, a homozygous mutation in the spermatogenesis-specific gene *SPATA 16*. This gene codes for a tetratricopeptide repeat (TPR) domain, highly conserved, which is a protein–protein interaction domain and a location for multiprotein assembly. Genetic analysis confirmed the switch between guanine and adenine (c.848G→A) in exon 4. *In vitro*, it has been confirmed that the mutation alters a splicing zone which disrupts the synthesis of the TPR domain and therefore the function of the protein (Dam *et al.*, 2007a). A first successful pregnancy in a globozoospermic patient having a new SPATA 16 mutation was described recently (Karaca *et al.*, 2014).

Regardless of the genetic origin, patients face difficulties in procreation. In total globozoospermia, absence of the acrosome makes fertilization impossible as round-headed spermatozoa are unable to bind to the zona pellucida and to fuse with the oocyte. Before the advent of ICSI, globozoospermic patients were considered sterile. However, the success rate using ICSI is still insufficient compared with the rates reported when other male indications were considered. In addition, in several cases, complete fertilization failure after ICSI has been reported indicating that other dysfunctions could be associated with globozoospermia.

One of the hypotheses to explain the weak rate of fertilization was the absence of a putative sperm-associated oocyte activating factor known as PLCζ, a spermatic protein involved in calcium oscillations during oocyte activation (Escoffier *et al.*, 2015).

Update of cases published between 1994 and April 2015

General presentation

Forty-five publications described 172 attempts. Among these 172 attempts, 131 used ICSI cycles alone, 32 used ICSI cycles with assisted oocyte activation (AOA), and nine IMSI attempts with four of them undertaken without AOA. These 172 attempts correspond to 94 infertile couples for which the male partner had total globozoospermia and for which primary infertility lasted from 1 to 23 years. This information was provided by 51% of the publications analyzed. Fertilization was obtained in 72.1% (124/172) of the attempts and the overall fertilization rate (number of oocytes fertilized/number of oocytes injected) was 35.2% (525/1490). Total fertilization failure was described in 27.9% of the attempts (48/172). Clinical pregnancy was obtained in 30.2% (52/172) of the attempts (Fig. 2). Pregnancy outcomes were unknown for 13 cases. Twenty-eight deliveries and 34 births were published (Fig. 3).

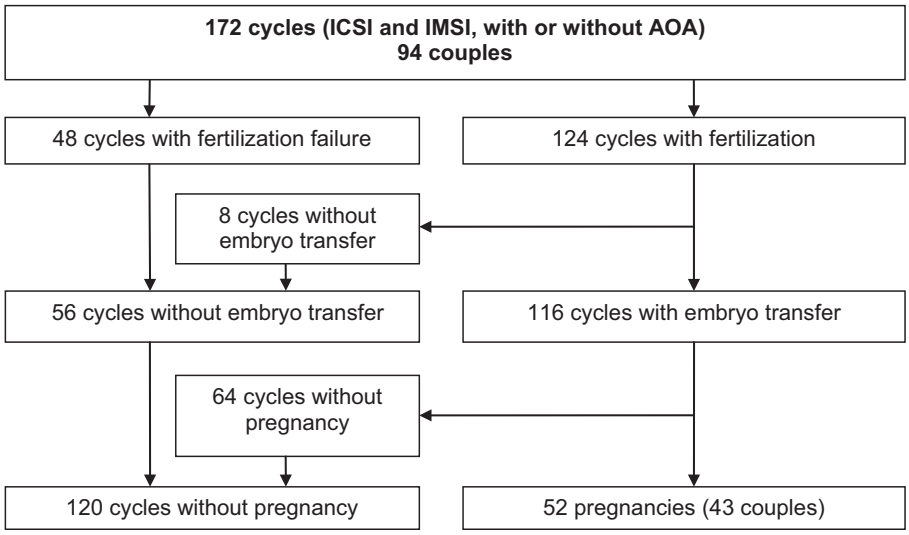


Figure 2 Results of *in vitro* fertilization (IVF) attempts published between January 1994 and April 2015. ICSI, intracytoplasmic sperm injection; IMSI, intracytoplasmic morphologically selected sperm injection, AOA, assisted oocyte activation.

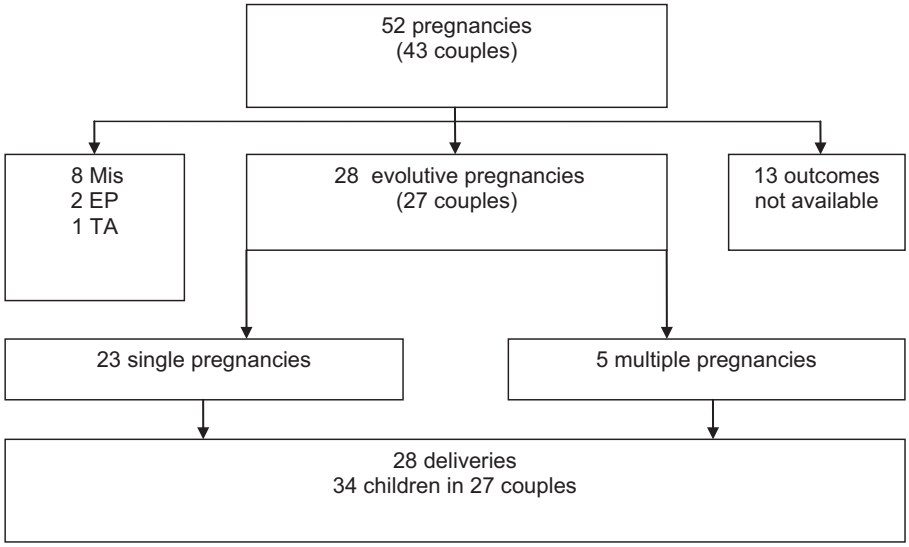


Figure 3 Globozoospermia and pregnancy outcomes published between January 1994 and April 2015. Mis, miscarriage; EP, ectopic pregnancy; TA, therapeutic abortion.

Attempts without assisted oocyte activation (AOA)

Conventional ICSI attempts without AOA. The results are presented in Tables 1, 2, and 3. The tables present the data depending on the years considered (Table 1: from 1994 to 2001; Table 2: from 2002 to 2011, and Table 3: from 2012 to April 2015). The first case was published in 1994 by Lundin *et al.* (1994) and a delivery (twin) occurred after the second attempt. All cases published described variable fertilization success and pregnancy rate. One hundred and thirty-one attempts were realized for 77 couples. The overall fertilization rate was 24.3% (262/1077, from 0% to 100%). The total number of embryos transferred was ≥174 (160 fresh plus 14 frozen/thawed embryos). Some data were missing in some publications. Twenty-nine pregnancies were described (pregnancy rate/cycle: 22.1%), which led to 16 deliveries with 21 child births. Four clinical pregnancy outcomes were missing. Complete fertilization failure was observed in 29.8% of the cycles analyzed (39/131).

IMSI attempts without AOA. Only four case reports of IMSI without AOA were published in 2011, 2012, and 2015 (Table 4). The global fertilization rate was 50% (9/18). Two pregnancies occurred with one leading to a child birth.

Attempts with assisted oocyte activation

Oocyte activation methods. Different techniques were used to activate the oocyte:

- Mechanical approach.
- Chemical approach (calcium ionophore and strontium chloride).
- Electrical approach.

Mechanical approach. Tesarik & Sousa (1995) described a modified ICSI procedure which mimics calcium ionophore effects. Indeed, a strong aspiration of the oocyte cytoplasm during sperm injection leads to an increase in the intracellular calcium concentration essential for the oocyte activation and so improves the fertilization rate. This technique was used by different teams thereafter (Rybouchkin *et al.*, 1997; Tesarik *et al.*, 2002; Dirican *et al.*, 2008).

Table 1 Cases published between 1994 and 2001 (ICSI without AOA)

References	Couples	Cycles	Oocytes		FR	ET	CP	Del	Outcome
			Injected	Fertilized		Fresh + frozen/ thawed			
	Nb	Nb	Nb	Nb	Min–Max	Nb	Nb	Nb	
Lundin *et al.* (1994)	1	2	28	12	38–47%	5	1	1	Twin
Liu *et al.* (1995a)	1	3	8	0	0%	0	0		
Liu *et al.* (1995a)	1	1	19	5	26%	3 + (1)	0		
Liu *et al.* (1995a)	1	1	25	1	4%	1	1	0	Mis
Liu *et al.* (1995a)	1	2	4	0	0%	0	0		
Liu *et al.* (1995a)	1	2	18	8	27–71%	6 + (4)	2	NA	EP, NA
Liu *et al.* (1995a)	1	1	5	0	0%	0	0		
Liu *et al.* (1995a)	1	1	6	0	0%	0	0		
Liu *et al.* (1995b)	1	1	15	0	0%	0	0		
Liu *et al.* (1995b)	1	2	12	0	0%	0	0		
Liu *et al.* (1995b)	1	1	3	0	0%	0	0		
Liu *et al.* (1995b)	1	2	7	0	0%	0	0		
Liu *et al.* (1995b)	1	1	6	0	0%	0	0		
Liu *et al.* (1995b)	1	1	3	0	0%	0	0		
Trokoudes *et al.* (1995)	1	1	6	3	50%	2	1	NA	NA
Bourne *et al.* (1995)	1	1	7	3	43%	2	0		
Battaglia *et al.* (1997)	1	2	32	3	7–12%	3	0		
Rybouchkin *et al.* (1997)	1	1	15	1	7%	0	0		
Khalili *et al.* (1998)	1	1	5	0	0%	0	0		
Khalili *et al.* (1998)	1	1	3	0	0%	0	0		
Khalili *et al.* (1998)	1	1	8	0	0%	0	0		
Khalili *et al.* (1998)	1	1	6	0	0%	0	0		
Kilani *et al.* (1998)	1	3	20	13	43–89%	9	1	1	Triplet
Edirisinghe *et al.* (1998)	1	1	24	2	8%	2	0		
Stone *et al.* (2000)	1	3	40	13	9–42%	7	1	1	1 Child
Viville *et al.* (2000)	1	2	12	0	0%	0	0		
Coetzee *et al.* (2001)	1	1	7	3	43%	3	1	NA	NA
Carrell *et al.* (2001)	1	1	9	1	11%	1	1	0	Mis

ICSI, intracytoplasmic sperm injection; AOA, assisted oocyte activation; Nb, number; FR, fertilization rate; ET, embryo transfer; CP, clinical pregnancy; Del, delivery; Mis, miscarriage; EP, ectopic pregnancy; NA, not available.

Chemical approaches

Calcium ionophore. The calcium ionophore was used by several teams to re-establish oocyte activation when fertilization failure occurred when injecting spermatozoa from globozoospermic patients (Eldar-Geva *et al.*, 2003). The first result of AOA with calcium ionophore in globozoospermia was described by Battaglia *et al.* (1997). In a patient with total globozoospermia, without ionophore, <10% (3/32) of the intact oocytes after ICSI fertilized. The remaining unfertilized oocytes were treated 20 h after ICSI with ionophore and another eight oocytes were treated with ionophore immediately after ICSI. A considerably higher fertilization rate (76%; 28/37) was obtained after oocyte activation. In all cases, all injected oocytes were of good quality (Battaglia *et al.*, 1997). AOA seemed to improve fertilization rate and allowed normal embryonic development.

Strontium chloride. Only one case of oocyte activation using strontium chloride (10 mM, 10 min) was published in 2012. Twenty percent of the oocytes fertilized and a twin birth occurred (Yang *et al.*, 2012).

Electrical approach. Egashira *et al.* (2009) described the first success with electric AOA in globozoospermia. The first cycle without AOA led to fertilization failure, whereas ICSI associated with AOA using electric pulse (750 V/cm during 50 microsec) succeeded. A fertilization rate of 100% was obtained, two embryos were transferred and a child birth occurred (Egashira *et al.*, 2009).

ICSI attempts associated with AOA. Thirty-two attempts were realized with ICSI + AOA in 20 couples. The global fertilization rate was 67.3% (241/358). Complete fertilization failure was observed in 6.3% of the cases. The total number of embryos transferred (ET) was ≥45 (some data were missing in some publications). Nineteen pregnancies were described (pregnancy rate per cycle: 59.4%). Twelve children were born, but the pregnancy outcome was not notified for 42.1% of the pregnancies reported (Table 5).

IMSI attempts associated with AOA. Between 2007 and 2012, five attempts of IMSI with AOA were published. These attempts corresponded to four couples among whom three benefitted from IMSI procedure alone (the three last publications in Table 6). The fertilization rate was 35.1% (13/37). Two pregnancies were described but no delivery occurred (Table 6).

Freezing–thawing cycles with or without AOA

Thirteen freezing–thawing cycles were described. Pregnancy rate was 61.5% (8/13), delivery rate was 30.8% (Table 7).

Table 2 Cases published between 2002 and 2011 (ICSI without AOA)

References	Couples	Cycles	Oocytes		FR	ET	CP	Del	Outcome
			Injected	Fertilized		Fresh + frozen/ thawed			
	Nb	Nb	Nb	Nb	Min–Max	Nb	Nb	Nb	
Nardo *et al.* (2002)	1	1	7	3	43%	3	1	1	1 Child
Nardo *et al.* (2002)	1	1	5	2	40%	2	0		
Tesarik *et al.* (2002)	1	1	8	0	0%	0	0		
Zeyneloglu *et al.* (2002)	1	2	22	7	31–33%	7	1	1	Twin
Kilani *et al.* (2004)	1	6	47	14	0–50%	14	0		
Kilani *et al.* (2004)	1	3	25	11	28–75%	8	0		
Kilani *et al.* (2004)	1	4	7	4	0–100%	4	0		
Kilani *et al.* (2004)	1	3	26	6	8–50%	6	1	0	Mis
Kilani *et al.* (2004)	1	4	24	14	28–100%	12	2	1	1 Mis, 1 Child
Schmiady *et al.* (2005)	1	3	37	2	≤11%	6	0		
Dirican *et al.* (2008)	1	1	11	1	9%	1	1	1	1 Child
Tejera *et al.* (2008)	1	1	14	5	36%	0	0		
Bechoua *et al.* (2009)	1	1	8	7	88%	2	1	1	1 Child
Bechoua *et al.* (2009)	1	2	8	0	0%	0	0		
Bechoua *et al.* (2009)	1	2	28	19	58–75%	2 + (6)	4	2	1 Mis, 1 TA, 1 Child, Twin
Banker *et al.* (2009)	1	2	20	6	29–31%	4 + (1)	1	0	Mis
Banker *et al.* (2009)	1	2	18	3	12–20%	2	1	1	1 Child
Egashira *et al.* (2009)	1	1	2	0	0%	0	0		
Sahu *et al.* (2010)	1	1	9	3	33%	2	1	1	1 Child
Huang *et al.* (2010)	1	1	19	4	21%	2 + (2)	1	1	1 Child
Zhioua *et al.* (2011)	1	4	19	1	5%	1	0		
Zhioua *et al.* (2011)	1	2	3	1	33%	1	0		
Zhioua *et al.* (2011)	1	1	10	1	10%	1	0		
Zhioua *et al.* (2011)	1	1	3	0	0%	0	0		
Harbuz *et al.* (2011)	6	7	NA	NA	13%	NA	1	NA	NA

ICSI, intracytoplasmic sperm injection; AOA, assisted oocyte activation; Nb, number; FR, fertilization rate; ET, embryo transfer; CP, clinical pregnancy; Del, delivery; Mis, miscarriage; TA, therapeutic abortion; NA, not available.

Table 3 Cases published between 2012 and April 2015 (ICSI without AOA)

References	Couples	Cycles	Oocytes		FR	ET	CP	Del	Outcome
			Injected	Fertilized		Fresh + frozen/ thawed			
	Nb	Nb	Nb	Nb	Min–Max	Nb	Nb	Nb	
Yang *et al.* (2012)	1	2	25	5	20%	2	0		
Kamiyama *et al.* (2012)	1	1	33	25	76%	NA (NA)	0		
Gatimel *et al.* (2013)	1	1	4	0	0%	0	0		
Gatimel *et al.* (2013)	1	1	9	1	11%	1	1	1	1 Child
Vozdova *et al.* (2014)	1	3	36	≥22	≥53–73%	16 (fresh + thawed)	0		
Yassine *et al.* (2015)	1	1	14	2	14%	2	1	1	1 Child
Yassine *et al.* (2015)	1	3	19	1	0–10%	0	0		
Yassine *et al.* (2015)	1	2	16	0	0%	0	0		
Yassine *et al.* (2015)	1	1	8	0	0%	0	0		
Yassine *et al.* (2015)	1	1	4	0	0%	0	0		
Yassine *et al.* (2015)	1	2	48	11	5–34%	4	NA	0	
Yassine *et al.* (2015)	1	1	10	1	10%	0	0		
Yassine *et al.* (2015)	1	1	11	1	9%	1	NA	0	
Yassine *et al.* (2015)	1	1	9	1	11%	1	1	1	1 Child
Karaca *et al.* (2015)	1	6	64	8	0–27%	7	1	0	EP
Chianese *et al.* (2015)	1	1	11	0	0%	0	0		
Chianese *et al.* (2015)	1	1	5	0	0%	0	0		
Chianese *et al.* (2015)	1	3	7	2	0–33–50%	2	0		
Zhang *et al.* (2015)	1	1	11	0	0%	0	0		

ICSI, intracytoplasmic sperm injection; AOA, assisted oocyte activation; Nb, number; FR, fertilization rate; ET, embryo transfer; CP, clinical pregnancy; EP, ectopic pregnancy; Del, delivery; NA, not available.

Table 4 IMSI attempts without AOA

References	Couples	Cycles	Oocytes		FR	ET	CP	Del	Outcome
			Injected	Fertilized		Fresh			
	Nb	Nb	Nb	Nb		Nb	Nb	Nb	
Sermondade *et al.* (2011)	1	1	5	3	60%	2	1	1	1 Child
Kashir *et al.* (2012)	1	1	6	0	0%	0	0		
Kashir *et al.* (2012)	1	1	7	6	86%	NA	1	NA	NA
Molelekwa & Kruger (2015)	1	1	NA	NA	NA	4	0		

IMSI, intracytoplasmic morphologically selected sperm injection; AOA, assisted oocyte activation; Nb, number; FR, fertilization rate; ET, embryo transfer; CP, clinical pregnancy; Del, delivery; NA, not available.

Table 5 Cases published between 1994 and April 2015 (ICSI with AOA)

AOA types and references	Couples	Cycles	Oocytes		FR	ET	CP	Del	Outcome
			Injected	Fertilized		Fresh + frozen/ thawed			
	Nb	Nb	Nb	Nb		Nb	Nb	Nb	
Mechanical AOA									
Rybouchkin *et al.* (1997)	1	1	3	0	0%	0	0		
Tesarik *et al.* (2002)	1	1	11	7	64%	3	1	1	1 Child
Tesarik *et al.* (2002)	1	1	3	2	67%	2	0		
Dirican *et al.* (2008)	1	1	6	2	33%	2	1	1	1 Child
Subtotal	4	4	23	11	47.8%	7	2	2	
AOA calcium ionophore									
Battaglia *et al.* (1997)	1	3	37	28	73–75–79%	7	0		
Rybouchkin *et al.*(1997)	1	1	5	5	100%	3	1	1	1 Child
Kim *et al.* (2001)	1	1	35	18	51%	5 (+4)	1	1	1 Child
Tesarik *et al.* (2002)	1	1	8	5	62%	2	0		
Tesarik *et al.*(2002)	1	1	4	3	75%	1	0		
Heindryckx *et al.* (2005)	6	12	167	128	77%	NA (NA)	7[a]	NA	NA
Tejera *et al.* (2008)	1	1	9	5	56%	2	1	1	1 Child
Kyono *et al.* (2009)	1	1	17	15	88%	0 (1)	1	1	1 Child
Taylor *et al.* (2010)	1	1	6	4	67%	2 (+2)	1	NA	NA
Kamiyama *et al.* (2012)	1	1	5	4	80%	2	1	1	1 Child
Karaca *et al.* (2014)	1	1	11	1	9%	1	1	1	1 Child
Karaca *et al.* (2015)	1	1	9	5	55%	2	1	1	1 Child
Subtotal	17	25	313	221	68%	–	15	–	
Mechanical AOA + ionophore									
Rybouchkin *et al.* (1997)	1	1	5	0	0%	0	0		
Electric AOA									
Egashira *et al.* (2009)	1	1	7	7	100%	2	1	1	1 Child
AOA SrCl2									
Yang *et al.* (2012)	1	1	10	2	20%	2	1	1	Twin

ICSI, intracytoplasmic sperm injection; AOA, assisted oocyte activation; Nb, number; FR, fertilization rate; ET, embryo transfer; CP, clinical pregnancy; Del, delivery; SrCl, strontium chloride; NA, not available. [a]Of which a twin pregnancy.

Table 6 IMSI attempts with AOA

References	Couples	Cycles	Oocytes		FR	ET	CP	Del	Outcome
			Injected	Fertilized		Fresh			
	Nb	Nb	Nb	Nb		Nb	Nb	Nb	
Check *et al.* (2007)	1	2	14	0	0%	0	0		
Sermondade *et al.* (2011)	1	1	6	4	66%	0	0		
Kashir *et al.* (2012)	1	1	8	6	75%	NA	2	0	2 Mis
Kashir *et al.* (2012)	1	1	9	3	33%	NA			

IMSI, intracytoplasmic morphologically selected sperm injection; AOA, assisted oocyte activation; Nb, number; FR, fertilization rate; ET, embryo transfer; CP, clinical pregnancy; Del, delivery; Mis, miscarriage; NA, not available.

DISCUSSION

This review can help professionals working in assisted reproductive medicine to manage patients with globozoospermia. Total globozoospermia is a genetic but rare pathology. With the emergence of ICSI, births were possible, nevertheless results are still not completely satisfactory. Fertilization rate with ICSI was low but once fertilization occurred, embryonic development happened properly and did not differ from embryos obtained

Table 7 Results of freezing–thawing cycles

Freezing-thawing cycles	ICSI	ICSI + AOA	IMSI	IMSI + AOA	Total
Number of cycles	8	4	0	1	13
Number ET	Data not available (1–4 embryos transferred per cycle)				
Pregnancy rate per cycle	37.5% (3/8)	100% (4/4)	–	100% (1/1)	61.5% (8/13)
Outcomes NA	0	2	–	0	15.4% (2/13)
Delivery rate per cycle	25% (2/8)	50% (2/4)	–	0	30.8% (4/13)
Number of children born	Twin+ 1 Child	2	–	0	5
Other outcome	1 TA	–	–	1 Mis	1 TA, 1 Mis

ICSI, intracytoplasmic sperm injection; IMSI, intracytoplasmic morphologically selected sperm injection; AOA, assisted oocyte activation; ET, embryo transfer; NA, not available; TA, therapeutic abortion; Mis, miscarriage.

when other male indications were involved. A detailed assessment is required in each case to evaluate the likelihood of having a child, to offer an adequate therapeutic strategy (Fig. 4) and to give all the information needed by the couples exposed to this type of infertility. ICSI or IMSI must be tried, with injection of selected spermatozoon: the one with the most oval form. Genetic counseling should be offered and strongly recommended to evaluate the risk of transmitting a chromosomal disequilibrium or a genetic mutation for couples undergoing microinjection. No recurrent constitutional chromosomal anomaly has been detected in globozoospermia yet. However, disruption in chromatin condensation, increase in DNA fragmentation, and aneuploidy (markers of molecular deterioration during spermiogenesis) have been reported. *DPY19L2* is the principal gene involved in globozoospermia. In patients for whom genetic explorations were negative, other genetic etiologies should be considered even if the genetic diagnosis does not yet provide any therapeutic indication or clear prognosis (Coutton *et al.*, 2015).

This review has limitations. Particularly, cases related to globozoospermia published during the last 20 years are few. Moreover, unlike pregnancies and births, the negative results were not published. This can lead to an underestimation of fertilization failure rate and an overestimation of success rate. Data on couples are incomplete, data on attempts imprecise, and pregnancy issues not detailed, which makes the analysis of the published cases difficult.

The capacity of round-headed spermatozoa to fertilize oocytes varies between patients. This review confirms that fertilization rate is less important to that obtained in other indications of ICSI. When considering all the techniques, the overall fertilization rate found for the 172 attempts was 35.2%. Total fertilization failure was reported in 27.9% of the cases. After AOA, three attempts ended in fertilization failure: one in ICSI with mechanical AOA, one with mechanical AOA + calcium ionophore, the third one with IMSI. Nevertheless, differences were really important according to the use or not of AOA which could improve considerably the pregnancy rate. Excluding all IMSI attempts

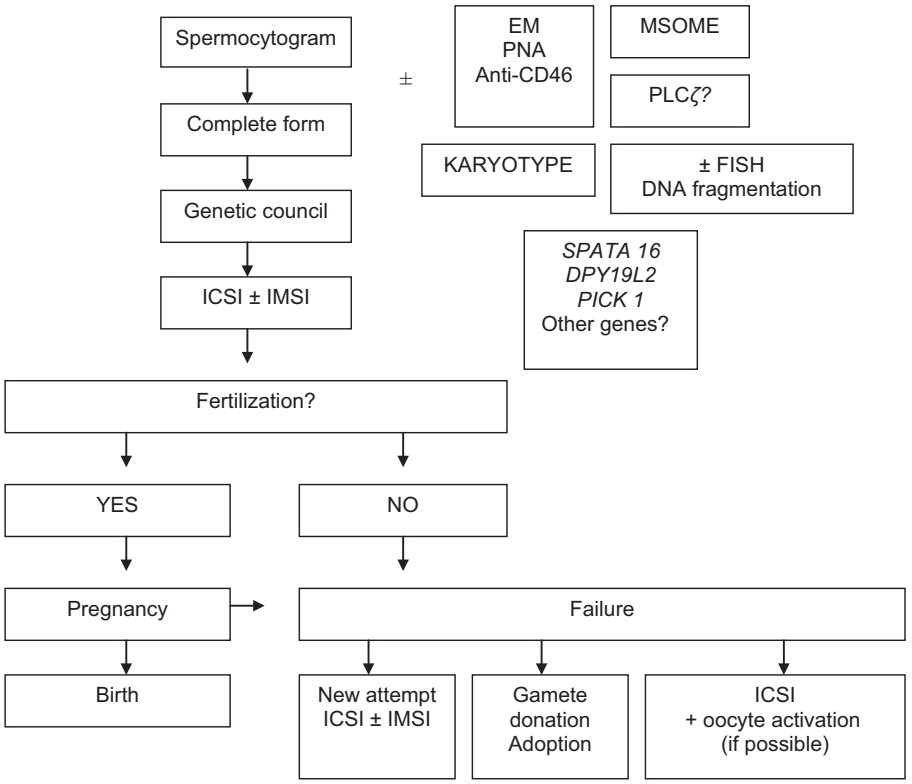


Figure 4 How to manage globozoospermic patients? ICSI, intracytoplasmic sperm injection; IMSI, morphologically selected sperm injection; EM, electron microscopy; PNA, peanut agglutinin, used to assess the sperm acrosomal status; CD46, cluster of differentiation 46, a marker of the inner acrosomal membrane; MSOME, motile sperm organelle morphological examination; PLCζ, phospholipase C zeta; FISH, fluorescence *in situ* hybridization; DNA, deoxyribonucleic acid; SPATA 16, spermatogenesis associated 16; DPY19L2, dpy-19-like 2 (*C. elegans*); Pick 1, protein interacting with C kinase 1.

(n = 9), when oocyte activation was associated with ICSI, the data indicated that: (i) the fertilization rate was higher (67.3% vs. 24.3%), (ii) the fertilization rate failure was lower (6.3% vs. 29.8%), (iii) the pregnancy rate per cycle was higher (59.4% vs. 22.1%), (iv) the delivery rate per cycle was higher despite all missing data on pregnancy issues in the ICSI + AOA group.

Unfortunately, AOA faces different problems. There is a partial efficacy of oocyte activation reestablishment, even if fertilization rate after AOA (around 70%) approaches the rate observed in other ICSI indications. But cases of total fertilization failure are more frequent (6.3% vs. 2–3% in other ICSI indications) (Mahutte & Arici, 2003; Palermo *et al.*, 2009). Oocyte activation is not always possible in total globozoospermia but we cannot exclude feminine factors or other sperm anomalies such as DNA fragmentation or aneuploidy. However, it has not been clearly established that globozoospermia is associated with a high rate of aneuploidy or DNA fragmentation in spermatozoa, as comparable rates were found in infertile patients, notably with oligoasthenoteratozoospermia, independently of the origins. Thus, these gametes can be used in ART, from a genetic aspect (Perrin *et al.*, 2013; De Braekeleer *et al.*, 2015) without performing necessarily FISH or DNA fragmentation. The hypothesis is that success with ICSI comes from the presence of a small acrosomal residue which can express enough factors needed for fertilization (Gatimel *et al.*, 2013). When acrosomal residue exists, IMSI could replace ICSI for a better selection of spermatozoa. Other publications on this topic are controversial. Indeed, after the use of IMSI without AOA and the absence of a rudimentary acrosomal structure, a normal fertilization rate was described (Kashir *et al.*, 2012). As data on IMSI are scarce, it seems difficult to draw a conclusion on the effectiveness of this technique in globozoospermia.

Despite results in favor of the use of AOA, attempts with AOA represent only 19.6% of all ICSI attempts published. This is probably linked to the potential teratogenic and mutagenic effects of the molecules used. Hence, their utilization is still limited, even forbidden in countries such as in France. However, most of the studies do not show any toxic effects of calcium ionophore on embryonic development or on pregnancy and the children born were in good health (no malformation or particular neonatal complication was observed) (Kim *et al.*, 2001; Heindryckx *et al.*, 2008; Tejera *et al.*, 2008; Kyono *et al.*, 2009). But the number of child births is limited, and no study can conclude on the harmless effect of AOA. Thus, calcium ionophore must be used with caution. After multiple failures, the only way to conceive is to resort to gamete donation. In the future, the use of recombinant PLCζ could be a promising biological tool to overcome fertilization failure (Amdani *et al.*, 2013).

CONCLUSION

For the past 20 years, deliveries and births have been published but the exact physiopathology of globozoospermia is still unknown. The globozoospermia literature in general lacks a standard definition of globozoospermia and there is a potential misclassification between total and partial globozoospermia. Reproductive medicine centers can offer appropriate therapeutical solutions to optimize fertilization rates. In many countries, oocyte activation can initiate the fertilization process and can be used as a first-line therapy before sperm donation could be envisaged. Hopefully, other effective and safe treatments will become available in a near future. Until then, the review of the literature we performed provides hope regarding the success of either ICSI or IMSI procedures for couples seeking babies.

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AUTHORS' CONTRIBUTION

LCD and SD performed the research and analyzed the data. LCD wrote the manuscript. SB and CJ revised critically the manuscript.

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