

Assisted oocyte activation overcomes fertilization failure in globozoospermic patients regardless of the *DPY19L2* status

P. Kuentz^{1,2,†}, F. Vanden Meerschaut^{3,†}, E. Ellnati¹, M.H. Nasr-Esfahani⁴, T. Gurgan⁵, N. Iqbal⁶, F. Carré-Pigeon⁷, F. Brugnon⁸, S.A. Gitlin⁹, J. Velez de la Calle¹⁰, Z. Kilani¹¹, P. De Sutter³, and S. Viville^{1,12,*}

¹Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), Institut National de Santé et de Recherche Médicale (INSERM) U964, Centre National de Recherche Scientifique (CNRS) UMR1704, Université de Strasbourg, 67404 Illkirch, France ²Service de Génétique Biologique Histologie du Développement et de la Reproduction (CECOS Franche-Comté Bourgogne), Centre Hospitalier Universitaire, F-25000 Besançon, France ³Department for Reproductive Medicine, University Hospital Ghent, 9000 Ghent, Belgium ⁴Department of Reproduction and Development, Reproductive Biomedicine Center, Royan Institute for Animal Biotechnology, ACECR, Isfahan, Iran ⁵Clinic Women Health, Infertility and IVF Center, Ankara, Turkey ⁶King Faisal Specialist Hospital and Research Center, 21499 Jeddah, Kingdom of Saudi Arabia ⁷Service de Gynécologie-Obstétrique, CHU Reims, Institut mère-enfant Alix de Champagne, 45 rue Cognacq-Jay, F-51092 Reims, France ⁸Laboratoire de Biologie de la Reproduction, Université Clermont I, UFR Médecine, EA 975, F-63001 Clermont Ferrand Cedex 1, France ⁹Department of Obstetrics and Gynecology, The Jones Institute for Reproductive Medicine, Eastern Virginia Medical School, Norfolk, 23507 VA, USA ¹⁰Unité FIV, Clinique Pasteur, 29200 Brest, France ¹¹Farah Hospital, 11183 Amman, Jordan ¹²Centre Hospitalier Universitaire, F-67000 Strasbourg, France

*Correspondence address. Tel: +33-3-88-65-33-22; Fax: +33-3-88-65-32-01; E-mail: viville@igbmc.fr

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STUDY QUESTION: Does *DPY19L2* status influence intracytoplasmic sperm injection (ICSI) outcomes with or without assisted oocyte activation (AOA)?

SUMMARY ANSWER: *DPY19L2* mutations have no major impact on ICSI outcomes in globozoospermic patients.

WHAT IS KNOWN ALREADY: Globozoospermia is a rare and severe teratozoospermia characterized by round-headed spermatozoa lacking an acrosome. Recently, it has been shown that *DPY19L2* mutations can be found in a vast majority of, but not all, globozoospermic patients (66.7%). These patients suffer from primary infertility due to a sperm-related oocyte activation deficiency secondary to the absence of an acrosome that can be overcome by the application of AOA.

STUDY DESIGN, SIZE, DURATION: Cohort study, retrospective, 34 patients, 83 cycles.

MATERIALS, SETTING, METHODS: Clinical and biologic data were collected from 29 patients mutated for *DPY19L2* and 5 non-mutated patients. In total, 35 ICSI cycles using AOA and 48 conventional ICSI cycles were included in the analysis. Patients were divided into groups according to whether or not they were mutated for *DPY19L2* and whether or not they received AOA.

MAIN RESULTS AND THE ROLE OF CHANCE: Regardless of the presence of a *DPY19L2* mutation, the fertilization rates with AOA are restored to normal when compared with conventional ICSI in our cohort of globozoospermic patients. Also, when performing ICSI plus AOA, both mutated and non-mutated cases have similar positive hCG rates, ongoing pregnancy rates and live birth rates per transfer. On the contrary, the fertilization rate in globozoospermic patients using conventional ICSI is correlated with the presence of a *DPY19L2* mutation, with slightly better, although still very low, fertilization rates in patients carrying a *DPY19L2* mutation. Nevertheless, when performing conventional ICSI, both mutated and non-mutated cases have similar very low positive hCG rates, ongoing pregnancy rates and live birth rates per transfer.

LIMITATIONS: A limitation of this study is the low number of included non-mutated cases.

† Equal first authors.

WIDER IMPLICATIONS OF THE FINDINGS: We propose a pathway for the clinical management of globozoospermic patients depending on the phenotype that includes several diagnostic and therapeutic steps.

STUDY FUNDING/COMPETING INTEREST(S): None.

Key words: globozoospermia / failed fertilization / ICSI / assisted oocyte activation / *DPY19L2* mutation

Introduction

Globozoospermia is one of the most often encountered forms of homogenous teratozoospermia. Nevertheless, it remains a rare condition, with an incidence of less than 0.1% (Dam *et al.*, 2007a). Total globozoospermia is diagnosed by the presence of 100% round-headed spermatozoa lacking an acrosome that is one of the major organelles involved in the fertilization process (Dam *et al.*, 2007a). The acrosome is essential for the spermatozoa to penetrate the zona pellucida, to reach the oocyte cytoplasmic membrane and to deliver the oocyte activation factor, suggested to be phospholipase C zeta (PLC ζ), into the oocytes cytosol (Saunders *et al.*, 2002; Ikawa *et al.*, 2010). Apart from the severe teratozoospermia, globozoospermic patients do not present any other symptom. Therefore, they are usually recruited via fertility centers. Because the spermatozoa of globozoospermic patients do not present chromosomal abnormalities (Carrell *et al.*, 1999; Viville *et al.*, 2000; Machev *et al.*, 2005), it is justified to propose intracytoplasmic sperm injection (ICSI). Unfortunately, the fertilization and birth rates obtained following conventional ICSI are extremely low in globozoospermic patients (Coetzee *et al.*, 2001; Kilani *et al.*, 2004; Dirican *et al.*, 2008; Banker *et al.*, 2009; Bechoua *et al.*, 2009). Recent reports suggest that globozoospermia is associated with a significant reduction in or even an absence of the sperm factor PLC ζ and perinuclear theca proteins (Heytens *et al.*, 2009; Oko and Sutovsky, 2009; Alvarez Sedó *et al.*, 2012). This might explain the very low or absent fertilization in globozoospermic patients because PLC ζ is suggested to be the main physiologic factor responsible for oocyte activation (Kashir *et al.*, 2010). Also, the perinuclear theca has been related to acrosomal development in mammalian species (Oko and Sutovsky, 2009).

The mouse oocyte activation test (MOAT), which is a heterologous ICSI model, was designed to assess the oocyte activation capacity of human spermatozoa from couples suffering from failed or low fertilization following ICSI (Rybouchkin *et al.*, 1996; Heindryckx *et al.*, 2005). For globozoospermic patients, the MOAT test has revealed a clear sperm-related activation deficiency, with mouse oocyte activation percentages usually below 20% (Heindryckx *et al.*, 2005).

To overcome oocyte activation failure and failed fertilization following ICSI, assisted oocyte activation (AOA) has been performed worldwide for more than a decade. Several activating agents have been shown to be able to overcome fertilization failure, such as electrical pulses, strontium chloride and calcium ionophores (Yanagida *et al.*, 1999, 2006; Eldar-Geva *et al.*, 2003; Chi *et al.*, 2004; Heindryckx *et al.*, 2005). These artificial activation agents cause a single prolonged calcium rise, but fail to elicit physiologic patterns of calcium release. Despite this, many healthy pregnancies were obtained so far (Rybouchkin *et al.*, 1996; Yanagida *et al.*, 1999; Tesarik *et al.*, 2002;

Araki *et al.*, 2004; Heindryckx *et al.*, 2005, 2008; Nasr-Esfahani *et al.*, 2010; Montag *et al.*, 2012; Vanden Meerschaut *et al.*, 2012). The most common type of AOA implies the use of a calcium ionophore that has been shown to improve fertilization and pregnancy rates (Taylor *et al.*, 2010). Also, in several globozoospermic patients, such a calcium ionophore activation treatment has been previously used successfully to restore fertilization and pregnancy rates (Heindryckx *et al.*, 2005, 2008). The latter achievements in globozoospermic patients are sound because AOA is known to be more efficient to overcome sperm-related activation deficiencies, rather than suspected oocyte-related activation deficiencies (Vanden Meerschaut *et al.*, 2012).

Descriptions of familial cases of globozoospermia led to the hypothesis of a possible genetic defect responsible for this phenotype. While studying family cases, we identified mutations in two genes, *SPATA16* and *DPY19L2* (Dam *et al.*, 2007b; Koscinski *et al.*, 2011). By analyzing an Ashkenazi Jewish family with three affected brothers, we identified a splice mutation in *SPATA16* leading to the deletion of exon 4. *SPATA16* is a protein involved in intracytoplasmic vesicle trafficking from the Golgi apparatus to the nascent acrosome. No other mutation was identified in a cohort of 21 patients (Dam *et al.*, 2007b). Nevertheless, by analyzing a consanguineous Jordanian family, we identified a large deletion (200 kb) encompassing the whole *DPY19L2* gene that was also found in three additional unrelated patients (Koscinski *et al.*, 2011). By studying a larger cohort of 64 patients including 54 genetically independent globozoospermic patients, we found that 36 of them were mutated for *DPY19L2* (66.7%). Out of these 36 mutated patients, 69.4% are homozygous deleted, 19.4% are heterozygous composite and 11.1% showed a homozygous point mutation (Elinati *et al.*, 2012).

The mechanism underlying the deletion is due to a non-allelic homologous recombination (NAHR) between two low copy repeats that share 96.5% identity and flank the *DPY19L2* locus. We characterized a total of nine breakpoints (BP) for the *DPY19L2* NAHR-driven deletion that clustered in two recombination hotspots (Elinati *et al.*, 2012). Therefore, globozoospermia can be considered as a new genomic disorder. The facts that the same BPs are shared by patients from completely different regions and that patients from the same country can show different BPs tend to exclude any founder effect, even a recent one, and strongly suggest that the deletion results from recurrent events linked to the specific genomic architectural feature of this locus. *DPY19L2*-mediated globozoospermia can also be due to mutations affecting its coding sequence (Coutton *et al.*, 2012; Elinati *et al.*, 2012).

Using *Dpy19l2* knockout mice, Pierre *et al.* (2012) demonstrated that the protein is expressed predominantly in spermatids with a very specific localization restricted to the inner nuclear membrane

facing the acrosomal vesicle. The absence of Dpy19L2 leads to the destabilization of both the nuclear dense lamina and the junction between the acroplaxome and the nuclear envelope. Consequently, the acrosome and the manchette fail to be linked to the nucleus leading to the disruption of vesicular trafficking, failure of sperm nuclear shaping and eventually to the elimination of the unbound acrosomal vesicle.

In uncapacitated human spermatozoa, the oocyte activation factor, suggested to be PLC ζ , is thought to be localized predominantly not only in the equatorial region but also in the acrosomal or post-acrosomal region (Saunders et al., 2002; Grasa et al., 2008; Ikawa et al., 2010). This pattern of localization is maintained following capacitation and ionophore treatment. Therefore, the DPY19L2 mutation indirectly prevents oocyte activation by preventing normal acrosome formation.

From our cohort of 64 globozoospermic patients, we were able to collect data concerning ICSI attempts for 34 couples and 83 cycles. All globozoospermic patients presented with primary infertility and were treated with ICSI with or without AOA. Male and female baseline characteristics were collected as well as cycle parameters.

The main objective of this retrospective cohort study was to confirm the efficiency of ICSI combined with AOA in patients with a sperm-related activation deficiency due to globozoospermia. We compared the fertilization and pregnancy outcomes of ICSI with or without AOA in globozoospermic patients and linked this with their DPY19L2 mutation status, to set a clinical guideline for such patients. In addition, we analyzed the semen parameters of DPY19L2-mutated patients to assess DPY19L2 function.

Materials and Methods

Patients

From our former cohort of 64 globozoospermic patients, we aimed to collect data concerning their fertility history and ICSI attempts. These data were eventually available for 34 patients of the original cohort. In total, 83 ICSI cycles could be included in the analysis. Patients were divided into 4 groups depending on their DPY19L2 status and the use or not of AOA: (i) DPY19L2+ and AOA+ (16 patients and 31 cycles), (ii) DPY19L2- and AOA+ (4 patients and 4 cycles), (iii) DPY19L2+ and AOA- (14 patients and 42 cycles) and (iv) DPY19L2- and AOA- (2 patients and 6 cycles). Two patients had cycles with and without AOA.

Data retrieval

Because the original cohort of 64 patients were recruited from 9 fertility centers, all the involved physicians were asked to fill out a form regarding clinical and biologic data of their patients who were previously selected and tested for mutations of DPY19L2 and SPATA16. Apart from fertility history and outcome of ICSI attempts, data were asked concerning comorbidity and lifestyle (smoking habits, alcohol consumption and BMI) to eliminate potential confusing factors. Unfortunately, for most of the cases, the latter provided data were not exhaustive. The completed forms were sent back by e-mail to the main investigators.

ICSI, AOA and outcome parameters

Conventional ICSI was performed as described originally by Palermo et al. (1992). For AOA, all included fertility centers used a calcium ionophore. ICSI-AOA was performed at least 6 h after oocyte retrieval, as previously

described by Heindryckx et al. (2008). Briefly, a spermatozoon was injected into the oocyte using conventional ICSI, together with a small amount of 0.1 mol/l CaCl₂ (corresponding to the diameter of the oocyte). Thereafter, the oocytes were incubated for 30 min at 37°C in a 6% CO₂ air atmosphere. Next, the oocytes were exposed 2-fold to a calcium ionophore (10 μ M Ionomycin, I9657, Sigma-Aldrich, Bornem, Belgium) for 10 min with 30 min incubation in between. Following each ionophore exposure, the oocytes were washed thoroughly with Cook's Cleavage medium (Cook Ireland Ltd, Limerick, Ireland) and were incubated again. Fertilization (the presence of two pronuclei) was evaluated 16 h after the ICSI procedure. Fertilization rate was defined by the number of normally fertilized oocytes when compared with the total number of injected and surviving MII oocytes.

Small differences between the fertility centers regarding ovarian stimulation protocols, laboratory media and procedures and culture conditions could not be excluded. The main outcome parameters were fertilization rate (defined by the amount of 2pn embryos over the total amount of injected mature oocytes 18 h post ICSI), positive hCG rate per transfer (defined by a positive hCG level 2 weeks following transfer), clinical pregnancy rate per transfer (defined by the presence of one or more heartbeats on ultrasound at 7 weeks of gestation) and life birth rate per transfer.

Semen analysis

Patients were selected by fertility centers through a semen analysis performed according to the World Health Organization's (2010) recommendations (Cooper et al., 2010). Globozoospermia was diagnosed using a spermcytogram after a Harris–Shorr coloration (presence of round-headed spermatozoa). In one case, electron microscopy was performed and globozoospermia was confirmed (data not shown).

Statistical analysis

To compare proportions between two groups (fertilization, pregnancy and birth rates), the Chi-square test or Fisher's Exact test was used. The Student's t-test was used to compare means between two groups (age, amount of mature oocytes and transferred embryos, sperm concentration and motility). In case equal variances were not assumed, alternatively, the non-parametric Mann–Whitney U-test was chosen (age). All statistical tests were performed using the statistical package SPSS (SPSS® Statistics 19, IBM corp., NY, USA).

Results

Demographics and fertility history

Clinical and biologic data were returned for 34 out of 64 globozoospermic patients (response rate 53.0%). All of them were formerly included in the mutation analysis cohort described by Elinati et al. (2012). Recruited patients are Caucasian (10 out of 34, 29.4%), Persian (11 out of 34, 32.4%) or Arabic (13 out of 34, 38.2%). All couples presented with primary infertility. Eight out of 34 men (23.5%) reported a history of consanguinity. Almost all men (32 out of 34, 94.1%) were diagnosed with total globozoospermia (100% round-headed spermatozoa), whereas the remaining 2 were diagnosed with partial globozoospermia, defined as the presence of both normal-shaped and round-headed spermatozoa in the semen sample. Sperm concentration ranged from severe oligozoospermia to normal sperm counts (range: 1–185 million/ml; mean: 53.4 \pm 41.6 million/ml).

DPY19L2 mutation analysis

Twenty-nine out of 34 (85.3%) patients were mutated for *DPY19L2*, which confirms the major role of this gene in globozoospermia. However, the prevalence of *DPY19L2* mutation might be overestimated in this cohort because we had to exclude several patients who were not mutated for this gene because for them, no clinical information was available. No patient was mutated for *SPATA16*.

Fertility treatment and outcome

For each couple, 1–5 ICSI cycles were included in the analysis (mean: 2.4 ± 1.28 ICSI cycles per couple). Patients were divided into four groups to compare the efficiency of AOA and the effect of *DPY19L2* mutation on the clinical outcome: (i) *DPY19L2+* and AOA+, (ii) *DPY19L2-* and AOA+, (iii) *DPY19L2+* and AOA– and (iv) *DPY19L2-* and AOA–. The mean male age at treatment was 32.6 ± 5.30 years (ranging from 19 to 43 years), and the mean female age was 29.2 ± 4.92 years (ranging from 17 to 39 years). The mean amount of MII oocytes retrieved per cycle was 9.6 ± 5.53 (ranging from 1 to 29). The mean amount of transferred embryos per transfer was 2.0 ± 1.0 (ranging from 1 to 5). Table 1 shows the numbers for each group individually. The average male and female age, the amount of MII oocytes and the mean number of transferred embryos were compared between the groups. One

difference was found, namely the amount of MII oocytes retrieved per cycle between groups (i) and (iii) (11.0 ± 6.4 versus 8.1 ± 4.3 ; $P = 0.026$). However, a fair amount of MII oocytes were available for all patients in the different groups; therefore, the influence of this difference on the results is probably absent or minor.

Semen parameters were compared between the different groups. The semen concentration is significantly lower in group (i) compared with group (ii) (41 ± 22.3 versus 95 ± 30.0 ; $P < 0.001$). This result is not confirmed by comparison between *DPY19L2*-mutated patients versus non-mutated patients regardless of AOA treatment, even if there is a trend that semen concentration is lower in *DPY19L2*-mutated patients (mean semen concentration = 52 ± 40.9 million/ml versus 76 ± 49.0 million/ml; $P = 0.100$).

The number of patients is very low in groups (ii) and (iv) (four and two patients, respectively) due to the high frequency of *DPY19L2* mutations in globozoospermic patients. Thus, comparisons implying these two groups are to be handled with care.

Considering only *DPY19L2*-mutated patients by comparing groups (i) and (iii), AOA allowed to normalize fertilization (64.4 versus 31.3%, $P < 0.001$) and positive hCG rates (43.8 versus 18.8%, $P = 0.03$) when compared with conventional ICSI. Both the ongoing pregnancy (34.4 versus 18.8%, $P = 0.181$) and the live birth rate per transfer (31.3 versus 15.6%, $P = 0.160$) were doubled following AOA treatment (Table 2), but this difference did not reach statistical significance.

Table 1 Initial characteristics of the included patients.

	(i) <i>DPY19L2+</i> and AOA+	(ii) <i>DPY19L2-</i> and AOA+	(iii) <i>DPY19L2+</i> and AOA–	(iv) <i>DPY19L2-</i> and AOA–
Number of patients	16	4	14	2
Mean number of cycles per patient \pm SD	2 ± 1.4	1 ± 0.0	3 ± 0.9	3 ± 0.0
Range	1–5	1–1	1–4	3–3
Mean male age \pm SD	32.3 ± 4.2	32.5 ± 10.8	31.8 ± 4.7	35.5 ± 9.2
95% CI	30.3–34.3	22.0–43	29.1–34.5	22.8–48.2
Range	22–39	19–43	24–40	29–42
Mean female age \pm SD	29.7 ± 5.1	30.8 ± 3.6	27.2 ± 4.4	32.0 ± 5.7
95% CI	27.1–32.3	27.3–34.3	24.6–29.8	24.2–29.8
Range	22–39	28–36	17–32	28–36
Mean amount of MII oocytes per cycle \pm SD	$11.0^* \pm 6.4$	10.8 ± 5.4	$8.1^* \pm 4.3$	11.0 ± 6.6
95% CI	8.8–13.2	5.5–16.1	6.8–9.4	5.7–16.3
Range	3–29	5–18	1–15	5–20
Mean amount of transferred embryos per transfer \pm SD	1.8 ± 0.8	2.0 ± 0.8	2.4 ± 1.1	1.2 ± 0.4
95% CI	1.5–2.1	1.2–2.8	2.0–2.8	0.8–1.6
Range	1–4	1–3	1–5	1–2
Semen concentration in 0.10^6 /ml \pm SD	$41.2^{**} \pm 22.3$	$95.0^{**} \pm 30.0$	60.1 ± 50.7	2.2
95% CI	33.5–48.9	65.6–124.4	43.1–77.1	
Range	4.4–80	80–140	1–185	
A + B semen motility in % \pm SD	29.7 ± 16.0	13.3 ± 11.6	27.1 ± 25.7	18.3 ± 4.5
95% CI	24.1–35.3	1.9–24.7	18.4–35.8	13.2–23.4
Range	10–60	0–28	0–71	14–23

AOA, assisted oocyte activation; CI, confidence interval.

* $P < 0.05$ (Student's t-test).

** $P < 0.001$ (Student's t-test).

Table II ICSI with or without AOA outcomes.

	(i) <i>DPY19L2+</i> and AOA+	(ii) <i>DPY19L2-</i> and AOA+	(iii) <i>DPY19L2+</i> and AOA-	(iv) <i>DPY19L2-</i> and AOA-
Fertilization rate (2pn/MII, %)	64.4%* (219 out of 340)	72.1% (31 out of 43)	31.3%*/** (107 out of 342)	10.6%** (7 out of 66)
Positive hCG rate per transfer (%)	43.8%*** (14 out of 32)	50.0% (2 out of 4)	18.8%*** (6 out of 32)	0.0% (0 out of 5)
Ongoing pregnancy rate per transfer (%)	34.4% (11 out of 32)	50.0% (2 out of 4)	18.8% (6 out of 32)	0.0% (0 out of 5)
Live birth rate per transfer (%)	31.3% (10 out of 32)	25.0% (1 out of 4)	15.6% (5 out of 32)	0.0% (0 out of 5)

AOA, assisted oocyte activation.

* $P < 0.001$ (Chi-square test).** $P < 0.001$ (Chi-square test).*** $P < 0.05$ (Chi-square test).

When comparing groups (i) and (ii), no association was found between the presence of *DPY19L2* mutation and the fertilization rate following AOA treatment (64.4 versus 72.1%, respectively, $P = 0.319$). The fertilization rates in our globozoospermic cohort are comparable with the rates obtained with conventional ICSI in non-globozoospermic patients (Palermo et al., 2009). Identically, positive hCG (43.8 versus 50%, $P = 1$), ongoing pregnancy (34.4 versus 50%, $P = 0.609$) and live birth rates per transfer (31.3 versus 25.0%, $P = 1$) were comparable between *DPY19L2*-mutated and non-mutated patients treated with AOA (Table 2).

On the contrary, a *DPY19L2* mutation is associated with higher fertilization rates using ICSI without AOA (conventional ICSI) in globozoospermic patients (31.3 versus 10.6%, $P < 0.001$). Nevertheless, positive hCG rates (18.8 versus 0%, $P = 0.281$), ongoing pregnancy rates (18.8 versus 0%, $P = 0.281$) and live birth rates per transfer (15.63 versus 0%, $P = 0.341$) were low and not different between *DPY19L2*-mutated and non-mutated patients when performing conventional ICSI (Table 2).

Two patients belonging to group (iii) (*DPY19L2+* and AOA-) were treated with intracytoplasmic morphologically selected sperm injection (IMSI) that implies the use of a powerful microscopy system to allow for the morphologic selection of the 'good' spermatozoon with strict criteria (Bartoov et al., 2002). Both patients underwent three IMSI cycles. One patient had two babies, and the other patient did not conceive. Importantly, the first patient was diagnosed with partial globozoospermia (69–76% of acrosomeless round-headed spermatozoa).

Discussion

Our former study allowed us to calculate an accurate prevalence of *DPY19L2* mutation involvement in globozoospermia, making the latter a new genomic disorder (Elinati et al., 2012). We analyzed a cohort of 54 genetically independent globozoospermic patients and found that 36 of them were mutated for *DPY19L2* (66.7%). Despite the identification of subtle mutations, the most frequent alteration remains the deletion of the whole gene. Indeed, in more than two-thirds (69%) of our patients, genes are homozygously deleted which also suggests that in one-third of the cases, a search for point mutations is justified. Globozoospermia is, thus, characterized by genetic heterogeneity which implies that unknown genes in some cases are neither secondary to a *DPY19L2* mutation nor a *SPATA16* mutation.

In case of globozoospermia, ICSI combined with AOA has proven to be a very efficient fertilization technique when compared with conventional ICSI (Heindryckx et al., 2005, 2008). This study confirms the good results obtained when ICSI is combined with AOA for treating globozoospermic patients regardless of their mutation status. Indeed, in our cohort, the fertilization rates following AOA are restored to normal when compared with conventional ICSI in both mutated and non-mutated patients. The average clinical pregnancy rate and live birth rate per transfer are also strikingly higher with AOA, but there is no statistical difference, even if it is close to significance. This is most probably due to the limited size of the cohort. Nevertheless, it is the largest cohort analyzed so far, and the increases in pregnancy and live birth rates have a positive clinical impact.

To our knowledge, this is the first study to link the efficiency of ICSI with or without AOA with the presence of a *DPY19L2* mutation in a large cohort of globozoospermic patients. The above-mentioned results are confirmed by the comparison between exclusively *DPY19L2*-mutated cases receiving conventional ICSI or ICSI combined with AOA. This comparison allowed us to assess the AOA efficiency in patients with a known *DPY19L2* mutation. In these patients, the fertilization rate is more than doubled using AOA. The same was true for clinical pregnancy and live birth rates per transfer, but still there was no statistical difference. Again, there is a clear indication in favor of treatment with AOA, which makes the result clinically relevant.

A mutation for *DPY19L2* does not seem to lead to a negative prognosis. Indeed, results are comparable between mutated and non-mutated cases. Interestingly, two Caucasian brothers mutated for *DPY19L2* were successfully treated with ICSI-AOA (one healthy child for each brother). In the same manner, two out of five brothers from a Jordanian family mutated for *DPY19L2* had a healthy child following conventional ICSI.

There is an indication that semen concentration is lower in *DPY19L2* mutated patients, even if there is no statistical difference, which is consistent with observations made by others (Coutton et al., 2012; Pierre et al., 2012). Still, most of the patients mutated for *DPY19L2* have normal semen concentrations in contrast with patients mutated for *SPATA16*, which suggests different functions of these two genes apart from acrosome biogenesis. In other words, *SPATA16* may be implied in spermatogenesis whereas *DPY19L2* may only be implied in spermiogenesis, which has been confirmed recently (Pierre et al., 2012). Unfortunately, we could not collect clinical data, outside the spermogram, from any patient mutated for *SPATA16*, which is

consistent with the rarity of this molecular etiology in this phenotype. Therefore, we were unable to compare results between these two different etiologies.

One patient mutated for *DPY19L2* and diagnosed with partial globozoospermia had two babies in three cycles with the so-called IMSI technique. This result is consistent with previous work by Sermondade *et al.* (2011) and confirms that IMSI can be helpful in partial globozoospermia. Indeed, it allows selecting for the absence of vacuoles and the presence of a (small) bud of acrosome. The fertilization rate is normal and comparable with or without AOA (Sermondade *et al.*, 2011). For these patients, it is thus possible to use IMSI, even if sperm selection is particularly tedious and time-consuming.

The prevalence of a heterozygous deletion in the surrounding region of *DPY19L2* is 1/222 (Koscinski *et al.*, 2011). Consequently, if we assist with artificial reproductive technologies in couples with men known to be mutated for *DPY19L2* without testing the woman, they are at an 1/444 risk for having a boy with the same mutation as his father. Heterozygous individuals can be detected by PCR testing. Therefore, if the woman is tested and not carrying a heterozygous mutation, the residual risk for a boy to be globozoospermic is extremely low corresponding to the incidence of neo-mutation. On the contrary, if the woman is tested and diagnosed to be a heterozygous carrier of a *DPY19L2* mutation, the risk is 1 in 2 to have an affected male child.

In conclusion, we propose a clinical pathway for globozoospermic patients depending on the phenotype, which is known to have an important impact on the therapeutic approach (Fig. 1). In case of complete or almost complete globozoospermia, patients should get genetic advice with testing for (i) *DPY19L2* homozygous deletion, (ii) *DPY19L2* point mutation and (iii) *SPATA16* mutation if no mutation is found in *DPY19L2*. The therapeutic approach should imply AOA, since it has proven its efficiency in sperm-related activation deficiency due to globozoospermia. In case of partial globozoospermia, when normal spermatozoa can be found, the therapeutic approach should imply IMSI instead of chemical AOA. Although the latter is cheaper and less technical, it implies the use of a non-physiologic agent which is still not considered to be a routine technique. The clinical use of calcium ionophores in assisted reproduction is limited by insufficient knowledge about their potential toxic effect on oocytes and embryos. However, to date, all pregnancies that went to term after AOA application gave rise to healthy infants with no major or minor malformations at birth (Heindryckx *et al.*, 2008). Nevertheless, calcium ionophore treatment should be applied cautiously and for well-defined indications (Vanden Meerschaut *et al.*, 2012) because the clinical experience is too limited to exclude cytotoxic, teratogenic and mutagenic effects on embryos and offspring.

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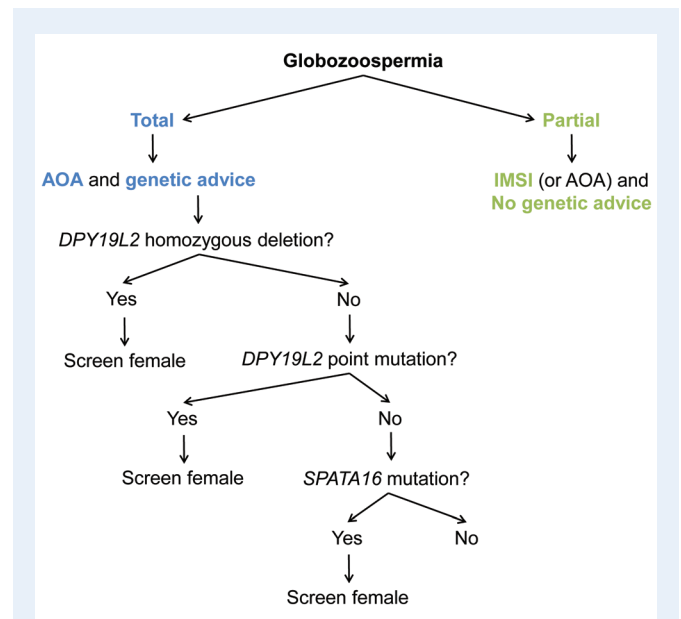


Figure 1 Clinical pathway for the management of patients diagnosed with globozoospermia. In case of complete globozoospermia, patients should be advised to be tested for (i) *DPY19L2* homozygous deletion, (ii) *DPY19L2* point mutation and (iii) *SPATA16* mutation. In case a mutation is found, the female has to be offered genetic screening for the same mutations to be able to give a risk estimation to have an affected male child. Regardless of the mutation result, the first-line therapeutic approach should imply AOA. In case of partial globozoospermia, genetic advice is not recommended. The first-line therapeutic approach should imply IMSI to identify morphologically normal spermatozoa or globozoospermic spermatozoa with a small bud of acrosome. AOA, assisted oocyte activation; IMSI, intracytoplasmic morphologically selected sperm injection.

Authors' roles

P.K. analyzed the data and wrote the manuscript; F.V.M. provided and analyzed the data and wrote the manuscript; E.E. performed some of the genetic analyses; M.H.N.E., T.G., N.I., F.C.P., F.B., S.A.G., J.V.C. and Z.K. provided clinical samples and data; P.D.S. and S.V. designed the study and approved the final draft.

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Conflict of interest

None declared.

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