Brief report

Savior siblings and Fanconi anemia: analysis of success rates from the family's perspective

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Abstract

Purpose: The only curative treatment of Fanconi anemia is hematopoietic stem cell transplantation, which has a higher rate of successful outcome when donors are compatible siblings. For this reason, a number of families opt to have a healthy and compatible baby after embryo selection by preimplantation genetic diagnosis with HLA typing to generate a donor for an affected sibling. The aim of this study is to provide an estimate of the success rate of this procedure from the family's perspective.

Methods: Forty two in vitro fertilization cycles were followed, with detailed genetic and embryology data from 38 cycles. Genetic reports with detailed embryology and genetic data per cycle were provided by the families for this study.

Results: From a total of 524 oocytes (14.1 oocytes per cycle), 299 embryos were generated (8.0 embryos per cycle) of which 16 were transferred to the uterus as they were non FA and HLA-matched. One baby was finally born. A younger couple delivered a healthy and HLA compatible baby after 4 cycles. Therefore, the success rate per cycle is below 5% (2 savior babies from 42 trials).

Conclusion: While FA *per se* does not worsen the probabilities of success, the critical factor in these families is advanced maternal age due to late diagnosis of FA leading to few non-FA and HLA-matched embryos and high aneuploidy rate. The families should be informed in advance of the many trials that they will probably need to undergo even if a haploidentical younger female relative is available as oocyte donor.

Introduction

The only curative treatment for many blood disorders is hematopoietic stem cell transplantation (HSCT), which has a higher rate of successful outcome when the donors are compatible siblings. For this reason, a number of affected families opt to have an HLA-compatible baby through embryo selection by preimplantation genetic diagnosis (PGD) with HLA typing[1] (PGD+HLA) in order to generate a donor for an affected child. In fact, this procedure was initially developed to provide a donor for a Fanconi anemia (FA) patient, and was later applied to other blood disorders when transplantation is non-urgent and parents are of reproductive age[2,3]. FA is a rare genetic syndrome characterized by bone marrow failure, congenital malformations, chromosome fragility, and cancer predisposition. Mutations in 16 genes (*FANCA*, *B*, *C*, *D1*, *D2*, *E*, *F*, *G*, *I*, *J*, *L*, *M*, *N*, *O*, *P*, and *Q*) are known to cause FA, including one recently identified by our team via whole exome sequencing[4]. Early genetic subtyping and mutational analysis of this and related disorders are extremely important, not only for proper diagnosis, genetic counseling, and management, as for any genetic disease, but also for the implementation of curative therapies including HSCT following embryo selection.

Most of the news and literature available on the topic of PGD and savior babies comes from *in vitro* fertilization (IVF) clinics with a potential bias towards positives outcomes given that unsuccessful trials are often not reported or announced in the mass media. As co-coordinator of the Spanish FA Research Network and reference laboratory in the genetic diagnosis of Spanish FA

patients[5], we are in contact with all Spanish FA families willing to undergo embryo selection. The aim of this study is to provide FA families and their clinicians and genetic counselors with an estimate of the success rate of this procedure from the family's perspective. For this purpose, we systematically collected the PGD experience of 8 Spanish FA families willing to have a savior baby.

Methods

In this study we followed 8 Spanish families with FA complementation group A (FANCA) over a period of 11 years. To avoid any selection bias, we included all FA families undergoing PGD with HLA typing without any selection based on procedure success, maternal age or gene. Collectively, these 8 families carried out 42 PGD+HLA typing selection trials. To our knowledge, this is the largest series of PGD+HLA individual cycles ever reported for FA. All cycles were performed in specialized reproductive clinics in the USA, Belgium, and Spain. The IVF reports with detailed embryology and genetic data per cycle were provided by the families for this study. The data collected was maternal age, number IVF cycles performed, number of oocytes retrieved per cycle, number of embryos obtained in each cycle, number of non-FA embryos (either carriers or non carriers of *FANCA* mutations), number of transferrable embryos (non-FA and HLA-matched), and number of transferred embryos. We followed up all the families to know the number of pregnancies and abortions, and number of babies born. Aneuploidy data was also collected from the genetic reports when available. Aneuploidy was tested by FISH, PCR, or aCGH depending on the date of the IVF cycle and the reporting IVF clinic.

Results

Of the 8 families and 42 IVF cycles followed in this study, detailed data on PGD+HLA procedures are available for 38 cycles in 7 families, with a maternal age of 39.3 ± 1.8 (range 37–41) (Table 1). As graphically shown in Fig. 1, from a total of 524 oocytes (14.1 ± 7.9 per cycle), 299 embryos (8.0 ± 6.1 per cycle) were generated by intracytoplasmic sperm injection. Of the embryos for which single blastocytes could be analyzed, 75 were non FA (including monoallelic carriers or non-carriers), 26 were non FA and HLA compatible, and 16 could be transferred to the uterus. Five pregnancies occurred (confirmed by ultrasound and hormonal testing), but only one baby was born. Therefore, the success rate per IVF cycle was 2.6% (1 baby born from 38 trials), and the live birth rate per embryo transferred was 6.25% (1 baby born from 16 embryos transferred). When considering the 8th family, who had a baby after 4 cycles, the success rate was still very low: 2 babies born from 42 trials (4.8%). However, it is important to highlight that this mother was 25 years old, which is much younger than the rest of the mothers included in this study.

The causes of the 4 documented abortions in this study are unknown. In addition to FA and HLA genetic status, 28 embryos were concurrently tested for chromosomal numerical abnormalities by FISH, PCR, or aCGH. In most cases, not all 23 chromosomes were assayed; however, 15 out of 28 embryos (53.6%) were found to be aneuploid, including a monosomy 6 that was indirectly detected in 1 embryo as a result of abnormal HLA markers. Of the 4 embryos that resulted in spontaneous abortion, none underwent aneuploidy testing.

However, given the advanced maternal age, the probability that these embryos were aneuploid for any of the 23 chromosomes is extremely high.

Discussion

In addition to the known low Mendelian probability (only 3 out 16 embryos are theoretically expected to be non FA and HLA-matched), there are a number of other factors that may lead to the observed low success rate. These include a low initial number of embryos; loss of embryos during culture, manipulation, and selection procedures; a low implantation rate; a low live birth rate per embryo transferred; and a high incidence of miscarriages most probably due to aneuploidy specially at advanced maternal age. In our cohort of FA families, the mean number of oocytes retrieved (14.1 per cycle), embryos (8 per cycle) and aneuploid embryos (53.6%) obtained is similar to that reported in larger non FA PGD cohorts of similar maternal age (39-40 years old)[6]. This observation suggests that the FA disease, *per se*, does not worsen the success rate of savior baby generation. The critical point in FA seems to be advanced maternal age. Unfortunately, advanced maternal age is unavoidable in most FA cases, given that the diagnosis of FA is rare in newborns. In fact, the mean age of onset of the hematological disease in FA is 7 years[5,7], and the majority of patients are diagnosed months to years after onset.

Given that an advanced maternal age negatively affects the success rate of IVF, one Spanish FA family who had unsuccessfully performed 4 IVF cycles (couple 6 in table 1) opted to use a haploidentical maternal cousin as an oocyte donor. The cousin was 10 years younger than the biological mother and was

not a carrier of the maternal mutation. In total 3 cycles were performed. No pregnancy occurred after the 2 first PGD+HLA trials. In the first trial, 2 out of 4 embryos were HLA-compatible, but 1 of these was determined by aCGH to be aneuploid. The matched embryo with normal ploidy was transferred to the uterus; however, no pregnancy occurred. In the second trial, only 2 embryos were analyzed for HLA, carrier status, and aneuploidy. Neither of these embryos was HLA-matched with the FA patient. In the third trial, 8 embryos were biopsied. Seven were not mutation carriers of which 5 were HLA-matched, and 3 of them were not aneuploid by aCGH. One non-FA, non-aneuploid and HLA-matched embryo was initially transferred to the uterus but it did not implant. The 2 remaining non-FA, non-aneuploid and HLA-matched embryos were transferred one month later and both implanted. The pregnancy with two fetal sacs currently goes on normally at week 21 (December 1st). This family's experience demonstrates the extreme difficulties that can arise during attempts to generate a savior baby. Three other families in our study underwent 10, 7, and 7 unsuccessful trials. Therefore, clinicians and FA families considering PGD+HLA must make informed decisions, taking into account that probably more than 95% of the IVF cycles will not result in a birth and that the probability worsens as the age of the mother approaches 40 years. Knowledge of the success rates is important for the families, not only in psychological terms but also for economic reasons, as PGD+HLA trials in private reproductive clinics can be extremely expensive for the family or the public health system. In addition, the families should be aware in advance of the many trials that they will probably need to undergo even if a haploidentical female relative is available as younger oocyte donor.

Despite the low success rate of savior baby generation, the outlook for FA patients has been improved by several recent advances. The clinical outcome of transplantation in FA using HLA-matched non-siblings as HSC donors has greatly improved in the last few years because of optimized HSCT drugs and protocols[8]. Additionally, FA gene therapy clinical trials are currently underway[9]. Finally, disease-free blood progenitors from skin fibroblasts of FA patients were recently generated via induced pluripotent stem cells, setting the path for a future cure through regenerative medicine for patients with FA and other blood disorders[10-12]. We hope that these therapeutic advances will make the generation of savior babies to cure FA and other blood genetic disorders unnecessary in the years to come.

Acknowledgements

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Conflict of interest disclosure:

The authors declare that there are no conflicts of interest in relation to the work

described in this article

Table 1: Data from 38 PGD+HLA typing IVF cycles in 7 Fanconi anemia families

	Couple number							mean	SD
	1	2	3	4	5	6	7		
FA gene mutated	A	A	A	A	A	A	A		
Maternal age (years)	37	42	38	38	40	41	39	39.3	1.8
Number of IVF cycles	6	7	2	10	7	4	2	5.3	2.9
Oocytes retrieved	60	59	37	236	92	34	6	14.1*	7.9*
Embryos	38	26	18	158	32	22	5	8.0*	6.2*
Non FA embryos**	15	7	9	22	10	9	3	2.2*	1.8*
Transferrable embryos	3	3	3	10	4	2	1	0.8*	0.8*
Embryos transferred	2	1	1	7	3	1	1	0.5*	0.6*
Pregnancies	2	0	1	0	0	1	1	0.2*	0.3*
Babies born	0	0	1	0	0	0	0	0.03*	0.2*

*Mean and SD (standard deviation) per cycle of all 38 individual IVF cycles
**Includes monoallelic carriers or non carriers of FANCA mutations

Legend to figure 1:

Graphical representation of accumulated embryology and genetic data from 38 PGD with HLA typing IVF cycles in 7 Fanconi anemia families