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Preimplantation Genetic Diagnosis for A Y; 14 Translocation; Consideration of Meiotic Outcomes and Embryo Choice

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Abstract

For couples where one partner carries a chromosome rearrangement, preimplantation genetic diagnosis (PGD) is an established treatment option. In most cases, the possible consequence of each meiotic translocation product, and likely outcome per embryo, is clear. Embryos are either genetically balanced or suitable for transfer, or genetically unbalanced and not suitable. However, for X/Y; autosome translocations, involving an autosome (one of chromosomes 1 to 22) and one of the sex chromosomes (X or Y), some of the genetically unbalanced outcomes may result in offspring with normal clinical phenotype. For some couples these embryos are the only chance of achieving a pregnancy. We describe such a case involving a balanced reciprocal whole arm translocation between the Y chromosome and chromosome 14 [46, X, t(Y;14)(p10; p10)] in a 35 year old male presenting with severe oligozoospermia (infertility). This couple underwent five cycles of PGD, with cleavage stage biopsy and fresh transfer, over a four year period, the fourth cycle resulting in the birth of a healthy baby boy. We discuss the difficult decisions facing this couple and potential benefits of employing trophectoderm biopsy and vitrification of embryos on day five or six, followed by biopsy analysis and subsequent warmed frozen embryo transfer in a further cycle.

Keywords: Preimplantation Genetic Diagnosis; Autosome Translocation; Oligozoospermia; FISH; Oligozoospermia

Introduction

Approximately one in every 300 people in the general population carries a balanced chromosome rearrangement. These are primarily balanced reciprocal translocations (prevalence approximately 1 in 500) and Robertsonian translocations (approximately 1 in 1000) but also include inter- and intra-chromosomal insertions, deletions, pericentric inversions and complex chromosome rearrangements [1]. With the exception of recurrent rearrangements such as the balanced reciprocal translocation between chromosome 11 and 22 [2], these are unique chromosome rearrangements confined to a single family. For couples presenting for PGD, the family history may include multiple miscarriages, neonatal death and/or the birth of children with profound medical and developmental problems. Alternatively, the balanced rearrangement may have arisen *de novo* in the carrier partner. The risk to each couple of producing a live born child with an unbalanced product of the chromosome rearrangement can be derived by taking into account family history, all possible meiotic segregation outcomes, the size of the unbalanced segments and the specific chromosome regions involved [3].

Each year, approximately 70 couples undergo PGD treatment at our centre because one partner carries a chromosome rearrangement. Approximately 20% involve Robertsonian translocations and approximately 75% are for carriers of unique, often familial, balanced reciprocal translocations; the remaining 5% comprise couples where one partner carries an inversion, deletion, duplication or a more complex chromosome rearrangement [4]. X/Y; autosome translocations form a special category, with complex outcomes, where couples are usually referred for infertility. PGD for these rearrangements is rarely reported [5]. From 1998 to 2014 our PGD Centre employed fluorescence in situ hybridization (FISH) techniques on interphase nuclei from day three embryos to carry out genetic testing for these couples resulting in more than 500 cycles and over 100 healthy live-births [6]. During this period we have treated three couples with X/Y; autosome translocations. PGD is performed at a number of specialist centres worldwide [7]; however we are not aware of any previous publication in the literature that describes a case similar to the one that we describe here.

We describe a couple where the male partner carries a Y; 14 translocation and presented with infertility. They underwent five cycles of PGD, with cleavage stage biopsy and fresh transfer, over a two year period. This paper details the outcome of each cycle, the difficult decisions that had to be made, and suggests ways in which the experience of the couple could have been improved.

Materials and Methods

Assisted Reproduction Technology

Ovarian stimulation, embryo culture and biopsy were performed as described previously [8-10]. In brief, a standard long stimulation protocol for controlled ovarian stimulation was followed by intracytoplasmic sperm injection (ICSI), then biopsy of one or two cells from cleavage-stage embryos three days after fertilization. One cell was biopsied from embryos with five or more cells on day three and a second cell was biopsied only if the first cell did not have a clear single nucleus after lysis and the embryo had six or more cells.

Genetic Testing

FISH probe selection, blastomere spreading, in situ hybridization and signal scoring protocols have been described in detail previously [11,12]. Blastomeres were spread using the Tween/HCl method [13]. Prepared nuclei were hybridized overnight and analysed using a fluorescence microscope suitably equipped with the appropriate filter sets for the probes being used. In the event of one cell being mononucleated and the second cell being multinucleated, only the mononculeated cell was analysed for diagnosis. FISH Probes used were: 14q subtelomere (D14S1420, LPT14QR, Texas Red, Cytocell, Cambridge, UK), X centromere (DXZ1, CEP X, Spectrum Aqua, Abbott Diagnostics, Maidenhead, UK), SRY (Yp11.3, LSI SRY,

Spectrum Orange, Abbott Diagnostics) and Y heterochromatin Yq12 (DYZ1, CEP Y satellite III, Spectrum Green, Abbott Diagnostics). Evaluation of this probe scheme on peripheral blood lymphocytes from the couple resulted in a risk figure of < 0.1% for the likelihood of transferring an unbalanced embryo with a normal test result following single cell biopsy. Embryos were transferred on day five if morphologically suitable.

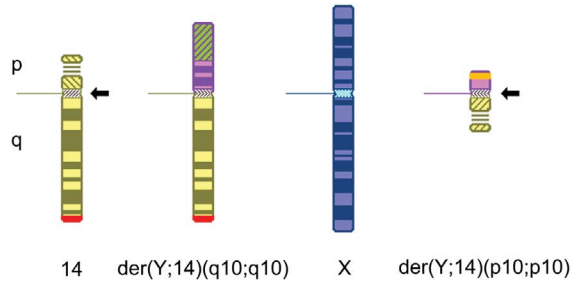

Figure 1: Partial ideogram of the apparently balanced reciprocal whole arm translocation carried by the male partner: 46,X,t(Y;14)(p10;p10). This ideogram indicates also the fluorescence in situ hybridization (FISH) probe scheme used for the PGD analysis. Probes used were: 14q subtelomere (D14S1420, LPT14QR, TexasRed, Cytocell), X centromere (DXZ1, CEP X, SpectrumAqua, Abbott), SRY (Yp11.3, LSI SRY, SpectrumOrange, Abbott) and Y heterochromatin Yq12 (DYZ1, CEP Y satellite III, SpectrumGreen, Abbott).

Clinical Details

This couple were initially referred for fertility treatment due to severe oligozoospermia, and subsequently for genetic counselling and testing. The female partner was 30 years old and the male 35 years old; he was found by karyotype analysis to carry a balanced reciprocal whole arm translocation between the Y chromosome and chromosome 14 [46, X, t(Y; 14) (p10; p10); Figure 1]. The results of all other investigations were normal. This chromosome rearrangement is consistent with the diagnosis of severe oligozoospermia.

Most Y-autosome translocation carriers experience infertility; this is thought to be the result of abnormal sex vesicle formation disrupting meiosis and causing spermatogenic arrest.

Chromosome Segregation Analysis

Following meiotic segregation and fertilization [14], this translocation has the potential to produce ten products that are considered to be frequent (A-D) or result in viable outcomes (E-J) (Figure 2) of which carrier male (A), which would be expected to

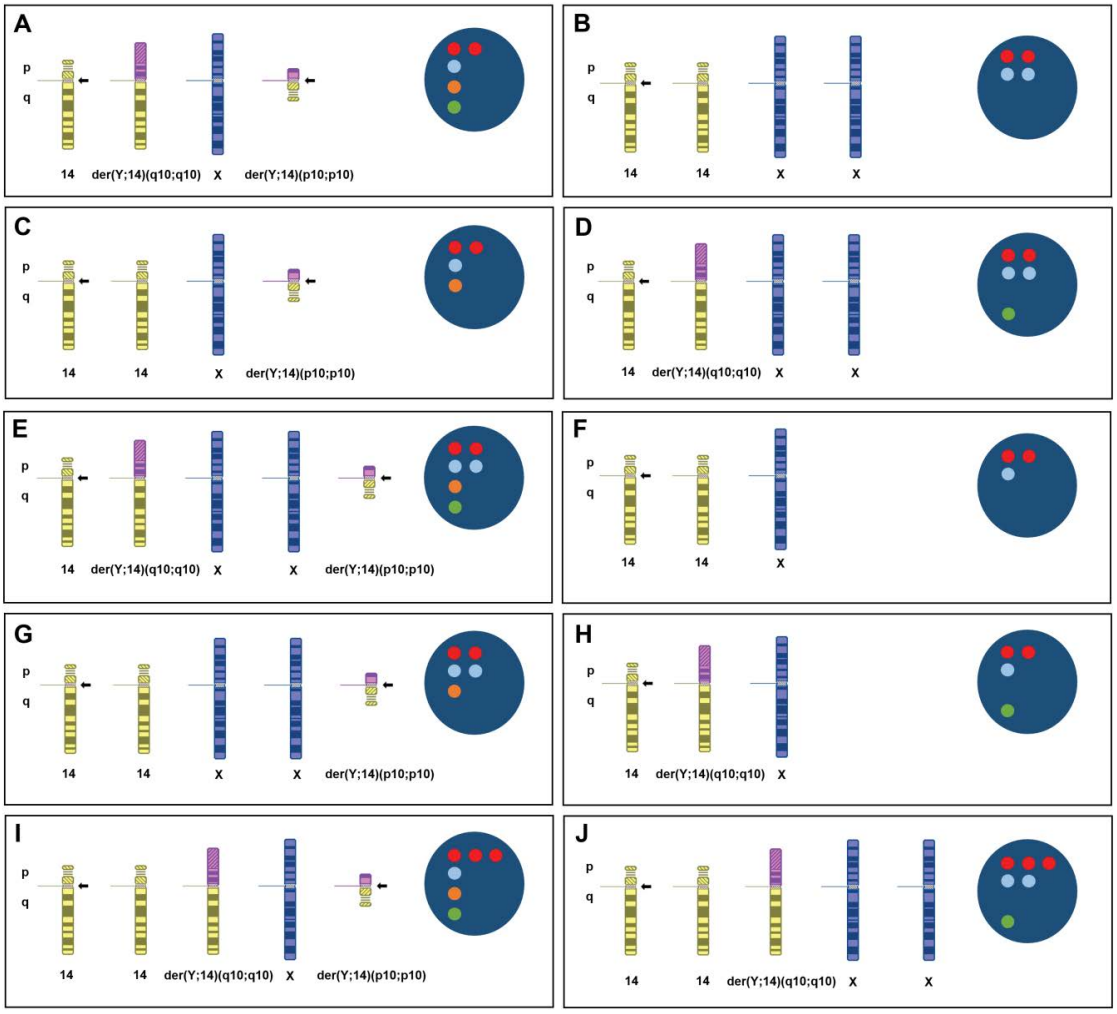

Figure 2: This translocation has the potential to produce ten outcomes that are considered to be frequent or viable. Each of these is shown alongside the expected blastomere nucleus signal pattern following FISH on day three blastomeres.

result in a male with subfertility, and normal female (B) embryos would be considered most suitable for transfer. However, two further embryos may also be considered: one male with Yq nullisomy (C), expected to result in a male with azoospermia, the other female (D) with two X chromosomes and one copy of Yq, expected to result in a phenotypically normal female; the SRY gene on Yp is required for testis formation and the AZFc genes resident in Yq are transcribed exclusively or predominantly in the testis [15]. The other six frequent or viable outcomes include: male with an extra X chromosome (E), expected to result in a clinical phenotype of Klinefelter syndrome, female with one X chromosome (F), expected to result in a clinical phenotype of Turner syndrome, male with two X chromosomes and Yq nullisomy (G), expected to result in a clinical phenotype of Klinefelter syndrome, female with one X chromosome and one copy of Yq (H), expected to result in a clinical phenotype of Turner syndrome, and male or female with trisomy 14 (I and J) expected to be non-viable but which may result in uniparental disomy (UPD) for chromosome 14 in the event of trisomy rescue.

Ethics Statement

Treatment (including embryo testing and storage) was conducted under license from the UK Human Fertilisation and Embryo Authority (HFEA). Consent to publish this work was received from the couple.

Results

This couple had five cycles of PGD (see Table 1); three unsuccessful cycles, with embryo transfer but no pregnancy, followed by one successful cycle that resulted in the birth of a healthy baby boy, and then one further cycle, again with embryo transfer but no pregnancy. Throughout this period of time, the couple changed their views regarding which embryos were acceptable for transfer, due to their increasing anxiety after successive unsuccessful cycles.

Cycle 1

Six embryos were suitable for biopsy (5 cells or more by day 3). Of these, five underwent biopsy of a single cell and one had two cells biopsied due to failure to visualize a nucleus in the first cell. Results are shown in table 1. The type C embryo (Grade 4 on day 3) and the type D embryo (Grade 3 on day 3) were not considered transferable by the couple. The type A balanced carrier male embryo was transferred but did not result in pregnancy.

Cycle 2

Nine embryos were suitable for biopsy (5 cells or more by day 3). Of these, all nine underwent biopsy of a single cell. Results are shown in table 1. The three type D embryos (all Grade 4 on day 3) were not considered transferable by the couple. The type B

Cycle	Embryo	Number of Cells Day 3	Embryo Quality at Biopsy	Likely Genetic Constitution as Shown by FISH Results	Embryo Quality Day 5
1	1	8	4	X,der(Y;14)(p10;p10),der(Y;14)(p10;p10),),14,14,14	BC3BC
	2	9	4	X,der(Y;14)(p10;p10),14	
	3	6	4	4n with loss of der(Y;14)(p10;p10)	
	4	6	3	Outcome D	
	5	10	4	Outcome C	
	6	5	4	Outcome A Transferred, no pregnancy	
2	2	8	4	Outcome H	Cellular
	3	8	4	Outcome D	
	4	5	4	Outcome D	
	5	7	3	1n or 4:0 segregation resulting in X,14	
	6	8	4	1n or 4:0 segregation resulting in X,14	
	7	7	3	Outcome B Transferred, no pregnancy	
	8	5	4	der(Y;14)(p10;p10),14	
	10	8	4	Outcome D	
	11	6	4	Outcome H	
3	11	5	4	Outcome F	Cellular
	15	5	4	Outcome C Transferred no pregnancy	
4	1	7	3	Outcome A but nucleus fragmented	Cellular
	2	8	4	Outcome D	Compacting
	3	9	4	Outcome D	BC1
	4	8	4	Outcome C	Morulla
	5	8	4	Outcome B but nucleus partially obscured	Cellular
	6	8	4	Outcome C Transferred, singleton pregnancy, healthy male delivered[a]	Cellular
	7	8	4	Outcome B	Cellular
	8	9	4	Outcome A	Cellular
	9	9	4	der(Y;14)(p10;p10)	Cellular
	10	6	4	Outcome A	
	11	8	4	X,der(Y)(p10;p10),14	
5	1	6	3	Outcome D Transferred, no pregnancy	BC2
	2	6	3	Outcome F	Cellular
	3	6	3	Outcome F	Cellular
	4	6	4	Outcome B	Compacting
	5	6	2	X;der(Y;14)(p10;p10)	Cellular
	7	6	3	Outcome C	Compacting
	8	5	3	Outcome H	Compacting

Table 1: Embryological details and genetic outcomes of PGD cycles 1 to 5. See figure 2 for chromosomal constitution of outcomes A to I. Embryo grades at biopsy are shown for all embryos. Embryo grades at day 5 for cycles 1 to 3 are only shown for those embryos considered transferable by the couple on the basis of chromosomal constitution. Embryo grades at day 5 for cycles 4 and 5 are shown for all embryos considered to be morphologically transferable. [a] Karyotype confirmed postnatally.

normal female embryo (Grade 3 on day 3 and cellular on day 5) was transferred but did not result in pregnancy.

Cycle 3

Two embryos were suitable for biopsy (5 cells or more by day 3). Both embryos underwent biopsy of a single cell and were analysed. The results are shown in table 1. The type C embryo was transferred but did not result in pregnancy.

Cycle 4

Eleven embryos were suitable for biopsy. Of these, eight underwent biopsy of a single cell and three underwent two-cell biopsy (due to unsuitable quality of the first biopsied nucleus). Results are shown in table 1. On the basis of morphology, only one type B and one type C were suitable for transfer. The couple decided to transfer the morphologically superior type C embryo with Yq nullisomy rather the type B normal female. This transfer resulted in a singleton pregnancy and a healthy male baby was delivered. Neonatal G-banded chromosome analysis confirmed the karyotype of 46, X, der(Y) t(Y; 14)(p10; p10) pat. The normal female embryo was not cryopreserved as it was not morphologically suitable.

Cycle 5

Eight embryos were suitable for biopsy. Seven embryos were biopsied and of these, all seven underwent biopsy of a single cell. Results are shown in table 1. Interestingly, the couple now considered the type D embryo to be amongst those suitable for transfer and opted to transfer the morphologically superior type D embryo (Grade 3 on day 3 and BC2 on day 5) rather than the type B normal female (Grade 4 on day 3 and compacting on day 5). However, no pregnancy resulted.

Discussion

Most balanced reciprocal translocations involve two autosomes, in which case the resulting embryos, following PGD analysis, can be clearly categorised either as genetically balanced and suitable for transfer, or as genetically unbalanced and not suitable for transfer. However, where the balanced chromosome rearrangement involves an autosome and a sex chromosome, the phenotypic consequences of some of the genetically unbalanced segregation outcomes may be acceptable. The majority of unbalanced outcomes can be categorised as not suitable for transfer but some unbalanced outcomes would be likely to result in a normal phenotype, and some a normal phenotype with infertility [16]. For most couples this latter would not be different from the phenotype of the carrier partner, and transferring one of these unbalanced embryos may be the only chance a couple will have of achieving pregnancy. Thus the decision has to be made whether a predicted phenotype of infertility is acceptable to the couple, which will be largely dependent on the impact that infertility has had on the affected individual's life and the experiences of the couple to date. They may take the view that offspring with infertility will have no worse an experience than they themselves have had, with inevitable improvements in clinical treatments in the years ahead. Conversely they may feel that their own infertility has been so detrimental to them that they could not inflict this on their own children. Either way, deciding which embryos to transfer amid the pressures of an ongoing PGD cycle and concern over increasing female age is difficult and stressful for such couples.

With regard to Outcome D (Figure 2), the presence of the Y chromosome long arm against a female background clearly caused unease for this couple, despite the very good chance that this would have no phenotypic effect. The resulting foetus would be expected to be female, as the SRY gene, required for testis formation and male sexual differentiation, was absent, and the AFZc genes located in Yq are expressed exclusively or predominantly in the cells of the testis [15]. The presence of the Yq is therefore considered to be without phenotypic effect.

At our centre, detailed counselling, including consideration of possible embryo results, takes place with all couples before a PGD cycle. However, in cases such as this, where the potential genetic outcomes are numerous and the expected phenotypic outcomes varied and complex, it is not always possible for the couple to make firm decisions prior to the cycle. To complicate matters further, couples who undergo successive cycles after failing to achieve a pregnancy may change their minds regarding which embryos they would consider for transfer, as in the case presented here. With cleavage stage biopsy on day three, diagnosis on day four and embryo transfer on day five, there is very limited time available for consideration of the complexities of the relative genetic and morphological characteristics of the available embryos.

Despite the predicted normal phenotype for type C and type D embryos, the couple had decided at their pre-test counselling that they were not prepared to accept these embryos for transfer. Embryos of outcomes A to D are predicted to result in normal phenotype, or normal with impaired fertility. In the first cycle, there were three such embryos (A, C and D), of which the balanced carrier male embryo (A, same karyotype as male partner) was chosen for transfer but did not result in pregnancy. In the second cycle there were four of these embryos (one B and three D). The couple still felt that outcome D was unacceptable due to the genetic imbalance, and therefore these three embryos were discarded, despite their better quality, and the normal female embryo was transferred, and again did not result in a pregnancy. In the third cycle, only one embryo was of type A to D; this was a male embryo with Yq nullisomy (outcome C). In the absence of any embryos of type A or B the couple opted to transfer this type C embryo, although previously this type had been considered not suitable for transfer. Pregnancy was not achieved. In the fourth cycle, seven embryos were of outcomes A to D (two A, one B, two C and two D). On this occasion, the couple opted to transfer the best quality embryo, which had outcome C. This transfer resulted in the establishment of a singleton pregnancy and the delivery at term of a healthy baby boy. Finally, in the fifth cycle, three embryos were of type A to D (one B, one C, and one D). The couple opted again to transfer the best quality embryo, which was the type D embryo, not chosen previously by the couple. This embryo was transferred but did not result in pregnancy.

Thus, throughout successive cycles, as the couple's experience of treatment increased they modified their views and gave the embryo quality greater consideration, to the extent where they opted to transfer superior quality embryos with what they considered the less desirable chromosomal constitution in order to increase the likelihood of establishing a pregnancy.

These difficult decisions would have been better made in less stressful conditions; this could be achieved by removing the time pressure associated with day 3 biopsy, day 4 diagnosis and day 5 transfer. Trophectoderm biopsy and vitrification of embryos followed by diagnosis and transfer of warmed embryos removes this time pressure. All couples have as much time as they required to decide which embryos to transfer, which is particularly beneficial for those couples with difficult decisions, as in this case. Evidence now exists for successful pregnancy outcomes following transfer of embryos that have been vitrified after biopsy [17,18]. Indeed, a recent review indicates that transfer of frozen thawed embryos may provide better obstetric and perinatal outcomes than fresh embryo transfers [19]. With more time to consider, this couple may have opted for the transfer of an embryo with outcome D in their second cycle.

PGD for chromosome rearrangements is amenable to trophectoderm biopsy, vitrification of embryos and diagnosis using array comparative genomic hybridization (aCGH); this is now the methodology of choice in our centre.

In summary, this case confirms the efficacy of PGD for Y-autosome translocations, as previously described [5] and underlines the complexity of these cases and the need for protocols that allow time for counselling and consideration of the diagnostic results.

Conflict of Interest

The authors declare that there is no conflict of interest.

Author Contributions

Conceived and designed the genetic test, PS and CO; analysed the results, AD, SB, PS and CO; drafted the manuscript, AD; contributed to reviewing and revising the manuscript, AD, SB, AL, PS, CO (cytogenetics), AL (genetic counselling), and YK (reproductive medicine and embryology).

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