

Preimplantation Genetic Diagnosis (PGD) for chromosome rearrangements

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Key Points

- Chromosome rearrangements include Robertsonian and reciprocal translocations (the most common form of chromosome abnormality in humans, present in approximately 1 in 500 individuals), peri- and paracentric inversions, inter- and intra-chromosomal insertions, deletions, duplications, and complex chromosome rearrangements (CCRs).
- In all cases presenting for PGD, the risk of viable abnormality should have been assessed and the couple counselled appropriately, as for many couples carrying chromosome rearrangements, the best chance of a successful pregnancy outcome may be by natural conception.
- A PGD test using commercially available FISH probes can generally be designed for all Robertsonian translocations, most reciprocal translocations and inversions, and some CCRs. Specific “home-grown” probes may be required for some insertions.
- By far the largest group presenting for PGD are couples where one partner carries a reciprocal translocation, which in nearly all cases is unique to that family.
- Most groups use combinations of probes for the different chromosome segments involved in the rearrangement in order to assess dosage of these regions in interphase nuclei or polar body preparations.

Introduction

Couples where one partner carries a balanced chromosome rearrangement or other structural chromosome abnormality are at risk of genetically unbalanced conceptions, which may result in failure to implant, early or late miscarriage, or liveborn children with physical and mental disability. The likely outcome of the conception will depend on the extent and severity of the genetic imbalance, which will in turn depend on the specific rearrangement. Testing embryos for genetic imbalance associated with the familial rearrangement will be appropriate where there is a history of liveborn children with genetic imbalance, an unacceptably large number of miscarriages, or where the couple requires IVF in order to conceive. For couples where there are no fertility or reproductive problems, and who have a “low risk” rearrangement (see below), spontaneous conception is likely to be the best option.

Chromosome rearrangements include Robertsonian and reciprocal translocations (the most common form of chromosome abnormality in humans,

present in approximately 1 in 500 individuals), peri- and para-centric inversions, inter- and intra-chromosomal insertions, deletions, duplications, and complex chromosome rearrangements (CCRs). A PGD test using commercially available FISH probes can generally be designed for all Robertsonian translocations, most reciprocal translocations and inversions, and some of the other more rare rearrangements. Specific “home-grown” probes may be required for some insertions. By far the largest group presenting for PGD are couples where one partner carries a reciprocal translocation, which in nearly all cases is unique to that family.

The material used for testing is usually either polar bodies or biopsied cells from cleavage stage embryos although it is also possible to use material from the trophoblast of blastocyst stage embryos. Most groups use combinations of locus-specific probes, (sometimes with whole chromosome paints) for the different chromosome segments involved in the rearrangement, in order to assess copy number of these regions in interphase nuclei or polar body preparations.

Reciprocal translocations

Balanced reciprocal translocations, which are typically an exchange of two terminal segments from different chromosomes, occur in approximately one in 500 live births (1), and are usually associated with a normal phenotype. (In rare cases, de novo reciprocal translocations may be associated with sub-microscopic imbalance or gene disruption at the breakpoints, leading to congenital abnormalities.) Reciprocal translocations may be associated with infertility in some carriers, while all carriers have reproductive risks due to abnormal segregation of the translocation chromosomes at meiosis, resulting in sperm or eggs with chromosome imbalance. The reproductive risks at conception and at term for a reciprocal translocation heterozygote are determined by the chromosomes involved, the different modes of segregation at meiosis, which occur with variable frequency, the number and position of chiasmata, and the size and genetic content of the chromosome segments of the products with chromosome imbalance (2, 3).

Translocation segment imbalance

Reciprocal translocations can be described in terms of four chromosome segments (see Figure 1): two centric segments that contain the centromeres, and two translocated segments comprising the exchanged material; segregation at meiosis can result in gametes with many different permutations of between zero and three copies of each of the four chromosome segments; deviation from two copies of any segment in the zygote results in chromosome imbalance (4). A zygote with translocation chromosome imbalance has monosomy and/or trisomy or tetrasomy for at least two of the translocation segments. For some translocations, very few or none of the conceptuses with chromosome imbalance

are compatible with an ongoing pregnancy; for other translocations, a pregnancy may be established that results in miscarriage, stillbirth, or the live birth of a child with significant mental and physical disability, which may result in early death.

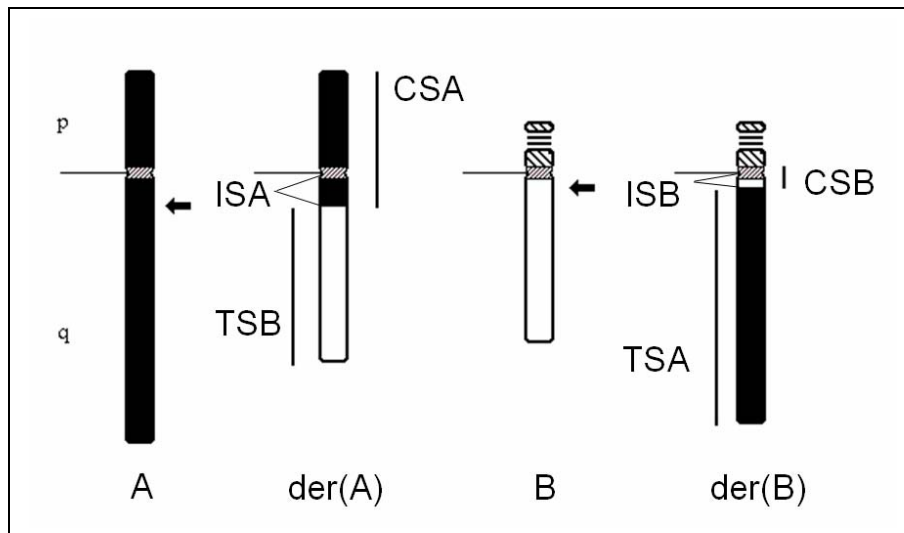


Figure 1: Reciprocal translocation segments. CSA(B) = centric segment of chromosome A(B), TSA(B) = translocated segment of chromosome A(B), ISA(B) = interstitial segment of chromosome A(B). An odd number of crossovers in the interstitial segments at meiosis results in recombinant products. All the different segregation products can be expressed in terms of the copy number of the centric and translocated segments (see Table 1).

Reproductive risk assessment

Couples presenting with reciprocal translocations may require assisted reproduction because they have male or female factor infertility. However, many couples have a reproductive history of recurrent miscarriage, termination of affected pregnancies, or an affected child with profound mental and physical disability. A history of infertility or recurrent pregnancy loss is not necessarily associated with the translocation, and it is often the case that products of conception have not been karyotyped; other potential contributing factors (e.g., antiphospholipid syndrome) should therefore be investigated thoroughly before considering subjecting a fertile woman to assisted reproduction. Similarly, although it is acknowledged that infertility in some male carriers may be due to their translocation, it is prudent to investigate other possibilities as appropriate (5). For a couple presenting with two or three miscarriages, and where a link has not been established with the translocation, and the risk of liveborn offspring with an unbalanced form of the translocation is low, the best chance of a successful outcome may be spontaneous pregnancy with PND for reassurance. In a case control study Franssen et al. (6) showed that in a two-year follow-up period 84% of couples are likely to have a successful outcome, although 54% had one or more spontaneous abortions. In favour of testing, a prospective study compared with an historic obstetric history of recurrent miscarriage and unsuccessful pregnancies (7) argued that testing significantly reduced the risk of miscarriage

and increased the chance of a viable pregnancy. Accurate reproductive risk assessment is vitally important in helping couples to decide if PGD is the right way forward. A decision to proceed with PGD should only be taken after all the available reproductive options have been considered.

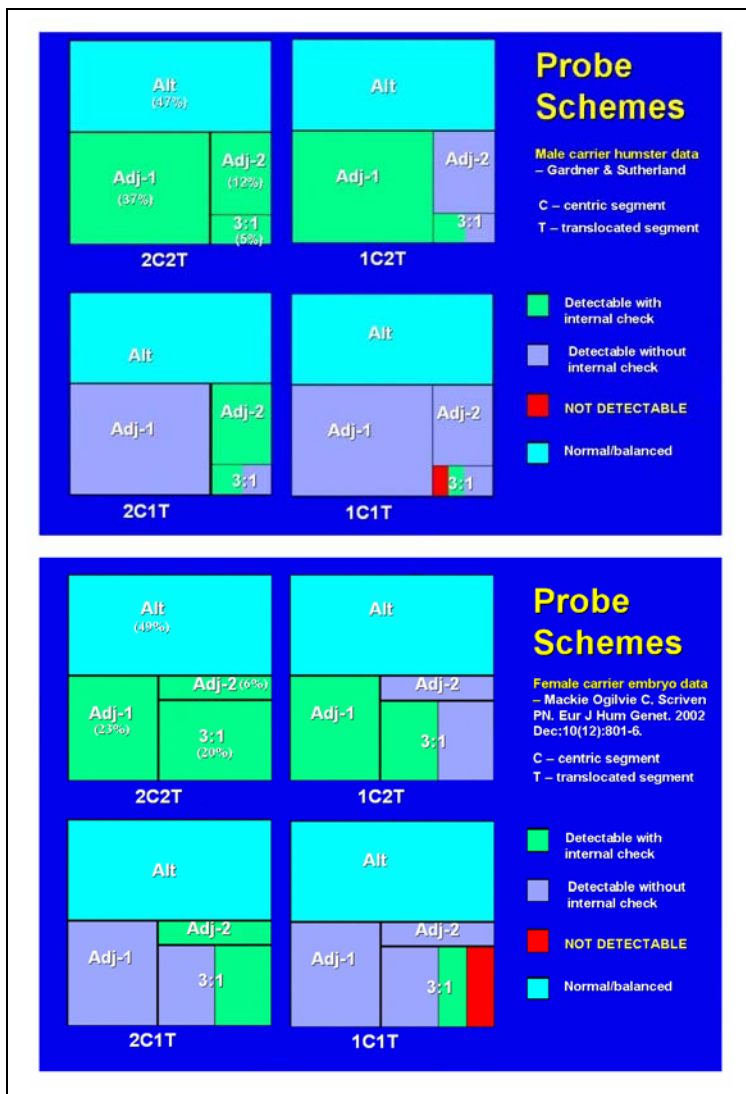
Gardner and Sutherland (3) describe an approach to risk assessment which includes empirical single-segment imbalance liveborn data (8-10), the predicted mode of segregation most likely to result in liveborn offspring with chromosome imbalance (11, 12), family genetic history, and a review of the literature (e.g. PubMed) (13). In addition to estimating the risk of liveborn offspring with chromosome imbalance, a 20% – 30% risk for fetal loss in relation to the general background risk of 15% is typically given for most reciprocal translocations (3, 10).

Testing for segment imbalance

In PGD clinical practice, one or both polar bodies can be sampled from oocytes, one or two blastomeres can be biopsied from cleavage stage embryos, or several cells can be sampled from the trophectoderm at the blastocyst stage. Polar body biopsy is only informative for female translocation heterozygotes; however, the other methods sample the zygote and can therefore be used for male or female translocation carriers. The primary technique used for preimplantation genetic testing is fluorescence in situ hybridization (FISH). The copy number of the different chromosome segments involved in reciprocal translocations (Table 1) is determined using target-specific DNA probes labelled with different fluorochromes or haptens. Clinical preimplantation genetic testing has employed chromosome paints and locus-specific probes. Chromosome paints applied to metaphase chromosomes from polar bodies (14) and after blastomere nucleus conversion (15, 16) can detect copy number as well as discriminate between normal and heterozygous chromosome complements; the translocation status of the oocyte can thus be inferred. Locus-specific probes applied to interphase nuclei from cleavage stage blastomeres (17-22) or cells sampled from the trophectoderm (23) are used primarily to detect copy number of the chromosome segments tested, and will not discriminate between normal and heterozygote chromosome complements unless probes are designed to span the breakpoints (24). The development of patient-specific breakpoint-spanning probes is time-consuming and expensive, and is therefore not generally undertaken for routine PGD testing. Commercially available probes specific for subtelomere and centromere regions and directly labelled with different fluorochromes offer the most straightforward approach to PGD for reciprocal translocations (4).

FISH Probe Selection

Initial selection of potentially informative FISH probes is made as part of the preliminary assessment as to availability of PGD. Theoretically, in order to maximise sensitivity, every FISH assay would incorporate four probes, one for each centric and translocated segment (see Figure 2), and the probe assay would have a hybridization efficiency of 100%. However, breakpoints are sometimes distal to available subtelomere probes, unique centromere probes are not available for all the acrocentric chromosomes, and suitable probes are not always available either indirectly labelled with an appropriate hapten, or directly labelled with the necessary fluorochromes. Probe efficiencies are rarely 100%, even for a single probe. Incorporating inefficient and possibly unnecessary probes into an assay can therefore significantly reduce the specificity of the PGD test. One probe in each of three out of the four centric and translocated segments is the minimum number of probes required to detect all the theoretical unbalanced forms of a reciprocal translocation (4), and is therefore



recommended for most reciprocal translocations. Given the practical limitations discussed above, probe selection should be based primarily on the predicted mode of segregation most likely to result in liveborn offspring with chromosome imbalance, and the segregation mode considered likely to be most frequent.

Figure 2. Reciprocal translocation probe schemes used in clinical practice. The proportions of segregation products are different for male and female carriers. An ideal probe mix would include a probe for both centric segments and both translocated segments (2C2T); two scoring errors are therefore required to misdiagnose an unbalanced product as normal/balanced; however, suitable probes may not be available. Probes for three out of the four segments are necessary to detect all the unbalanced products. A 1C2T mix, with careful selection of which centric segment, is preferred for single cell biopsy to ensure two informative probes for the unbalanced products most likely to be frequent and viable; two-cell biopsy is recommended for 2C1T and 1C1T probe schemes. Some 3:1 segregation products are not detectable with a 1C1T probe scheme.

Adjacent-1 segregation, or alternate segregation following an odd number of crossovers in the interstitial segment (see Figure 1), results in zygotes with monosomy and trisomy for the translocated segments. Empirically, this is the most frequent reciprocal translocation imbalance found in zygotes (25). For translocations where products of adjacent 1 segregation are likely to lead to embryos with viable segment imbalance, three probes, one for each of the translocated segments and one for one of the centric segments, will give a FISH assay ensuring that only in the case of two signal scoring errors will an embryo with segment imbalance following adjacent-1 segregation be diagnosed as balanced (this requirement for two scoring errors can be described as an “internal check”, see Figure 2). This therefore provides a robust test with a very low false normal error rate.

Viable chromosome imbalance consistent with 3:1 segregation is usually associated with monosomy or trisomy for the smallest chromosome (12). For translocations with a risk of viable 3:1 segregation products, the smallest chromosome usually therefore dictates the choice of centric segment probe, and a second probe on the smallest chromosome will provide two probes for potentially viable imbalance (see above).

Very few reciprocal translocations result in viable offspring with imbalance due to adjacent-2 segregation (monosomy and trisomy for the centric segments); however, where this is a possibility, probes for both centric segments, with a single probe in one of the translocated segments, should be incorporated into the assay.

Available commercial FISH probes include those produced by Abbott (formerly Vysis) and Cytocell. The FISH assays may combine directly labelled and indirectly labelled probes, and probes from different manufacturers. Probes with known polymorphisms, or those known to cross-hybridize significantly with other chromosomes (26), should be avoided where possible as first choice probes. It is not always possible to design assays using three probes directly labelled with different fluorochromes. Where Abbott CEP probes labelled with SpectrumAqua are not available, biotinylated alphasatellite and classical-satellite probes detected with Cy-5 streptavidin (Amersham) and visualised using a FarRed filter provide an alternative strategy. However, other groups choose to mix red and green probes or use a second round of hybridization (27). An elegant solution to the third colour problem exploits the observation that Abbott SpectrumOrange probes can be visualised using a TexasRed Filter and a SpectrumGold filter, whilst Cytocell TexasRed labeled probes can only be seen using the TexasRed filter; probes labeled with these fluorochromes can therefore be used together in an assay (see Fig. 3).

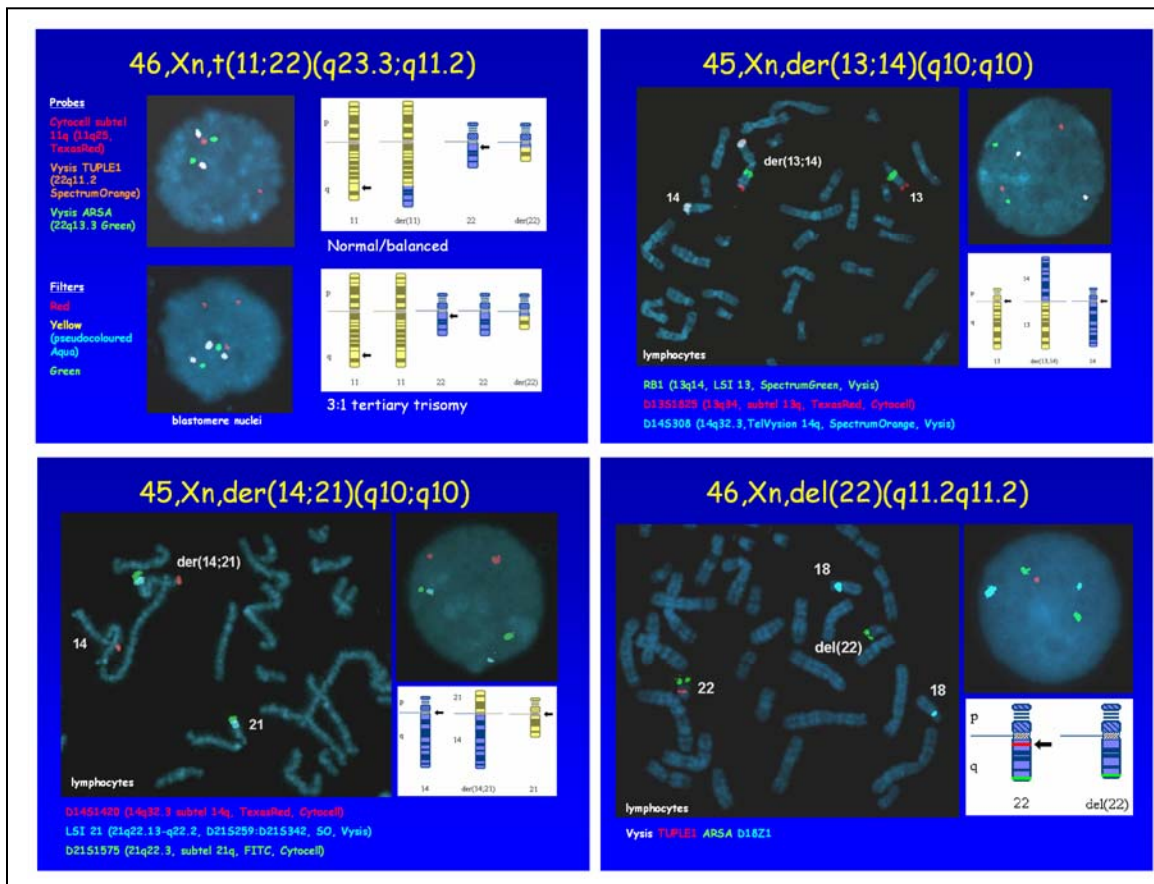


Figure 3: Recommended FISH probe combinations for:

- the “common” 11;22 translocation: 46,Xn,t(11;22)(q23.3;q11.2); 11qsubtel (11q25; TexasRed, CytoCell), TUPLE1 (22q11.2; SpectrumOrange, Abbott), ARSA (22q13.3; SpectrumGreen, Abbott). Top row: normal/balanced blastomere nucleus and ideogram of balanced rearrangement; bottom row: 3:1 tertiary trisomy product (blastomere and ideogram), demonstrating “internal check” for the supernumerary chromosome.
- Robertsonian translocation between chromosomes 13 and 14: 45,Xn,der(13;14)(q10;q10); RB1 (13q14; SpectrumGreen, Abbott), D13S1825 (13q34; TexasRed, CytoCell), D14S308 (14q32.3; SpectrumOrange, Abbott). Metaphase spread and interphase nucleus from carrier lymphocytes, and ideogram of the balanced rearrangement.
- Robertsonian translocation between chromosomes 14 and 21: 45,Xn,der(14;21)(q10;q10); D14S1420 (14q32.3; TexasRed, CytoCell), LSI21 (21q22.13-q22.2; SpectrumOrange, Abbott), D21S1575 (21q22.3; FITC, CytoCell). Metaphase spread and interphase nucleus from carrier lymphocytes, and ideogram of the balanced rearrangement.
- 22q11 deletion (46,Xn,del(22)(q11.2q11.2)): TUPLE1 (22q11.2; SpectrumOrange, Abbott), ARSA (22q13.3; SpectrumGreen, Abbott); and to confirm diploidy D18Z1 (cen 18, SpectrumAqua, Abbott). Metaphase spread and interphase nucleus from carrier lymphocytes, and ideogram illustrating the deletion.

Lymphocyte FISH Work-up

It is necessary to assess each probe combination prior to clinical treatment (Thornhill et al. 2005). Metaphase spreads and interphase nuclei should be analysed from each of both reproductive partners. Metaphase spreads should be analysed from the translocation carrier to ensure that the probes selected hybridize as expected and are informative for the translocation segments.

Metaphase spreads should be analysed from the non-carrier reproductive partner to ensure that the probes hybridize normally. Interphase nuclei should be scored to make a quantitative assessment of the assay and a qualitative assessment of FISH signal intensity and discreteness. The quantitative data collected may be used to assess the potential analytical performance of the assay. The false abnormal and false normal error rate can be estimated to assess the test specificity and sensitivity. The negative predictive accuracy (the likelihood that a normal test result represents a normal or balanced chromosome complement) and the positive predictive accuracy (the likelihood that an abnormal test result represents segment imbalance) can be used to decide if the probe combination is suitable for a clinical PGD cycle (Scriven 2003). Test analytical performance is a function of individual probe efficiencies, the prevalence of the 32 different reciprocal translocation segregation products, and the fidelity of embryo sampling.

For the purpose of assessing the analytical performance of a probe assay, it is assumed that random sampling of a cell from a day 3 embryo is representative. Efficiencies of at least 95% are realistically achievable for individual probes. The prevalence in day 3 embryos of the different translocation forms can at best only be estimated. Each translocation, with very few exceptions, is unique to one family, and therefore pedigree information is usually very limited. Although it is possible to undertake sperm-FISH studies for male carriers, it may be impractical to offer this on a routine basis. The inaccessibility of the female germline means that, for practical purposes, only estimation is possible. However, pachytene shape analysis appears useful not only in predicting the mode of segregation associated with liveborn imbalance, but also in predicting which translocations are likely to produce a high prevalence of 3:1 or adjacent-2 segregation products (25).

Careful probe selection, with two informative probes for potentially viable chromosome imbalance (28), and an estimated negative predictive value of at least 95% and a positive predictive value of at least 85%, are generally considered acceptable in order to offer a clinical PGD cycle using single cell biopsy (29). Figure 3a shows the recommended FISH probe combination for the "common" 11/22 reciprocal translocation.

Number of blastomeres cells to test

Mosaicism (the presence in an embryo of two or more genetically different cell lines) is well described (30-32). Some groups therefore choose to biopsy and test two cells from cleavage-stage embryos, only transferring those embryos where there is a concordant result between the two cells tested. However, this may in many cases be overcautious and result in unnecessary exclusion of embryos from the cohort suitable for transfer. Inherent errors in the test may result in a false abnormal result for one of the two cells. In addition, for most translocations, mosaicism for the chromosomes involved is unlikely, and cells carrying such

imbalance are likely to be selected against in favour of the normal cell line. However, where the FISH assay has no internal check for a potentially viable product, or where a small derivative chromosome may be lost, giving rise to a viable minor cell line, two-cell biopsy and testing is prudent.

Robertsonian translocations

Introduction

Robertsonian translocations (centric fusion of two acrocentric chromosomes) occur with a prevalence of 1 in 1000 in the general population (3). By far the most common are the nonhomologous forms, i.e. those involving two different acrocentric chromosomes—either two different D group chromosomes (chromosomes 13, 14 and 15), two different G group chromosomes (21 and 22), or a D group and a G group chromosome. At meiosis, these rearrangements form trivalents, segregation of which may result in gametes nullisomic or disomic for one of the chromosomes involved in the rearrangement and consequently to a zygote with trisomy or monosomy for one of the chromosomes involved. Zygotes with monosomy are not compatible with life and most translocation trisomy conceptuses are expected to result in first trimester loss or earlier; however, some survive beyond the second trimester and to term.

Reproductive risks

The most common Robertsonian translocation is between chromosomes 13 and 14. This D/D translocation makes up ~75% of all Robertsonians (3) The potential liveborn chromosomally unbalanced outcome of this is translocation trisomy 13 (Patau syndrome); there is an empirical risk of occurrence at second trimester prenatal diagnosis of <0.4% (3, 33). There is also potential for uniparental disomy (UPD) for chromosome 14 following trisomy rescue, with an estimated risk of ~0.1–0.5% (3). Translocation trisomy 14 is expected to result in first trimester loss. For der(13;14) carriers the overall risk of miscarriage is not expected to be significantly different from the background risk of 15% (Harris et al., 1979) (up to two miscarriages); however, some individuals with a der(13;14) present with infertility or recurrent spontaneous abortions. The empirical reproductive risks for male carriers of 13;14 Robertsonian translocation carriers are low. Sperm FISH using probes for the translocation chromosomes can be used to establish the level of aneuploidy, and in the case of a normal sperm count and low aneuploid levels, PGD may not be indicated (34). For some males who present with oligozoospermia and a Robertsonian translocation, ICSI may be necessary to overcome the infertility, in which case PGD would be indicated, to exclude the possibility of transferring abnormal embryos, and therefore to increase the chances of establishing a normal pregnancy.

Other D/D Robertsonians are much less frequent and specific risks have not been derived; however, der(13;15) and der(14;15) might be expected to have similar risks to the der(13;14) (3).

The most common Robertsonian after the der(13;14) is the der(14;21). The potential liveborn unbalanced outcome of this D/G Robertsonian is translocation trisomy 21 resulting in Down's syndrome; for female carriers, the empirical risk of occurrence at second trimester prenatal diagnosis is 15%, with a 10% risk of liveborn trisomy 21 plus a small risk of UPD 14, as before. For male carriers, the second trimester risk of translocation trisomy 21 is <0.5% (3, 33).

Other D/G Robertsonians which involve chromosome 21 may be expected to have similar reproductive risks to the der(14;21); those involving chromosome 22 have a lower risk since trisomy 22 has very limited potential to be viable.

FISH probe selection

Any combination of two probes specific for the long arms of the two chromosomes involved in the Robertsonian translocation (for instance a subtelomere probe for each chromosome) can be used as a FISH test; however, where there is a likely viable outcome, such as for Robertsonian translocations involving chromosomes 13 or 21, the use of two different probes for the viable trisomy, plus a probe for the other chromosome involved in the rearrangement, is considered prudent, as this gives an internal check (see above). See Figure 3b and 3c for the recommended FISH probe mixes for the two most common Robertsonian translocations.

Indications and counselling

For infertile couples undergoing assisted conception, where one partner carries a Robertsonian translocation, the screening out of embryos with an unbalanced translocation prior to transfer is likely to be beneficial, regardless of the chromosomes involved. However, where a couple is referred for recurrent miscarriages, careful counselling is required prior to PGD; the translocation may not be causative of the miscarriages, and subjecting a fertile couple to invasive procedures in these cases should be avoided. This is particularly the case for the der(13;14) translocations, where empiric data suggests that the translocation is unlikely to be associated with recurrent miscarriage. Karyotyping products of conception may be a worthwhile pathway to follow for the couple and their counsellor. Further discussion of this issue, and a description of cases tested for Robertsonian translocation, can be found in Scriven et al (34).

Other chromosome rearrangements

Carriers of balanced pericentric inversions are at risk of pregnancies with imbalance for both the non-inverted segments. This risk will depend on the length of the inverted segment, which will be related to the likelihood of an odd number of cross-overs within the inversion loop. Imbalance for the non-inverted segments can be ascertained using subtelomeric probes for the relevant segments (19, 35)

Melotte et al (36) describe the design and execution of a PGD cycle for an insertional translocation, demonstrating the care that is needed in considering all the possible outcomes of such rearrangements, and the need to design a PGD strategy that adequately detects chromosome imbalance in these cases. For complex rearrangements such as insertional translocations, custom probes may need to be worked up, such that informative probes are available for segments at risk of imbalance. Three- or four-way translocations may be approached either by selection of probes labeled with appropriate fluorochromes, or, if this is not possible, re-hybridization of biopsied nuclei with a second probe set. Carriers of deletions or duplications have a 50% risk of transmitting the abnormal chromosome to their embryos. Where the effect of the imbalance may be variable (for instance the 22q11 microdeletion syndrome), PGD may be sought to avoid a child more severely affected than the parent. In these cases, there may be only a single probe available for the deleted or duplicated segment, in which case two-cell biopsy would be recommended, with transfer only of embryos with a concordant normal result. Figure 3d shows the FISH work-up for a clinical diagnostic cycle for a carrier of a 22q11 microdeletion.

Conclusion

There is considerable heterogeneity in the approach to this application of PGD: some centres use polar body analysis or blastomere conversion (14, 16, 37), whilst others use labelled probes to ascertain copy number of rearrangement segments in blastomere nuclei. For reciprocal translocations, some groups favour a two centromere and one telomere combination (21), or even one centromere and one telomere (Lim et al. 2004) rather than the two telomere, one centromere approach described above. Additionally, the routine biopsy and testing of two cells for every chromosome rearrangement is carried out at some centres (21, 38). The ESHRE PGD Consortium data collection (39) indicated that from within the Consortium, 33 centres routinely carried out PGD for chromosome abnormalities, with an average clinical pregnancy success rate of 24% per embryo transfer. Overall, individual success rates for different centres ranged from 0% to 100% (median 12.5%); the variation is likely to be due to small numbers, the differences in patient groups and practice described above, and it may reflect the quality of the ART performance in the centre in which PGD is based. Our own experience based on a large number of cycles is 33% per ET (40); outside the Consortium other large centres have reported a similar success rate of 35% per ET (23, 41).

Segregation mode	Zygote 2n complement	Segment count			
		TSA	TSB	CSA	CSB
4:0	A,B	1	1	1	1
3:1 Tertiary monosomy	A,der(A),B	1	2	2	1
3:1 Interchange monosomy	A,B,B	1	2	1	2
2:2 Adjacent-2 cross-over in A	A,der(A),der(A),B	1	3	3	1
2:2 Adjacent-1	A,der(A),B,B	1	3	2	2
2:2 Adjacent-2 cross-over in B	A,B,B,B	1	3	1	3
3:1 cross-over in A	A,der(A),der(A),B,B	1	4	3	2
3:1 cross-over in B	A,der(A),B,B,B	1	4	2	3
3:1 Interchange monosomy	A,A,B	2	1	2	1
3:1 Tertiary monosomy	A,B,der(B)	2	1	1	2
2:2 Adjacent-2	A,A,der(A),B	2	2	3	1
2:2 Alternate (normal)	A,A,B,B	2	2	2	2
2:2 Alternate (balanced)	A,der(A),B,der(B)	2	2	2	2
2:2 Adjacent-2	A,B,B,der(B)	2	2	1	3
Anaphase II nondisjunction	A,A,der(A),der(A),B	2	3	4	1
3:1 Tertiary trisomy	A,A,der(A),B,B	2	3	3	2
3:1 cross-over in A	A,der(A),der(A),B,der(B)	2	3	3	2
3:1 Interchange trisomy	A,der(A),B,B,der(B)	2	3	2	3
3:1 cross-over in B	A,A,B,B,B	2	3	2	3
Anaphase II nondisjunction	A,B,B,B,der(B)	2	3	1	4
2:2 Adjacent-2 cross-over in A	A,A,A,B	3	1	3	1
2:2 Adjacent-1	A,A,B,der(B)	3	1	2	2
2:2 Adjacent-2 cross-over in B	A,B,der(B),der(B)	3	1	1	3
Anaphase II nondisjunction	A,A,A,der(A),B	3	2	4	1
3:1 Interchange trisomy	A,A,der(A),B,der(B)	3	2	3	2
3:1 cross-over in A	A,A,A,B,B	3	2	3	2
3:1 Tertiary trisomy	A,A,B,B,der(B)	3	2	2	3
3:1 cross-over in B	A,der(A),B,der(B),der(B)	3	2	2	3
Anaphase II nondisjunction	A,B,B,der(B),der(B)	3	2	1	4
4:0	A,A,der(A),B,B,der(B)	3	3	3	3
3:1 cross-over in A	A,A,A,B,der(B)	4	1	3	2
3:1 cross-over in B	A,A,B,der(B),der(B)	4	1	2	3

Table 1. Reciprocal translocation segment combinations

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