Translocations, inversions and other chromosome rearrangements

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Chromosomal rearrangements have long been known to significantly impact fertility and miscarriage risk. Advancements in molecular diagnostics are challenging contemporary clinicians and patients in accurately characterizing the reproductive risk of a given abnormality. Initial attempts at preimplantation genetic diagnosis were limited by the inability to simultaneously evaluate aneuploidy and missed up to 70% of aneuploidy in chromosomes unrelated to the rearrangement. Contemporary platforms are more accurate and less susceptible to technical errors. These techniques also offer the ability to improve outcomes through diagnosis of uniparental disomy and may soon be able to consistently distinguish between normal and balanced translocation karyotypes. Although an accurate projection of the anticipated number of unbalanced embryos is not possible at present, confirmation of normal/balanced status results in high pregnancy rates (PRs) and diagnostic accuracy. (Fertil Steril® 2017;107:19–26. ©2016 by American Society for Reproductive Medicine.)
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Robertsonian translocations were first described in 1916 by American biologist William Rees Brebner Robertson (1) while studying grasshoppers. Soon thereafter, a group of *Drosophila* geneticists (2) observed the phenomenon of "crossing over" of chromosomes and alluded to the requirement for chromosomal breakage to facilitate recombination. In 1921, A.H. Sturtevant (3) observed that pieces of a chromosome could not only recombine with their homologous chromosome, but occasionally attach to a separate chromosome—the first description of a reciprocal translocation. Subsequent population-based studies observed that these structural imbalances were associated with an increased risk of cancer and developmental delay.

Identification of chromosomal translocations as a cause of recurrent pregnancy loss (RPL) did not occur until 1962, when Schmid (4) first characterized an inherited translocation in a couple with multiple pregnancy losses. Additional studies (5, 6) followed and the incidence of balanced translocations was ultimately determined to be 1 in 500 in the general population. The incidence is, however, substantially higher in patients with RPL. A large database study (7) in Quebec estimated that a translocation is present in 2.2% of couples after one miscarriage, 4.8% after two miscarriages, and 5.7% after three miscarriages.

Obtaining a karyotype is now a basic and essential component of the RPL work-up. Historically, however, couples carrying a translocation had no therapeutic options for reducing the risk of pregnancy loss. Furthermore, couples carrying a translocation associated with developmental delay or fetal anomalies were left with the prospect of invasive prenatal testing and a decision regarding whether or not to terminate their pregnancy if an unbalanced translocation was detected. The development of IVF and advanced preimplantation molecular diagnostics has allowed many patients to actively manage their risk of conceiving a pregnancy carrying a translocation. The increased sensitivity of these technologies and the more liberal use of the preconception genetic evaluation have created new challenges for practitioners and patients. More patients are being diagnosed with rearrangements and these clinical scenarios are stretching the genetic vocabulary and counseling acumen of clinicians. With these issues in mind, this review seeks to summarize important concepts in chromosomal rearrangements and review the state of the art in molecular diagnostics.

TERMINOLOGY

Reciprocal Translocations

Reciprocal translocations occur when two nonhomologous chromosomes exchange segments. If no genetic material is gained or lost and if the breakpoints do not result in truncation of a gene, patients can be phenotypically normal.

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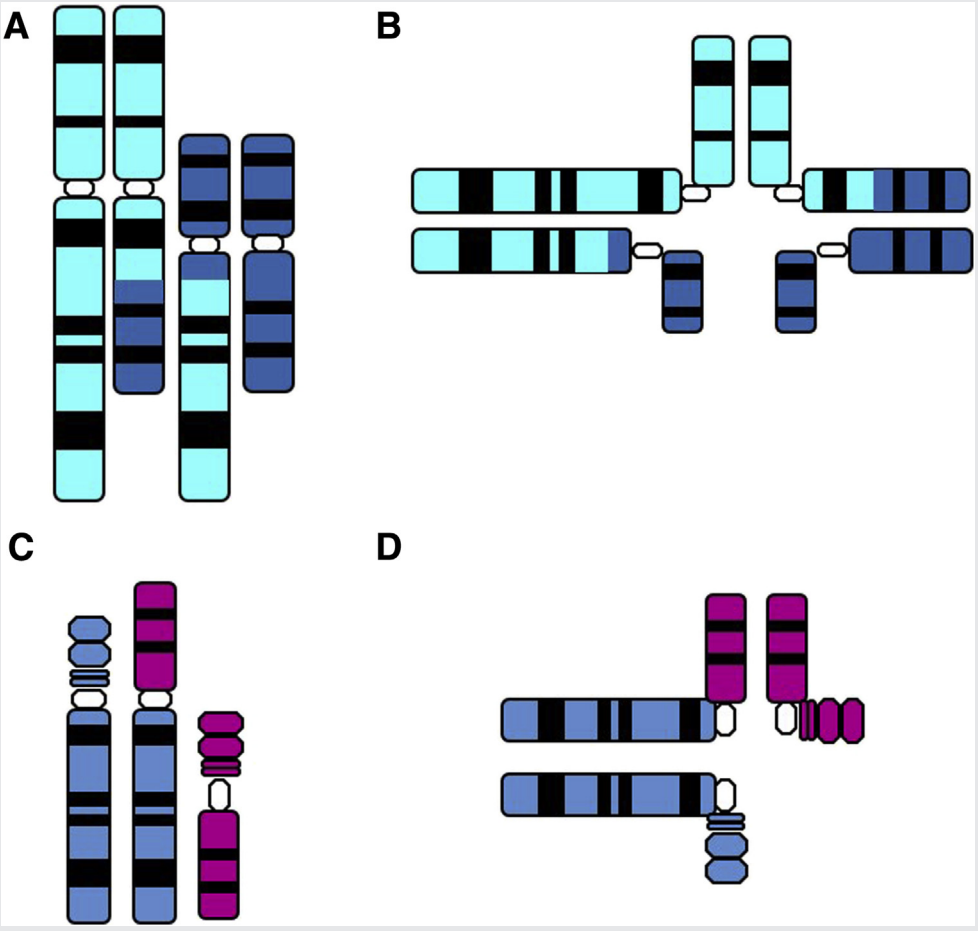
FIGURE 1



Alignment of translocations before segregation. Balanced translocations (**A**) form a quadrivalent structure during meoisis (**B**), before segregation and formation of gametes. Robertsonian translocations (**C**) form structures known as trivalents (**D**).

Morin. Translocations and other rearrangements. Fertil Steril 2016.

However, patients with reciprocal translocations are at a significantly increased risk of infertility and RPL. Although most reproductive failures are due to the generation of unbalanced gametes, additional mechanisms can significantly impact translocation carriers' fertility. For instance, men with translocations involving the X chromosome are prone to complete spermatogenic arrest due to incomplete inactivation of the X chromosome (7, 8).

Certain rearrangements between highly homologous regions at increased risk of recombination are seen with greater frequency in the general population. Hot-spots can be found at 11q23, 17q11, and 22q11, thus leading to the recurrent translocations t(11;22) and t(17;22) (9). Of note, the site on chromosome 22 that is often implicated in translocations is the same locus associated with DiGeorge/velocardiofacial syndrome (22q11 deletion disorder).

Robertsonian Translocations

Robertsonian translocations occur when two acrocentric chromosomes (13, 14, 15, 21, and 22) fuse at the centromere (10, 11). The incidence of Robertsonian translocations is 0.1% in the general population, 1.1% in patients with RPL, and 3% in infertile men (12, 13). These translocations can occur between homologous chromosomes, but they are more commonly observed between nonhomologous pairs (14). Although the short p-arms of the translocated chromosomes also fuse, the resulting rearrangements are often lost with early cell divisions. This loss has little impact on cell function as these p-arms are redundant and no unique genes are found in these regions. However, at meiosis the fused long arms persist and form trivalents (Fig. 1), which have the potential to produce nullisomic or disomic gametes. Resultant fertilizations produce either monosomic or trisomic zygotes.

Rearrangements involving chromosomes 13 and 14 (der(13;14)(q10;q10)) account for 75% of Robertsonian translocations (12). Pregnancies from carriers of this translocation can produce viable gestations with Patau syndrome (trisomy 13). However, most conceptions in der (13; 14) carriers result in early pregnancy loss (14), with only 0.4% of second trimester prenatal diagnostic tests demonstrating an

unbalanced result. The risk of a viable, unbalanced gestation is much higher for carriers of Robertsonian translocations involving chromosome 21. However, this risk is dependent on the parental origin of the translocation. Pregnancies with a maternal origin of the translocation demonstrate a significantly increased chance of a positive second trimester screen than those with a paternal origin (15% vs. <0.5%) (15).

Inversions

An inversion occurs when a piece of a chromosome breaks at two points and reinserts within the same chromosome. There are two types of inversions: pericentric and paracentric. Pericentric inversions involve the short and long arms (p-arm and q-arm, respectively) of the chromosome and include the centromere. Paracentric inversions occur in one arm of the chromosome and do not include the centromere (11).

Unbalanced paracentric inversions produce gametes that have either no centromere (acentric) or two centromeres (dicentric) and are thus not viable. Any efforts in preimplantation genetic testing serve to improve reproductive efficiency and not to prevent the birth of an anomalous child. However, unbalanced pericentric inversions can result in live born children with birth defects due to the presence of partial trisomy or partial monosomy. The overall risk for a carrier for pericentric inversion to have a child with an unbalanced chromosome rearrangement is estimated at 5%–10% (11). Further risk estimates can be calculated based on the size of the inverted region, as the chance of meiotic imbalances correlates with the size of the inverted segment in proportion to the length of the chromosome. The risk of recombinant chromosomes becomes a factor if the inverted segment constitutes >30% of the total chromosome length and risks are considered significant once the inverted segment exceeds 50% (16, 17).

Complex Rearrangements

Complex chromosomal rearrangements refer to structural rearrangements involving more than two breakpoints and often more than two chromosomes. With fewer than 300 cases reported in the literature, complex chromosomal rearrangements resulting in viable offspring are rare (18). Complex chromosomal rearrangements are generally divided into three classes: [1] three-way rearrangements involving the formation of three derivative chromosomes from three original chromosomes that have broken and exchanged segments, [2] independent reciprocal translocations involving two different sets of chromosomes, and [3] other complex rearrangements that include various derivative chromosomes and multiple breakpoints, which are referred to as exceptional complex chromosomal rearrangements (19).

Carriers of a complex rearrangement are at a significantly increased risk of spontaneous abortion and fetuses with congenital anomalies due to the multiple opportunities for unbalanced chromosomal configurations. However, specific risk figures are difficult to ascertain as most complex chromosomal rearrangements occur as de novo events (20). Thus, estimates are empiric in nature. When familial rearrangements are identified, a reproductive risk assessment can be tailored to the specific family history. In most of reported cases, the incidence of RPL and intellectual disability are significantly increased (21).

Marker Chromosomes

Supernumary marker chromosomes are structurally abnormal chromosome fragments that cannot be characterized fully by conventional cytogenetic techniques. These centric chromosomes can originate from any of the 24 chromosomes and their frequency in the general population is estimated to be approximately 0.04% (22). However, the incidence is increased three-fold in infertile couples and they are more commonly found in the male partner (23). Most cases (70%) occur as de novo events and involve acrocentric chromosomes. In the case of familial inheritance, maternal transmission occurs more frequently than paternal transmission (24).

Approximately two-thirds of supernumary marker chromosome carriers exhibit no identifiable phenotype. The remainder exhibit a variety of clinical features, ranging from subfertility, such as azoospermia or oligospermia in men, to birth defects and/or intellectual disability including defined syndromes such as Pallister-Killian syndrome (25). When a marker chromosome is detected by conventional G-banding karyotype, microarray or FISH analysis is the next appropriate follow-up to determine the chromosome origin of the extra material, prior to referral to a PGD laboratory.

Ring Chromosomes

Ring chromosomes occur when two breaks are created in one chromosome and the resulting ends fuse to form a ring. These are rarely seen and typically occur as de novo events. Most individuals with ring chromosomes exhibit a phenotype associated with that specific ring chromosome, which may include dysmorphic features and intellectual disability, and therefore are less likely to present for fertility treatment (10).

Common Polymorphisms

Polymorphic inversions involving heterochromatic regions of chromosomes 1, 2, 9, 10, 16, and Y are frequently seen. The most common is inv(9)(p11q12)/(p11q13), which is present in 1%–3% of the population (26). Although these normal variants are not associated with unbalanced rearrangements in offspring (10), it is important to note the breakpoints of these inversions (Table 1), as other inversions on these chromosomes could be associated with reproductive pathology. Some investigators have reported an increased risk of infertility in carriers of these variants, but further study is needed to make a more definitive determination (27, 28).

PGD TECHNOLOGIES

Platforms for Detection of Unbalanced Rearrangement Products

Although a standard G-banding karyotype is typically used to detect gross chromosome rearrangements from peripheral blood and prenatal samples (chorionic villi and amniocytes),

TABLE 1

Common chromosome inversion polymorphisms.

inv(1)(p11q12)
inv(2)(p11.2q13)
inv(3)(p11q11)
inv(3)(p11q12)
inv(3)(p13q12)
inv(5)(p13q13)
inv(9)(p11q12)
inv(9)(p11q13)
inv(10)(p11.2q21.2)
inv(16)(p11q12)
inv(16)(p11q13)
inv(Y)(p11q11)

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G-banding requires cells to be cultured to obtain a metaphase spread. This method is not applicable to preimplantation embryos, as most cells are caught in interphase and banding cannot be detected on chromosomes. Thus, the development of different strategies for detecting unbalanced chromosome derivatives was required for the application of PGD for rearrangements. These methodologies have evolved in the past 25 years from targeted hybridization to next-generation sequencing (NGS). This section will review the shift in technology during this time and discuss the current state of the art.

The first successful clinical application of PGD for translocation detection was published in 1998 when Munne et al. (29) reported two deliveries after polar body (PB) biopsy and FISH-based selection of normal/balanced embryos for two maternal carriers of Robertsonian translocations. However, performing FISH on polar body biopsies proved technically challenging and early reports indicated a failed diagnosis rate of up to 24% due to poor fixation and chromosomal clumping (30). Paternal translocations were also unable to be detected with this methodology. As a result, subsequent attempts at FISH-based translocation detection focused on testing blastomeres from cleavage-stage embryos (31, 32).

However, several technical limitations continued to significantly impact the diagnostic accuracy of FISH-based techniques. Many investigators raised concerns that inconsistent fixation, signal splitting and failed hybridization compromised the fidelity of results (33, 34). These concerns were confirmed when more advanced molecular diagnostics were compared directly with FISH. One study reanalyzed 50 embryos previously diagnosed as abnormal by cleavage-stage FISH with single nucleotide polymorphism (SNP) microarray and found that 58% were euploid in each of four separate blastocyst biopsies. However, the most important technical limitation of FISH for translocation detection was its inability to simultaneously evaluate all 24 chromosomes for aneuploidy, thus leaving open the possibility of a concomitant copy number error in a chromosome unrelated to the translocation.

The importance of simultaneous comprehensive aneuploidy screening was illustrated first by Treff et al. (35) in 2010. In a study of embryos from 15 translocation carriers evaluated with SNP microarray, standard FISH analysis of chromosomes 13, 16, 18, 21, and 22 would have missed 70% of aneuploidies unrelated to the targeted translocation. A separate study (33) compared pregnancy rates (PRs) for translocation carriers whose preimplantation diagnostics were performed with FISH versus SNP microarray. Ongoing PRs were significantly improved with SNP microarray for both reciprocal (69% vs. 38%; $P<.01$) and Robertsonian translocation carriers (74% vs. 39%; $P<.01$). Using extended culture and trophectoderm biopsy, the percentage of samples with an interpretable result is also significantly improved versus FISH. This has been reported at 93%–98% in different trials (32, 36).

Array comparative genomic hybridization (CGH) emerged soon after SNP microarray as an option for concomitant comprehensive aneuploidy assessment during preimplantation translocation evaluation. Two reports (37, 38) of successful clinical application of array CGH were published in 2011. Fiorentino et al. (37) reported successful diagnosis in 93.5% (187/200) embryos from 28 couples harboring a translocation. Clinical pregnancy was achieved after 70.6% of ETs in this trial. In a series of 16 translocation carriers whose embryos underwent either PB, cleavage stage, or trophectoderm biopsy, Alfarawati et al. (38) reported achieving a diagnosis in 91.7% of embryos. However, the delivery rate was only 27% per ET.

An important consideration when assessing modern strategies for diagnosing chromosomal translocations is the resolution of each platform to detect these small segmental imbalances. Resolution has been reported for CGH (10–20 Mb and 25–100 Mb) (39, 40), array CGH (2.5 and 2.8 Mb) (37, 38), and SNP microarrays (2.4 and 5 Mb) (41, 42). However, the resolution for a given translocation is heavily dependent on the location of that translocation, thus limiting generalizability to subsequent cases. Diagnostic laboratories must assess each couple presenting for chromosome rearrangement PGD to ensure that their platform can detect all possible abnormalities that can be derived from the rearrangement.

As more laboratories shift to NGS for whole chromosome aneuploidy screening, a natural evolution to simplify laboratory workflow would be to also use NGS to detect segmental abnormalities associated with chromosome rearrangements. However, there are limited data on the success of this approach at present. Bono et al. (43) used the same whole genome amplification (WGA) products from 145 embryo biopsies (from 33 couples carrying chromosome rearrangements) initially tested by array CGH and prepared NGS libraries that were subsequently run on Ion Torrent PGM sequencers. They reported the smallest fragment detected by NGS was 5 Mb, and a 2.8-Mb fragment previously detected by array CGH was missed by NGS. In addition, a recent publication (44) noted the success of one NGS platform, referred to as CNV-Seq. In this study, PGD was performed for 21 translocation carriers. Nine patients proceeded to ET and the investigators reported a clinical PR of 62%. All karyotypes from invasive prenatal testing matched the preimplantation diagnoses in this cohort (44).

Additional Considerations

Because Robertsonian rearrangements involve whole chromosome imbalance, any platform validated to detect whole

chromosome aneuploidy is sufficient to screen embryos in a couple that carries a Robertsonian rearrangement. This typically allows Robertsonian translocation carriers to enter treatment more expeditiously, as the precycle PGD work-up is not required. An added benefit in these patients is the opportunity to perform a fresh ET if the standard aneuploidy screening workflow allows for rapid turnaround of results.

The increased utilization of aneuploidy screening may have the unexpected effect of increasing the diagnoses of inherited chromosomal rearrangements. Treff et al. (45) reported the incidental finding of suspected familial chromosome rearrangements while performing aneuploidy screening in three cases. In each case, the tested embryos in a single cohort displayed patterns of reciprocal imbalance, which prompted parental karyotyping and confirmation of translocation carrier status. These cases can be expected to become more frequent as utilization of aneuploidy screening increases and as the resolution for diagnosing segmental imbalances improves with NGS platforms. Thus, validation of thresholds for accurate diagnosis of segmental imbalances is essential to establish cost effective criteria for karyotype evaluation of parents and embryos based on these results.

TECHNICAL CHALLENGES IN PGD FOR TRANSLOCATIONS

Uniparental Disomy

Due to the complex segregation patterns associated with chromosomal rearrangements, there is an increased risk of uniparental disomy (UPD) in the offspring of translocation carriers (46). Uniparental disomy is defined as the inheritance of both homologues of a chromosome from a single parent and no contribution from the other parent. This phenomenon is thought to be primarily the result of trisomy rescue, an attempted self-correction mechanism that occurs when a trisomic cell attempts to restore disomy by excluding one of the three homologues present. This can result in inheriting two copies of the same chromosome from one parent (isodisomy) or inheriting a chromosome pair from a single parent (heterodisomy).

Uniparental disomy is well established as the mechanism mediating many genetic syndromes, particularly those involving chromosomes 6 (transient neonatal diabetes mellitus), 7 (Russell-Silver syndrome), 11 (Beckwith-Wiedemann syndrome), 14 (Temple syndrome), and 15 (Prader-Willi and Angelman syndromes). The American College of Medical Genetics and Genomics recommends that UPD testing should be offered on a fetus or child for which prenatal testing detected mosaicism of one of the clinically relevant chromosomes, or a Robertsonian translocation that involves chromosome 14 or 15; thus, in similar fashion, PGD laboratories should also evaluate the presence of UPD in embryos with rearrangements involving the clinically relevant chromosomes.

It is important to note the limitations of the various PGD platforms in detecting UPD. Array CGH and quantitative polymerase chain reaction (PCR) are not capable of detecting either isodisomy or heterodisomy (47); however, SNP microarray has been reported to detect both (48–51). Although most laboratories are transitioning to NGS for whole chromosome aneuploidy detection, none of the current NGS platforms are capable of detecting UPD at a single or partial chromosome resolution. Thus, development and validation of UPD detection warrants careful attention before NGS platforms are applied to translocation cases. Patients with embryos that have been deemed "normal" or "balanced" should be counseled appropriately on the risk of UPD, and the limitations on the detection of UPD on the platform being utilized for PGD.

Telomeric or Subtelomeric Translocations

One limitation of all modern platforms is the inability to reliably detect unbalanced derivatives from rearrangements that have breakpoints in the telomere or subtelomere. Fluorescent in situ hybridization is currently the only technique available to address proper coverage and probe hybridization to the subtelomeric region; however, the standard limitations of FISH apply and misdiagnoses have been reported (52). Given these limitations it is important to allow the PGD laboratory to evaluate each rearrangement's breakpoints against the current methodology to ensure that all unbalanced products can be accurately detected (often referred to as a work-up).

BALANCED VERSUS NORMAL

Until recently, no molecular technique has been able to reliably distinguish embryos that carry a balanced translocation from those with a normal karyotype. Although there is no expected phenotype difference between a balanced and a normal embryo, many couples would prefer to transfer a normal embryo versus a balanced one so that the child can be relieved of any future reproductive problems related to the rearrangement.

Treff et al. (41) reported the first case to demonstrate successful classification of an embryo as normal, with the karyotype of the child confirmed at birth. Subsequently, the same group of investigators (53) expanded the strategy further to use SNPs near translocation breakpoints that are informative between the balanced translocation carrier and the embryos to track which chromosomes are inherited by balanced/normal embryos. If present, unbalanced embryos produced in the same cohort can also be used to determine which of the two alleles are linked to the chromosomes involved in the translocation. Using this approach, the assigned balanced versus normal calls correctly predicted karyotypes in 10 babies born through translocation preimplantation assessment. If these results are confirmed in prospective studies, couples who previously have had their embryos tested by SNP microarray and still have unused embryos frozen could potentially have those embryos reanalyzed to distinguish between balanced and normal. The SNP microarray is currently the only platform with any published data demonstrating this capability. It is likely that most NGS platforms will be too limited by shallow sequencing to perform a similar analysis.

OUTCOMES AFTER PGD

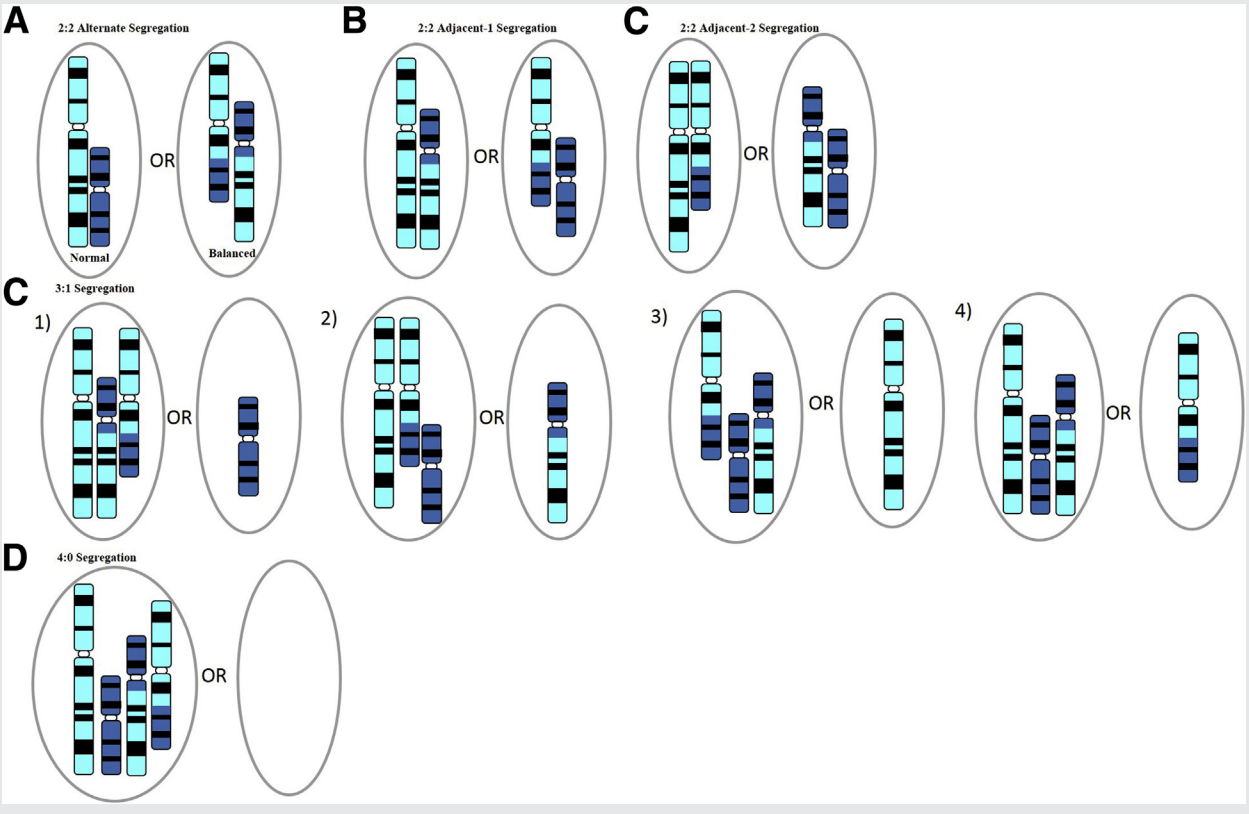
How Many Embryos Will be Normal?

Patients pursuing PGD for reciprocal chromosomal translocations frequently ask clinicians and genetic counselors to estimate the proportion of normal/balanced embryos anticipated in a given cycle. Comprehensive counseling of patients in this scenario requires a working understanding of segregation dynamics in chromosomes containing rearrangements. During meiosis I, the four chromosomes involved in a balanced translocation align in a cross-shaped configuration referred to as a quadrivalent (Fig. 1). Homologous centromeres then separate based on one of three characterized modes of separation: 2:2, 3:1, or 4:0 (Fig. 2). Only alternate 2:2 segregation can result in normal/balanced gametes. Adjacent 2:2 segregation generates unbalanced gametes because a derivative chromosome is inherited, which can lead to a fetus with multiple abnormalities. Both 3:1 and 4:0 segregation produce unbalanced gametes only capable of trisomic or monosomic conceptions. These segregation patterns have been observed in PGD cases, but never in pregnancies (10). Thus, of the 16 possible outcomes of segregation of translocation chromosomes, only two produce balanced or normal gametes.

However, the proportion of gametes that fall in each category cannot be accurately predicted for a given reciprocal translocation. In sperm, the incidence of an unbalanced result of meiosis varies widely from 18%–82% (54, 55). In general, the proportion of unbalanced sperm generated is typically lower than unbalanced oocytes due to more stringent cell cycle checkpoint mechanisms that reduce the production of unbalanced gametes in men (56).

The proportion of normal/balanced embryos depends on the timing of embryo biopsy, as unbalanced embryos are more likely to arrest before the blastocyst stage (63% vs. 36%; $P<.05$) (32). Studies using cleavage stage biopsy for reciprocal translocation PGD have reported proportions of unbalanced embryos as high as 82% (57). Reciprocal translocation studies using trophectoderm biopsy have typically reported rates between 52% and 67% (33, 51). The incidence in Robertsonian translocation carriers is even lower, reported at 23%–33% in recent studies (33, 51). Although a so-called interchromosome effect resulting in an increased incidence of aneuploidy in chromosomes unrelated to the translocation has been proposed, contemporary studies using comprehensive aneuploidy screening techniques have failed to demonstrate this association (32). Clinical PRs after transfer of a

FIGURE 2



Segregation of chromosomes from balanced translocations. Graphic representation of all possible outcomes in gametes produced by a balanced translocation carrier, as described in the text. (**A**) 2:2 alternate segregation results in normal or balanced gametes. (**B**) Adjacent-1 segregation and (**C**) adjacent-2 segregation result in unbalanced gametes, each with one derivative chromosome. (**C**) 3:1 egregation can occur in four different ways (**C1**-**C4**), with all outcomes leaving gametes unbalanced. (**D**) 4:0 segregation also leads to unbalanced gametes where either all chromosomes involved segregate to one gamete, or none, which would cause nullisomy of both chromosomes.

Morin. Translocations and other rearrangements. Fertil Steril 2016.

normal/balanced embryo diagnosed with 24-chromosome platforms are equivalent to euploid embryos produced from nontranslocation carriers, with multiple studies reporting PRs approaching 70% (33, 34).

In conclusion, chromosome translocations and other rearrangements are commonly encountered in modern infertility practice. Preimplantation genetic testing and transfer of a normal/balanced embryo has been demonstrated in multiple trials to improve live birth rates and decrease miscarriage rates. Modern platforms offer the added advantage of concomitant aneuploidy screening of chromosomes unrelated to the translocation, thus significantly improving clinical outcomes. These technologies also offer the potential to assess uniparental disomy and may be able to consistently distinguish between balanced and normal embryos in the near future. However, the application of advanced molecular techniques has also highlighted the need for better characterization of the reproductive risk associated with less frequent segmental rearrangements. A combination of improved understanding of the population-based incidence of segmental abnormalities and more precise diagnostic techniques will allow optimization of the reproductive care of these patients.

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