# Chapter 5
# The Genetics of Pregnancy Failure

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Having a normal genetic composition is a necessary, but not sufficient, requirement for an embryo to implant and progress to a healthy delivery. By testing products of conception, it has been known for decades that whole chromosome aneuploidy, primarily trisomy, is the leading cause of failure of clinically recognized pregnancies. Clarifying the role of aneuploidy and other genetic abnormalities in the failure of embryos to implant has been more elusive but was assumed to be an important factor based on the strong association of advancing reproductive age and infertility. The development and rapid utilization of assisted reproductive technologies (ART) have provided invaluable insight into the genetic causes of failed embryonic implantation. The application of robust genetic testing platforms—from microarrays to real-time polymerase chain reaction (PCR) to next-generation sequencing (NGS)—to test the genetic status of gametes and preimplantation embryos has improved our understanding of the genetic contribution to an ongoing conceptus. Recent advances have focused on the impact of segmental imbalances and mosaicism on implantation and progression to normal deliveries. Future ART research will focus on other genetic causes that influence the ability of a euploid embryo to implant.

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## The Role of Genetics in Early Pregnancy Failure

### *Cytogenetic Findings in POCs*

Approximately 20% of clinically detected pregnancies result in a loss, with over 50% of losses being attributed to a whole chromosome abnormality. An early study reported in 1975 used Giemsa staining (G-banding) to analyze the karyotypes of nearly 1500 miscarriage specimens and found that over 61% of samples had an abnormal karyotype, which included monosomies, trisomies, double trisomies, triploidy, and tetraploidy [1]. As this study and many subsequent studies showed, trisomies are overwhelmingly responsible for first trimester pregnancy loss, most commonly trisomy 16 and trisomy 22. Monosomies and polyploidy account for the majority of the remaining abnormalities. Turner syndrome (45,X) is the most common monosomy finding in first trimester miscarriages. Although Turner syndrome is a viable aneuploidy, nearly 99% of 45,X fetuses spontaneously abort [2].

While G-banding is able to identify the majority of abnormalities, the method is not able to detect maternal cell contamination (MCC), which could cause a false-negative result in the case of an apparently normal female (46,XX) miscarriage. More recently, several studies have been published examining the utilization of newer molecular technologies to diagnose products of conception [3–6]. Microarrays using comparative genomic hybridization (CGH) and single nucleotide polymorphisms (SNPs) and NGS generate higher-resolution data, allowing for increases in reportable results and diagnostic yield. Similar to earlier findings, across all four studies (Table 5.1), approximately 50% of products of conception had at least one whole chromosome abnormality detected. Of the remaining samples, 40–48.4% were left with a normal diagnosis, 2.3–7.5% were triploid, and <0.5% were tetraploid. Now with the ability to detect partial chromosomal abnormalities, the studies reported that 1.3–5.3% had at least one segmental aneuploidy detected (in the absence of a translocation history in a parent). Mosaicism was reported at a very low frequency (0.67%) in the 1975 study, but not in the noted molecular studies.

### *Chromosome Rearrangement History*

Chromosome rearrangements, including balanced translocations, inversions, and Robertsonian translocations, are often implicated in the etiology of recurrent pregnancy loss [7–9]. In the general population, approximately 1 in 500 individuals is likely to carry an apparently balanced chromosome rearrangement [10, 11]. Carriers of a balanced rearrangement typically are asymptomatic but present with fertility issues generally in the form of recurrent pregnancy loss due to the risk of a fetus inheriting an unbalanced derivative of the rearrangement. Thus, the recurrent

**Table 5.1** Genetic characterization of products of conception

| | Boue (1975) | Levy (2014) | Maslow (2015) | Shen (2016) | Sahoo (2016) |
|---|---|---|---|---|---|
| No. of samples | 1498 | 2392 | 62 | 436 | 8118[a] |
| Platform | G-banding | SNP microarray (ILMN) | SNP microarray | aCGH (ILMN) and NGS (WGS on PGM) | SNP microarray (81.6%), array CGH (BAC and oligo) (18.4%) |
| Fresh or paraffin POC? | Fresh | Fresh | Paraffin | Fresh | Fresh and FFPE |
| Result rate | NR | 99.9% (2389/2392) | 71% (44/62) | 100% | 91.1% (7396/8118) |
| Resolution of segmental aneuploidy | | >10 Mb | 5 Mb (1–5 Mb clinically relevant) | ~4 Mb to 111 Mb | >2.4 Mb (BAC aCGH), 112 kb (oligo aCGH), 20 kb (SNP array) |
| *Genetics* | | | | | |
| Maternal cell contamination | NR | 22% (528/2392) | 24% (15/62) | NR | NR |
| Normal | 38.5% (577/1498) | 40.6% (755/1861) | 43% (19/44) | 48.4% (211/436) | 44.3% (3272/7396) |
| Aneuploid | 42.5% (636/1498) | 50.8% (945/1861) | 54.5% (24/44) | 43.1% (188/436) | 42.9% (3176/7396) |
| Partial aneuploidy (no hx of translocation) | NR | 1.3% (24/1861)[b] | NR | 5.3% (23/436) | 1.7% (127/7396) |
| Triploid | 12.2% (183/1498) | 6.1% (114/1861) | 2.3% (1/44) | 3.2% (14/436) | 7.5% (554/7396)[c] |
| Tetraploidy | 3.8% (57/1498) | 0.2% (4/1861) | NR | NR | 0.03% (2/7396) |
| Uniparental disomy (UPD) | NR | 0.16% (3/1861) | NR | NR | 0.5% (37/7396) |
| Mosaicism | 0.67% (10/1498) | NR | NR | NR | NR |

*NR* not reported, *ILMN* illumina
[a]Includes 99 non-POC samples
[b]Includes marker and isodicentric chromosomes
[c]FISH used on fresh, non-SNP array cases

pregnancy loss population likely has a higher incidence of chromosome rearrangements, and a recurrent pregnancy loss work-up usually includes obtaining a karyotype on the patient and partner, and if a rearrangement is found, miscarriage can be avoided by using preimplantation genetic diagnosis to select for balanced or normal embryos [12]. Robertsonian translocations are the products of the fusion of two acrocentric chromosomes (13, 14, 15, 21, and 22) and are found at an increased frequency in patients with recurrent pregnancy loss [13, 14].

## *Single Gene Disorders and Recurrent Pregnancy Loss*

While relatively rare in comparison to aneuploidy in pregnancy, there are several single gene disorders (SGD) that are associated with recurrent pregnancy loss or fetal demise.

Some autosomal recessive disorders that present with multiple congenital anomalies can have lethal presentations in utero. Smith-Lemli-Opitz (SLO) is caused by deficiency in an important component of cholesterol metabolism, 7-dehydrocholesterol (7-DHC). Mutations in the *DHCR7* gene, which encodes 7-DHC, cause SLO, and approximately 1 in 30 to 1 in 70 individuals in the general population are thought to be carriers of a single mutation in *DHCR7*. Congenital disorder of glycosylation type Ia (CDG-Ia) is caused by mutations in the *PMM2* gene and has a carrier frequency of approximately 1 in 70 European Caucasians.

Interestingly, the reported carrier frequencies of these disorders are much higher than expected if calculated using the disease incidence. For example, SLO incidence in Canadian and European populations ranges from 1/60,000 to 1/20,000, which would suggest that the carrier frequency in these populations is approximately 1/120 to 1/70, respectively. However, laboratories performing carrier screening for SLO are finding the carrier frequency closer to 1/40 to 1/50 [15]. Keeping in mind that most labs screen for only common mutations, this suggests that the carrier frequency could be even higher and that either the disease is significantly variable and underreported or there is a significant amount of fetal demise associated with the disorder. The W151X mutation in *DHCR7*, when homozygous, has been reported in first trimester miscarriages [16]. The same can apply to CDG—the R141H mutation in *PMM2* is also thought to be lethal when homozygous [17], and to date, no homozygotes have been reported [18]. Both of these mutations can be screened for on most expanded carrier screening panels, and this testing could be considered during a recurrent pregnancy loss work-up.

There are also genes involved in chromosome segregation that, when mutated, can be implicated in pregnancy loss. *SYCP3* is a gene primarily involved in homologous chromosome pairing and recombination. Loss of SYCP3 in mice is associated with male infertility and decreased fertility in females. In humans, the T657C variant in SYCP3 has been very strongly associated with recurrent pregnancy loss [19].

Complete (CM) and partial (PM) hydatidiform molar pregnancies are typically isolated events for a patient; however, some patients have been found to have recurrent molar pregnancies. CM most often arise from the inheritance of all 46 paternal chromosomes and no maternal chromosomes. PM have a different pathology and are typically due to triploidy (69,XXX or 69,XXY). Mutations in either NLRP7 or KHDC3L are associated with recessive inheritance of recurrent molar pregnancies [20].

It is well known that some losses can be attributed to mutations or polymorphisms in coagulation pathway genes, such as Factor V, prothrombin, and Factor II. A recent meta-analysis was performed and found 37 genes that have strong associations with pregnancy loss due to hyperactive immune response, thrombophilia, abnormal placental function, and disruption in the regulation of metabolism [21].

## The Role of Genetics in Implantation

Whereas the genetic contribution to pregnancy failure has been well established by studying miscarriage specimens, understanding the role of the genetics in the preimplantation embryo's ability to successfully implant has been more elusive. Though challenged in recent years by animal and preliminary human studies proposing the presence of oogonial stem cells [22], the established dogma of human oocyte physiology remains that women are born with their lifetime endowment of approximately 1–2 million follicles and oocytes. While the menopause and the complete exhaustion of the follicle pool herald an absolute barrier to successful pregnancy, there is a well-established age-related decline in fertility, likely related to the decline in oocyte quantity and quality. The gradual decline in oocyte quantity, which accelerates after age 37, has been documented by studying tissue specimens at the time of oophorectomy [23].

Although some markers, such as an elevated serum follicle-stimulating hormone (FSH) levels on day 3 of the menstrual cycle, have been correlated with a reduced chance for a viable pregnancy, there is no definitive assay for oocyte quality. A good-quality oocyte can be considered one of the sufficient qualities to complete meiosis, achieve cytoplasmic and nuclear maturity to allow for normal fertilization after activation by viable spermatozoa, and then develop into an embryo capable of implantation and progression to a normal viable neonate. Several lines of evidence support the proposition that oocyte quality declines with increasing age and that this decline accelerates in the late 30s and even more rapidly in the early 40s. In historical populations that predated contraception and family planning, there was a clear decline in fertility rate with increasing maternal age [24]. While compelling, this association does not prove that the aging oocyte, and likely chromosomal aneuploidy, fully explains this decline in fecundity. Several other possible explanations exist, including a decline in sperm quality and function, decreased coital activity, increased risk of uterine abnormalities such as leiomyomas and synechiae, and increased risk of other medical comorbidities.

One model that could correct for several of these variables is women seeking to conceive with timed intrauterine insemination using thawed sperm from fertile donors. This population includes presumably fertile women requiring the use of donor sperm because they are single, are lesbian, or have a partner with azoospermia. The CECOS study evaluated 2193 married French women who underwent donor sperm and timed insemination because their husbands were azoospermic [25]. This study found a significant decline in the chance for pregnancy, with 73% of women under age 31 conceiving within 12 cycles, compared with 54% over age 35 ($P < 0.001$). The decline would likely be even sharper if women over age 40 were analyzed separately. This diminution in live birth rate most likely reflects an increase in the chance of mature oocytes being released that are not of sufficient quality to implant and progress to delivery. Other studies suggest that the decline in fecundity is primarily related to an age-related decline in oocyte quality, independent of quantity. One study from Ottawa found that women using timed donor insemination had

a similar chance of conceiving whether they had low or high antral follicle counts, a marker of ovarian reserve [26]. In women attempting to conceive, a low AMH level—another reliable marker of ovarian reserve—does not appear to be predictive of natural fertility [27], indicating that the decline in oocyte quality is mostly related to advanced reproductive age rather than simply depletion of the follicular pool.

While the aging oocyte is less likely to result in a viable offspring, there are several potential causes for this including genetic (increased risk of aneuploidy, mosaicism, de novo segmental imbalances or mutations, epigenetic changes), cytoplasmic (increase in mitochondrial dysfunction, perhaps due to accumulation of reactive oxygen species from dysfunctional recycling of organelles/autophagy), or reduced ability of the uterus to facilitate implantation of a viable embryo. The advent of ART and its clinical application has shed light on these factors, confirming the pivotal role of genetics in embryonic competence.

Even before there was the ability to reliably assess the chromosomal status of preimplantation embryos, the relationship between increased maternal age and decreased rates of successful implantation became apparent. The first successful application of in vitro fertilization performed by the late Sir Robert Edwards (Nobel Laureate 2010) and Patrick Steptoe was in a 29-year-old woman, at the peak of her fertility, who had tubal factor infertility. The early practitioners of IVF attempted to compensate for diminished oocyte and embryo quality by stimulating multiple follicles to mature with the use of exogenous gonadotropins extracted from human menopausal urine. Since the average embryo was not capable of progressing to delivery, multiple embryos would routinely be transferred to the uterus. Even still, pregnancy rates in women of advanced reproductive age remained dismal, and miscarriage rates increased with increasing age. Schieve et al. found that miscarriage rates after IVF increased from 10.1% among women in their 20s to 39.3% for women older than 43 [28]. Similar to the prior spontaneous abortion literature, the most common cause of clinical miscarriage after ART appears to be aneuploidy, accounting for more than half of the losses in most reviews of products of conception after ART [33, 29–33]. The rate of aneuploid losses after ART does not appear to differ from natural conceptions, though one review reported a higher risk from intracytoplasmic sperm injection (ICSI) as compared to conventional insemination to achieve assisted fertilization [34]. Similar to natural conceptions, autosomal trisomy accounts for most of the aneuploid miscarriages after ART [35].

The introduction of donor oocyte programs further proved the primary role of the aging oocyte's contribution to the age-related decline in fertility. When transferred to the uterus of women of advanced reproductive age, even into the late 40s, embryos created from oocytes donated by women typically in their 20s resulted in successful implantations at rates commensurate with the oocyte donor rather than the recipient age [36]. Thus, it appears unlikely that there is an intrinsic decline in uterine receptivity with increasing maternal age, at least through the mid-40s. Unlike the well-established increase in miscarriage risk with increasing age, pregnancies conceived after oocyte donation had a 13.1% miscarriage risk that did not significantly vary with the age of the recipient. Similarly, delivery rates from egg donation remained high independent of paternal age, mitigating the causative role of sperm

in the age-related decline in fertility [37]. The risk of de novo autosomal dominant mutations, however, appears to increase with increasing paternal age [38], a finding thought to relate to exposure of the paternal genome to reactive oxygen species over time.

Still, while the decline in oocyte quality with age is now well established, the ability to reliably test the genetics of preimplantation embryos was required to determine the relative contribution of genetics to implantation failure.

## Preimplantation Genetic Screening (PGS): First Generation, Limited by Suboptimal Biopsy and Testing Methodology

Given the decreased implantation rates from transferred embryos in older women and the higher risk of aneuploid miscarriages, it seemed reasonable that testing embryos and selecting against aneuploid embryos would enhance IVF success rates. The first attempt at this strategy, given the technology available at the time, relied on biopsy of a single blastomere at the cleavage stage (day 3) of embryo development with subsequent fixation for fluorescence in situ hybridization (FISH) analysis [39]. While intriguing, there were several limitations of this approach. To facilitate biopsy of a single blastomere, embryos had to be placed in a magnesium-calcium-free media that could impact their further development into competent blastocysts. Next removal of one or two out of an embryo with typically six to ten cells was required, representing a substantial portion of the embryo that could impact its developmental competence. Furthermore, the accuracy of FISH, though proven in other clinical settings such as after chorionic villus sampling, was not reliably validated on single blastomeres since there is not a gold standard to retest the same blastomere. Finally, only the chromosomes most often found in clinically recognized abnormal pregnancies (including 13, 16, 18, 21, X, Y) were probed for. It is now known that errors can occur on any chromosome and, therefore, some embryos may have been misdiagnosed as normal. In addition, a reanalysis of embryos predicted to be abnormal by FISH found that 58% were euploid when analyzed by a more robust microarray platform at the blastocyst stage [40], indicating a high false-positive rate.

Retrospective, nonrandomized studies of the application of FISH-based preimplantation genetic screening (PGS) appeared to show benefit, especially for women of advanced reproductive age. However, several randomized trials failed to show benefit, and some even showed a detrimental effect. A meta-analysis reviewed nine randomized trials, five limited to women of advanced reproductive age, and concluded that FISH-based PGS resulted in a lower chance for delivery after IVF (26% vs. 18% per cycle) [41]. The most significant trial was led by Mastenbroek et al. and effectively dealt the death knell to FISH use in clinical ART [42]. In this trial of 408 women who underwent 836 total IVF cycles, those randomized to PGS had a lower live birth rate (24% vs. 35%). A later trial by the group at Instituto Valenciano de Infertilidad (IVI) used day three biopsy and nine chromosome FISH (13, 15, 17, 16, 18, 21, 22, X, Y) and found benefit in women of advanced reproductive age, but not

in those with recurrent implantation failure (≥3 IVF failures) [43]. By the time this trial was published, the field had already advanced to employ a different biopsy technique and more robust genetic screening technologies.

## Preimplantation Genetic Screening: Second Generation, Improved Biopsy, and Comprehensive Testing Platforms

While FISH-based PGS was unable to improve IVF success, it did not invalidate the general principle that selecting chromosomally normal embryos could improve the chance of live birth after IVF. Efforts then focused on using more sophisticated methodologies including SNP microarrays, array CGH, real-time PCR, and then NGS, to better diagnose embryos with aneuploidy by using a new method of PGS, called comprehensive chromosome screening (CCS), to detect the copy number status of all 22 autosomes and the sex chromosomes.

Many CCS platforms begin with whole genome amplification (WGA), which can be performed with any number of commercially available kits such as REPLI-g, GenomiPhi, GenomePlex, SurePlex, or MALBAC. The basic concept is random amplification of the genome such that the resulting product represents the relative quantity and genotypes present in the original sample. Of course, none of the methods of WGA provide a perfect representation, and thus downstream methods of analysis with highly parallel testing of the genome, such as SNP array or array CGH, have been applied in order to help overcome WGA inaccuracies. NGS has also been developed as a downstream analysis method that along with molecular barcoding has helped reduce the costs associated with CCS.

Preclinical studies showed that these technologies could reliably detect chromosome imbalance in samples from cell lines and then from embryos. Given the experience with FISH, the SNP array platform was validated with a "nonselection" trial in which embryos were biopsied and transferred with the clinicians not being privy to the PGS prediction [44]. The biopsies were then analyzed and the result correlated with the clinical outcome of the transferred embryo (using DNA fingerprinting in the case of multiple embryo transfer). The result clearly demonstrated that euploid embryos had a higher chance of implanting successfully than unselected and aneuploid embryos (41.4% vs. 28.2% vs. 4%, $P < 0.001$). The low false-positive rate was low enough to justify discarding abnormal embryos in an effort to enhance outcomes with the selective transfer of euploid embryos. The predictive value of euploid blastocyst implanting was significantly higher than a euploid day 3 cleavage stage embryo (48.2% vs. 29.2%, $P < 0.01$).

A paired randomized trial from the same group was performed to assess the safety of embryo biopsy at the cleavage vs. blastocyst stage. A double embryo transfer was performed in 116 women with one embryo undergoing biopsy and one not biopsied. The biopsy was used to later perform DNA fingerprinting to confirm which embryo is implanted in the case of a singleton delivery. The removal of a single cell on day 3 of development resulted in a significant 39% decrease in implan-

tation potential, whereas removal of approximately five cells from the outer trophectoderm layer of the blastocyst did not significantly impair the chance of the embryo implanting and progressing to delivery [45]. Clinical studies of embryo biopsy for PGD evaluation of a monogenic disorder (beta-thalassemia) found that significantly more blastomere biopsies did not yield a result (25%) compared with trophectoderm biopsy (4%) [46] and the embryos undergoing trophectoderm biopsy were more likely to implant. Polar body biopsy has been proposed as a less invasive form of biopsy [47] since the polar bodies are naturally extruded during oocyte maturation and fertilization. When applied to preimplantation screening for aneuploidy using the SNP arrays, however, analysis of both polar bodies was found to disagree with the subsequent embryo biopsy 30% of the time and was less predictive of implantation potential [48]. Since premature separation of sister chromatids has been shown to be the predominant cause of meiotic errors in the oocyte, an embryo originating from an oocyte with reciprocal errors in the polar bodies often is actually euploid [49, 50]. Thus, it appears that trophectoderm biopsy at the blastocyst stage is the optimal stage for preimplantation analysis [51].

Given the high predictive values of these tests, the next step was to demonstrate clinical benefit in a randomized controlled trial. A trial comparing transfer of a single untested blastocyst vs. a biopsied euploid blastocyst by array CGH demonstrated improved success in a relatively young (<35 years old) patient population [52], with ongoing pregnancy rates of 41.7% vs. 69.1% ($P = 0.009$). Another study compared the transfer of two untested vs. two euploid blastocysts as determined by a validated real-time PCR assay [53]. This randomized trial also showed significant improvement in delivery rates with 84.7% of cycles delivering after transfer of euploid embryos compared with 67.5% of cycles transferring untested embryos [54]. Finally, the Blastocyst Euploid Selective Transfer (BEST) trial demonstrated that in women with normal ovarian reserve up to age 42, transferring one euploid blastocyst was not inferior to transferring two untested blastocysts (60.7% vs. 65.1% ongoing pregnancy rate to 24 weeks gestation) but had a much lower risk of multiples (0% vs. 53.4%) [55]. A follow-up study determined that those women randomized to transfer of a single euploid blastocyst had a much lower risk of having a baby with low birth weight and preterm delivery or requiring NICU admission [56]. A meta-analysis [57] and systematic review [58] both conclude that trophectoderm biopsy and comprehensive chromosome screening to select euploid blastocysts for transfer result in improved outcomes, particularly in good-prognosis patients with normal ovarian reserve.

The increased utilization of PGS clinically has provided a large body of data providing insight into the origins and prevalence of aneuploidy in preimplantation embryos. Retrospective analysis of outcomes using array CGH to screen for aneuploidy found that transferring euploid embryos corrected for the expected age-related decline in IVF pregnancy rates, at least up until age 42 [59]. An analysis of 247 blastomere biopsies from cleavage stage embryos using microarray and parental genotyping confirmed that the origin of aneuploidy can mostly be traced to errors in maternal meiosis [60].

A large clinical experience of the real-time, quantitative PCR CCS platform by Franasiak et al. evaluated 15,169 consecutive trophectoderm biopsies from blastocysts

and found that the rate of aneuploidy remained stable in the low 30% range in the early 30s age group, rising rapidly in the late 30s and reaching 75% by age 42 [61]. The majority of errors in women in their 30s involved a single chromosome error, with the proportion of monosomies and trisomies being roughly equivalent. However, the incidence of multiple chromosome errors increased with age, and more than two-thirds of affected embryos in women over age 43 had more than one abnormal chromosome. In addition, the relative proportion of trisomies increased with advancing maternal age. Another study using the same dataset found an increase in the incidence of abnormalities involving chromosomes that are known to be found in clinically recognized pregnancies resulting in miscarriage [62].

While the array-based and PCR platforms demonstrated benefit in prospective trials, there are limitations. An analysis of 2354 clinically recognized pregnancies achieved after the transfer of euploid embryo testing with PCR found that there was a 0.13% error rate with resulting aneuploid pregnancies [63]. Follow-up testing revealed some of these pregnancies exhibited mosaicism, which is a known limitation of PGS since a prediction of the whole embryo has to be made from a small biopsy. A similar evaluation of pregnancies achieved after aCGH PGS found an error rate of 1.5% in clinical pregnancies [64].

Improvements in massive parallel sequencing technology allowed for the development of NGS at lower cost with the ability to barcode embryos and run dozens of samples on one sequencing chip [65]. A nonselection study of a targeted NGS approach again demonstrated high predictive values with euploid embryos implanting ~58% of the time and none of the predicted aneuploid embryos implanting. The development of NGS also led to the identification of segmental aneuploidy and mosaicism, i.e., a predicted mix of normal and abnormal cells. A nonselection trial for segmental aneuploidy demonstrated a significantly lower implantation rate for embryos harboring a >5 Mb deletion or duplication. Clinical studies have also shown lower chance of ongoing pregnancy from predicted mosaic range embryos and a higher risk of miscarriage [66].

Clinical CCS studies have also demonstrated reduced miscarriage rates. For example, Forman et al. found a significant decrease in clinical pregnancies resulting in a miscarriage when embryos were first screened by CCS (10.5%) compared to untested embryos (24.8%), Sher et al. found a significant reduction from 12% to 4% when incorporating CCS [67], and Keltz et al. found an 11% miscarriage rate in CCS tested embryos compared to 26% in untested embryos [68].

Interestingly, there remains a subset of cases where miscarriage occurred despite the transfer of a chromosomally normal embryo. While there are many possible explanations for this observation, there may be additional genetic causes other than whole chromosome uniform aneuploidy to consider. For example, mosaicism may contribute to some extent. Mosaicism originates from mitotic nondisjunction errors resulting in an embryo with cell lines with differing chromosomal makeup. Some evidence suggests that embryos predicted to be mosaic from a trophectoderm biopsy may possess reduced reproductive potential.

In addition, segmental aneuploidy may also represent a genetic factor that reduces reproductive potential. Many CCS methods have demonstrated the ability

to detect segmental aneuploidy associated with inheritance of unbalanced chromosomes from carriers of a balanced translocation. These same methods may be capable of detecting de novo segmental imbalances. Preliminary data suggests that the majority of de novo segmental aneuploidies are of mitotic origin, making it important to demonstrate the ability to detect mosaic range segmental imbalances in a trophectoderm biopsy.

While these factors are among the most obvious targets for selection of competent embryos, there remains an enormous amount of uncharacterized molecular biology. For example, the preimplantation stage of embryo development represents the most dynamic period of time with respect to epigenetic modification of the embryonic genome. Characterizing the methylome during preimplantation development will undoubtedly improve our understanding of normal embryogenesis and potentially lead to new biomarkers of reproductive potential.

## Conclusion

The evidence is clear that genetics plays an essential role in the ability of a fertilized embryo to progress to delivery of a healthy newborn. Decades worth of data studying products of conception from clinical miscarriages proved that chromosomal aneuploidy is the single largest factor contributing to the failure of established pregnancies to progress to delivery. Historical data also demonstrated that the aging oocyte is the major cause of the age-related decline in fertility, in large part due to the rapid increase in aneuploidy. The development of ART has provided valuable insight, conclusively demonstrating that aneuploidy increases dramatically with age. By using a safe biopsy technique, embryos can be selected for transfer that are chromosomally normal, resulting in a higher chance of delivery and lower risk of miscarriage and ongoing aneuploid gestation. Still, these testing platforms are not perfect, and there are other causes of failed implantation beyond whole chromosome aneuploidy. In addition, there are no proven interventions to reduce the prevalence of age-related aneuploidy in oocytes and embryos. Future developments will likely focus on improved methods of embryo selection to optimize the outcomes with transfer of genetically normal, competent embryos.

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