Embryo selection versus natural selection: how do outcomes of comprehensive chromosome screening of blastocysts compare with the analysis of products of conception from early pregnancy loss (dilation and curettage) among an assisted reproductive technology population?

Jorge Rodriguez-Purata, M.D.,[a] Joseph Lee, B.A.,[a] Michael Whitehouse, B.A.,[a] Rose Marie Moschini, M.S.,[a] Jaime Knopman, M.D.,[a,b] Marlena Duke, M.S.,[a] Benjamin Sandler, M.D.,[a,b] and Alan Copperman, M.D.[a,b]

[a] Reproductive Medicine Associates of New York and [b] Department of Obstetrics, Gynecology, and Reproductive Science, Mount Sinai School of Medicine, New York, New York

Objective: To compare the incidence of numerical chromosomal abnormalities (NCAs) reported after preimplantation genetic screening (PGS) analysis compared with that reported after cytogenetic analysis of products of conception after spontaneous abortion.
Design: Retrospective study.
Setting: Private academic in vitro fertilization center.
Patient(s): Cytogenetic reports of patients who underwent an IVF cycle with PGS of at least one biopsied embryo were compared with cytogenetic analysis reported from patients who had dilation and curettage (D&C) for the treatment of a spontaneous abortion after assisted reproductive technology (ART) treatment.
Intervention(s): None.
Main Outcome Measure(s): Frequencies for each numerical chromosomal abnormality from both groups were compared.
Result(s): A total of 1,069 NCAs were reported after PGS (trisomy 54.3%, monosomy 45.7%, no polyploidies), resulting in a trisomy/monosomy ratio of 0.82. A total of 447 NCAs was reported after D&C (trisomy 83%, polyploidy 10.7%, monosomy 6.3%). The aneuploidies most frequently identified were similar in both groups and included 15, 16, 18, 21, and 22. Monosomies (n = 28, 6.3%) were rarely observed in the group that underwent D&C after ART.
Conclusion(s): This review provides an analysis of the most commonly identified NCAs after PGS and in first-trimester D&C samples in an infertile population utilizing ART. Although monosomies comprised >50% of all cytogenetic anomalies identified after PGS, there were very few identified in the post-D&C samples. This suggests that although monosomies occur frequently in the IVF population, they

Received March 18, 2015; revised August 1, 2015; accepted August 6, 2015; published online September 8, 2015.
J.R.-P. has nothing to disclose. J.L. has nothing to disclose. M.W. has nothing to disclose. R.M.M. has nothing to disclose. J.K. has nothing to disclose. M.D. has nothing to disclose. B.S. has nothing to disclose. A.C. has nothing to disclose.
Reprint requests: Jorge Rodriguez-Purata, M.D., Reproductive Medicine Associates of New York, 635 Madison Avenue, 10th Floor, New York, New York 10022 (E-mail: jrodriguez@rmany.com).

Fertility and Sterility® Vol. 104, No. 6, December 2015 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2015.08.007

commonly do not implant. Despite this difference, this study demonstrated that the specific NCAs observed after PGS analysis and D&C were comparable. (Fertil Steril® 2015;104:1460–6. ©2015 by American Society for Reproductive Medicine.)
Key Words: In vitro fertilization, preimplantation genetic screening, products of conception, dilation and curettage, numerical chromosomal abnormalities

It has been more than 50 years since the identification of the first human with aneuploidy, a term used to describe a loss or gain of genetic material of a chromosome(s) (1, 2). Since this initial observation, aneuploidy has been demonstrated to be an incredibly common event, accounting for no less than 15%–20% of all clinically recognized pregnancies. The majority of aneuploid embryos will never result in a clinical gestation and terminate in utero, making aneuploidy the leading cause of miscarriage, but some are compatible with live birth, making aneuploidy the leading cause of congenital birth defects and mental retardation.

Molecular genetic analysis of parental markers in such products of conception (POC): miscarriage, abnormal pregnancies, and live births) has established that most aneuploidies are female in origin (3). Although there are a variety of segregation events, including mitotic and meiotic errors of both the gametes and embryos, evidence suggests that the majority of segregation errors arise during maternal meiotic divisions (4, 5). Given the biology of the human egg, this is not entirely unexpected. The first stage of female meiosis initiates in the fetal ovary and is followed by a long "arrest" phase that lasts until the time of ovulation. Thus, the first meiotic division is amazingly protracted, taking at least 10–15 years, but elapsing for as many as 45–50 years to completion. Furthermore, observations gleaned from IVF have demonstrated that errors in female meiosis not only occur in non-disjunction but also when two sister chromatids separate prematurely in meiosis I (MI) (6, 7).

In the field of assisted reproductive technology (ART), aneuploidy has been identified as a significant factor contributing to IVF cycle failures, specifically implantation failure and/or spontaneous miscarriage (8). However, recent advances in reproductive medicine and molecular cytogenetics have revolutionized the treatment protocol designed for infertile couples struggling with recurrent aneuploidy losses. Formerly, genetic testing such as chorionic villus sampling, amniocentesis, and cell-free fetal nucleic acids from maternal blood were only available prenatally. With such techniques, if a nonfavorable result was reported, a subsequent termination of pregnancy at varying gestational ages would still be necessary. The advent of preimplantation genetic screening (PGS), particularly trophectoderm biopsy and techniques such as array comparative genomic hybridization (CGH) (9) and quantitative polymerase chain reaction (qPCR)–based comprehensive chromosome screening (CCS) (10), have allowed patients to go "back to the future" by providing important information before pregnancy is achieved, which often averts the need for termination of pregnancy due to aneuploidy. These techniques allow scientists to analyze the copy number in all 23 pairs of chromosomes after whole-genome amplification from single cells and to select euploid embryo(s) before transfer, improving dramatically overall IVF cycle success rates.

Chromosomal abnormalities (CAs) can either be the result of a numerical chromosomal abnormality (NCAs) or a structural chromosomal defect, and may involve either one or multiple autosomal chromosomes or one of the sex chromosomes. They are a common event and are estimated to occur in 20%–50% of human conceptions (11). An NCA can be caused by either a selective loss or gain of an individual chromosome (monosomy and trisomy, respectively) (12) or by the gain of one or more complete haploid chromosome sets (polyploid karyotype). Structural chromosomal abnormalities comprise those modifications coming from one or more fractures in a chromosome (13). For more than 30 years researchers have been studying the prevalence of particular chromosomal aberrations. In 1980 Hassold et al. (14) reported that the risk of having an NCA after a spontaneous pregnancy loss was 50%. The majority of such NCAs resulted from three autosomal trisomies (trisomy 21, trisomy 18, and trisomy 13) and four types of sex chromosomal aneuploidies: Turner syndrome (usually 45X), Klinefelter syndrome (47XXY), 47XYY, and 47XXX. More recently, Werner et al. (15) analyzed the cytogenetics of abortuses in an infertile population and reported a 76% prevalence of chromosomal abnormalities in patients who underwent dilation and curettage (D&C) after treatment with ART (15), a number consistent with a 63% frequency reported by Bettio et al. (16). Moreover, in a large, retrospective cohort study analyzing patients who underwent IVF with PGS/preimplantation genetic diagnosis for aneuploidy, Franasiak et al. (17) demonstrated that in 9,889 aneuploidies identified, 4,513 were monosomies and 4,376 were trisomies. This resulted in a trisomy/monosomy ratio of 0.97, displaying a near equal portion of an embryo's vulnerability to either type of aneuploidy.

The frequency of NCAs in preimplantation embryos has been described in previous studies of preimplantation embryos (after PGS) and postimplantation embryos (after spontaneous abortion); however, to date the prevalence of specific NCAs has not been compared and contrasted among patients in a similar population. Thus, the objective of our study was twofold: first, to assess the incidence of specific aneuploidies both before implantation after PGS, as well as after implantation with cytogenetic analysis of first-trimester D&C specimens from early pregnancy losses; and second, to compare these two groups for the specific aneuploidies identified.

MATERIALS AND METHODS

A retrospective cohort analysis was conducted from July 2002 to July 2014 at a large, academic IVF center. The cytogenetic reports of patients who underwent an IVF cycle with a conclusive PGS result with at least one biopsied embryo (group 1, from January 2012 to July 2014) and those patients who underwent surgical management of a miscarriage with D&C (group 2, from July 2002 to July 2014) were reviewed. No cytogenetic analysis was identified from patients who underwent PGS. With the objective of accounting for frequency, for complex, chaotic numerical abnormalities, each NCA was accounted as one (i.e., a result with 47XX,+16,+18,−21 was summed as three data points). Mosaicism (more than one cell line) reports were excluded. Frequencies for each NCA from both groups were compared. Additionally, frequencies were registered according to the patient's age group: group A <35 year old (yo), group B 35–38 yo, group C 39–40 yo, group D 41–42 yo, group E >42 yo.

Group 1: Cytogenetic Analysis of Biopsied Embryos

Patients underwent an IVF cycle with PGS for aneuploidy screening. Successful fertilization results seen as two-pronuclei zygotes on day 1 were cultured to the cleavage stage. Embryos with at least six cells and <40% fragmentation at the cleavage stage were cultured to day 5/6. Only embryos reaching the blastocyst stage with a grade ≥3BB were biopsied. Trophoectoderm biopsy was performed as previously described (18) on day 5/6 of embryonic life, and embryos were screened using a qPCR–based CCS (10). Embryos were reported to be euploid, aneuploid, or undetermined according to screening results. Only conclusive aneuploid results were included.

Group 2: Cytogenetic Analysis of POC

Specimens evaluated included reproductive losses from treatment cycles, including ovulation induction with or without intrauterine insemination, IVF, or frozen embryo transfer. Ovum donation cycles were excluded. Samples were pre-prepared for analysis in conical tubes, with transport media composed of basal medium, fetal bovine serum, L-glutamine and antibiotics. All samples were packaged and sent to Integrated Genetics (Esoterix Genetic Laboratories) for chromosome analysis (karyotyping) throughout the entire study period.

Because of the retrospective nature of this study, informed consent was not necessary. The study protocol and analysis was approved by the Western Institutional Review Board.

RESULTS

Preimplantation Genetic Screening

For group 1, 557 patients (average [±SD] age 36.9 ± 4.7 years; range, 22.1–46.5 years) with 2,054 embryos were analyzed (day 5: 52.6%; day 6: 47.4%) (3.7 biopsied embryos per patient) (Supplemental Table 1, available online). Of those, 1,178 embryos (57.0%) were reported as normal (day 5: 57.8%; day 6: 42.2%), 818 embryos (40.2%) were reported as abnormal (day 5: 46.2%; day 6: 53.8%), and 58 embryos (2.8%) were reported as undetermined (i.e., non-concurrent) (day 5: 40.1%; day 6: 59.9%) (Supplemental Table 2). From the 818 aneuploid embryos, a total of 1,069 numerical abnormalities were identified: 489 (45.7%) were monosomies, and 580 (54.3%) were trisomies (Table 1, Fig. 1), resulting in a trisomy/monosomy ratio of 0.82. No polyploidies were identified through PGS. The frequency for each abnormality is described in Table 1 and Figure 2.

TABLE 1

Detected numerical chromosomal aneuploidies after PGS.

	PGS			
	Monosomy (n = 489)		Trisomy (n = 580)	
Chromosome	n	%	n	%
1	14	1.3	4	0.4
2	22	2.1	12	1.1
3	14	1.3	1	0.1
4	7	0.7	15	1.4
5	16	1.5	10	0.9
6	8	0.7	14	1.3
7	17	1.6	17	1.6
8	22	2.1	11	1.0
9	29	2.7	6	0.6
10	18	1.7	14	1.3
11	13	1.2	8	0.7
12	10	0.9	4	0.4
13	19	1.8	15	1.4
14	14	1.3	22	2.1
15	56	5.2	42	3.9
16	66	6.2	64	6.0
17	16	1.5	14	1.3
18	25	2.3	32	3.0
19	30	2.8	30	2.8
20	22	2.1	10	0.9
21	55	5.1	46	4.3
22	76	7.1	86	8.0
X	10	0.9	12	1.1
Y	1	0.1	0	0

Rodriguez-Purata. Chromosomal abnormalities with PGS & D&C. Fertil Steril 2015.

FIGURE 1

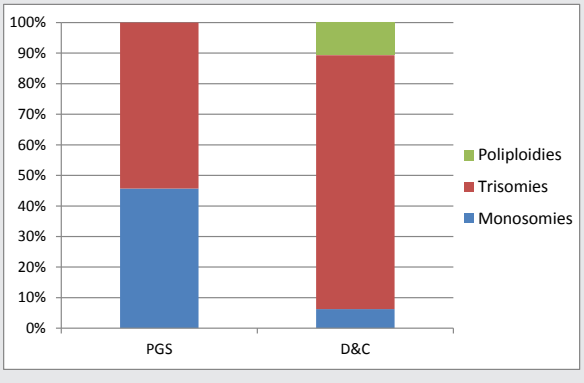
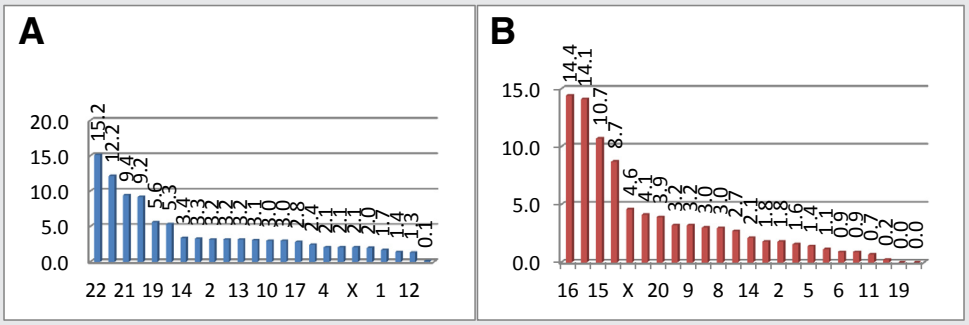


Diagram showing the frequency of aneuploidies.

Rodriguez-Purata. Chromosomal abnormalities with PGS & D&C. Fertil Steril 2015.

FIGURE 2



Frequencies of aneuploidy after (**A**) PGD and (**B**) D&C. Most-frequent to less-frequent chromosomes affected (monosomy and trisomy included).

Rodriguez-Purata. Chromosomal abnormalities with PGS & D&C. Fertil Steril 2015.

Additionally, the aneuploidy rate per age group after PGS was as follows: group A: 27.6%; group B: 35.7%; group C: 50.7%; group D: 72.9%; group E: 78.1% (Supplemental Table 2). In group A we observed 57% trisomies and 43% monosomies; the most frequent trisomies seen were 22, 16, 19, 21, and 8, and for monosomies 22, 16, 13, 19, and 21 (Supplemental Table 3). For group B we observed 50.0% trisomies and 50.0% monosomies; the most frequent trisomies seen were 21, 16, 22, 15, and 9, and for monosomies 22, 16, 21, 19, and 15 (Supplemental Table 4). For group C we observed 52.2% trisomies and 47.8% monosomies; the most frequent trisomies seen were 22, 15, 16, 21, and 19, and for monosomies 16, 22, 21, 18, and 14 (Supplemental Table 5). For Group D we observed 57.4% trisomies and 42.6% monosomies; the most frequent trisomies seen were 16, 21, 15, 22, and 20, and for monosomies 15, 22, 16, 18, and 21 (Supplemental Table 6). For Group E we observed 56.6% trisomies and 43.4% monosomies; the most frequent trisomies seen were 21, 22, 15, 16, and 9, and for monosomies 22, 15, 16, 21, and 18 (Supplemental Table 7). A correlation of frequencies of trisomies and monosomies per age group can be observed in Supplemental Figures 1 and 2.

Products of Conception

During the study period we identified 1,398 patients (age 37.0 ± 4.9 years; range, 20.7–48.8 years) who experienced a clinical loss. From these, 49.6% (n = 693) were analyzed cytogenetically, 61.5% (n = 426) after a miscarriage after ovulation induction/controlled ovarian stimulation, and 38.5% (n = 267) after IVF. We identified 83 samples (11.4%) with inconclusive results and 610 samples (88.7%) with conclusive results: 255 samples were reported to be euploid (41.8%) and 355 to be aneuploid (58.2%) (Supplemental Table 2). The 355 aneuploid POC comprised 438 different numerical abnormalities. The observed results included 362 trisomies (83%), 48 polyploidies (10.7%), and 28 monosomies (6.3%) (Fig. 1). Of polyploidies, 25 (5.6%) were triploidies, and 23 (5.1%) were tetraploidies. Among triploidies detected, 14 (56%) were 69XXY, and 11 (44%) were 69XXX; tetraploidies were 12 (52.1%) 92XXYY, and 11 (47.9%) were 92XXXX. The frequency for each NCA is described in Table 2 and Figure 2.

Additionally, we realized a subanalysis according to age groups. The aneuploidy rate per age group after D&C was as follows: group A: 38.3%; group B: 47.3%; group C: 57.7%; group D: 75.8%; group E: 62.5% (Supplemental Table 2). For group A we observed 90.7% trisomies and 9.3% monosomies; the most affected chromosomes were 22, 16, 15, and 21 (Supplemental Table 3). For Group B we observed

TABLE 2

Detected numerical chromosomal aneuploidies after D&C.

	Monosomy (n = 28)		Trisomy (n = 362)	
Chromosome	**n**	**%**	**n**	**%**
1	0	0	1	0.2
2	0	0	8	1.8
3	0	0	5	1.1
4	0	0	13	3.0
5	0	0	6	1.4
6	0	0	4	0.9
7	1	0.2	13	3.0
8	0	0	12	2.7
9	0	0	14	3.2
10	1	0.2	7	1.6
11	0	0	3	0.7
12	0	0	4	0.9
13	0	0	18	4.1
14	0	0	9	2.1
15	0	0	47	10.7
16	0	0	63	14.4
17	0	0	7	1.6
18	0	0	12	2.7
19	0	0	0	0
20	0	0	17	3.9
21	7	1.6	31	7.1
22	1	0.2	61	13.9
X	18	4.1	2	0.5
Y	0	0	0	0
mar			5	1.1
Triploid	25		5.7	
Tetraploid	23		5.3	

Rodriguez-Purata. Chromosomal abnormalities with PGS & D&C. Fertil Steril 2015.

83.1% trisomies, 6.2% monosomies, 4.6% tetraploidies, and 1.6% mar; the most affected chromosomes were 16, 22, 15, and 21 (Supplemental Table 4). For Group C we observed 85.6% trisomies, 4.8% monosomies, 4.8% triploidies, 3.8% tetraploidies, and 1.0% mar; the most affected chromosomes were 16, 22, 15, and 13 (Supplemental Table 5). For Group D we observed 91.1% trisomies, 5.4% monosomies, and 3.5% triploidies; the most affected chromosomes were 21, 15, 22, and 16 (Supplemental Table 6). For Group E we observed 89.7% trisomies, 2.9% monosomies, and 7.3% tetraploidies; the most affected chromosomes were 22, 15, 20, and 21 (Supplemental Table 7).

DISCUSSION

It is widely recognized that aneuploidy is the leading cause of implantation failure and miscarriage in both fertile and infertile couples seeking to achieve a pregnancy. Cytogenetic analysis of previous miscarriages is an important component in the assessment of couples with a history of pregnancy loss because it can guide subsequent treatment. Furthermore, the field of PGS for aneuploidy screening has also provided an opportunity to understand cell division errors, which has eliminated a potential implantation failure due to aneuploidy. Additionally, the use of PGS has created a positive impact on IVF success rates in certain cases, a worldwide push towards single-embryo transfer, and a reduction in multiple births after ART.

This study provides descriptive data of the most common NCAs that occur both before implantation (after IVF/PGS) (Supplemental Fig. 3) and after implantation (after D&C) (Supplemental Fig. 4) in an infertile population pursuing pregnancy (Fig. 1). Although the incidence of monosomies and trisomies observed before implantation was equivalent (ratio is 0.82) in group 1 patients (Table 1, Fig. 2, and Supplemental Figs. 3 and 5), group 2 patients showed fewer occurrences of monosomies (ratio 0.07) and rarely observed implantation of these embryos (Table 2, Fig. 2, and Supplemental Figs. 4 and 5). These results advocate PGS technology as an advantageous facilitator that helps circumvent the inheritance of potentially adverse conditions, such as aneuploidy. These data demonstrates that performing PGS will significantly reduce the incidence of NCA, thereby decreasing the likelihood of implantation failure and/or miscarriage after IVF. With this knowledge, implantation and pregnancy rates can be expected to be increased after an IVF cycle(s) by means of preventing implantation failure and early miscarriages.

Many researchers have analyzed the frequency of NCA among human fetuses by analyzing chorionic villi after a miscarriage (14, 15, 19, 20) and report an incidence of NCA ranging between 40% and 80% (depending on the culture method adopted for karyotyping analysis). However, in general, these reports are biased by the high spontaneous loss rate of chromosomally abnormal pregnancies before a pregnancy is clinically recognized, as well as the lack of patients universally electing for cytogenetic analysis of their POCs. Such limitations were addressed by Fragouli et al. (21), in one of the first publications analyzing PGS technology. In this study, ploidy status was analyzed throughout the preimplantation period—at oocyte, embryo cleavage, and blastocyst stages. Overall, the same chromosomal abnormalities were shown irrespective of the development phase. In oocytes the most frequent NCAs were 21, 22, 15, 20, and 19; during the cleavage stage the most frequent NCAs were in chromosomes 22, 16, 15, 19, and 21; and in the blastocyst stage the most frequent NCA were 22, 16, 15, 21, and 22. Interestingly, the same chromosomes were affected throughout all stages analyzed, even in the oocyte, when the sperm was not involved. This present study had an additional advantage, in that patients in both groups were from the same infertile population. Results showed that after PGS performed at the blastocyst stage, the most frequently affected chromosomes with a copy number gain were 22, 16, 21, 18, and 15 (order reflects frequency) and with copy number loss were chromosomes 22, 16, 15, 21, 18, and 19 (order reflects frequency), which is consistent with the results of the Fragouli et al. study (21). Remarkably, when these results were compared with tissue collected after D&C, monosomies were rarely observed and trisomies most frequently were shown in chromosomes 22, 16, 21, 15, and 19 (order reflects frequency) (Tables 1 and 2). The study demonstrated a more advanced understanding of the prevalence of aneuploidy across embryo development, gaining better insight into the biology of human reproduction.

The present study's results confirm previous reports that NCAs are present at a high frequency, rooting from early development. Previous molecular genetic analyses of CAs occurring in miscarriages have revealed that most aneuploidy events arise during female meiosis (22), usually as a consequence of nondisjunction in the first meiotic division. Direct observation of female meiotic divisions (via polar body analysis) and early embryonic stages has shown that, before implantation, a wide range of aneuploidies are present. Historically most investigations have focused on trisomies, especially those compatible with live birth. On the basis of those analyses, three "rules" of human nondisjunction were formulated: first, regardless of the specific chromosome, most trisomies originate during oogenesis; second, for most chromosomes, maternal MI errors are more common than maternal meiosis II (MII) errors; and third, the proportion of cases of maternal origin increases with age (23). Additionally, recent studies have combined those findings with others to focus on commonly overlooked NCAs that require more insight, such as trisomy 22 (24). It seems that chromosome-specific patterns are in place and that ultimately there are three different types of nondisjunction mechanisms: those that affect all chromosomes (maternal MI errors, because oocytes are arrested in MI for 10–50 years), those that affect a group of chromosomes ("acrocentric" chromosomes: 13, 14, 15, 21, and 22), and those that affect individual chromosomes.

Preimplantation genetic screening continues to evolve, and its technique is not without flaws. The current PGS technique does not detect certain polyploidies. Chorionic villi collected via D&C showed an incidence of 10.7% of

polyploidies (Table 2). Overall, polyploidies are rarely observed in humans. Triploidy has been identified in 1%–3% of human pregnancies and in 15% of miscarriages (25); tetraploidy on the other hand has only been observed in 1%–2% of early miscarriages (26). In this context, a limitation of PGS is that it can only detect copy number gain or loss, which means it cannot detect some triploidies, such as 69XXX, and tetraploidies, such as 92XXXX or 92XXYY, given that no gain or loss can be read. Conversely, it could detect a gender trisomy in a polyploidy such as triploidy 69XXY, tetraploidy 92XXXY, or tetraploidy 92XYYY. Notwithstanding, any polyploidy not detectable by PGS that is also abnormal for aneuploidy (93XXXX+22) will be classified as aneuploid and therefore discarded as abnormal regardless. In this study, from the 10.7% (n = 48) polyploid embryos detected in POC, 29.2% (n = 14) were heterogeneously polyploid (69XXY) vs. 70.8% (n = 34) homogeneously polyploid (69XXX, 92XXYY, 92XXXX); therefore, only 2.7% of the total embryos analyzed would have been missed if done by means of preimplantation technology. Additionally, a non-concurrent or inconclusive result after PGS is sometimes obtained after biopsy when an embryo's karyotype is not clearly diagnosed as disomic, monosomic, or trisomic. We observed an inconclusive result in 2.8% of samples (n = 58), which is comparable to previous reports (27). This variation may be explained by the conservative interpretations of genetic data, technical issues, or sample collection techniques, and not necessarily to chaotic chromosomal abnormalities.

There are limitations to the present study. First, the retrospective nature of the study creates a selection bias. Retrospective designs establish the temporal relationship between exposures and outcomes, thus ensuring that the measurement of the exposure is not biased by the outcome and reducing the likelihood that an association is "effect–cause." Nevertheless, confounding must be considered as a possible threat to validity.

Second, we acknowledge that not all patients are able to undergo PGS; therefore, only patients with at least one screened blastocyst were included in this analysis. Nevertheless, given that the indication for CCS was either routine infertility care (60%) or gender selection (25%, 50% [n = 70] of which gender selection was not the primary reason for PGS), we believe these results are generalizable among patients who are able to make blastocysts for biopsy, but more research is required to confirm this impression. Of 4,958 patients who underwent an IVF cycle during the study period, 476 patients underwent 628 cycles of PGS (2012–2013, www.sart.org; 2014 unpublished data).

Third, we understand the impact and its disadvantages of structural chromosomal rearrangements (28) within our population, and although this was not a focus in this study, it will be in a future analysis.

Fourth, D&C is only carried out in certain cases, because many patients who experience pregnancy termination present complete passage of all fetal tissues and therefore do not require surgical management; hence a cytogenetic result is not available. During the study period we identified 1,398 patients who experienced a loss after a gestational sac was seen; from these, 49.6% (n = 693) had karyotyping of POCs, 90% of whom had experienced at least one previous loss.

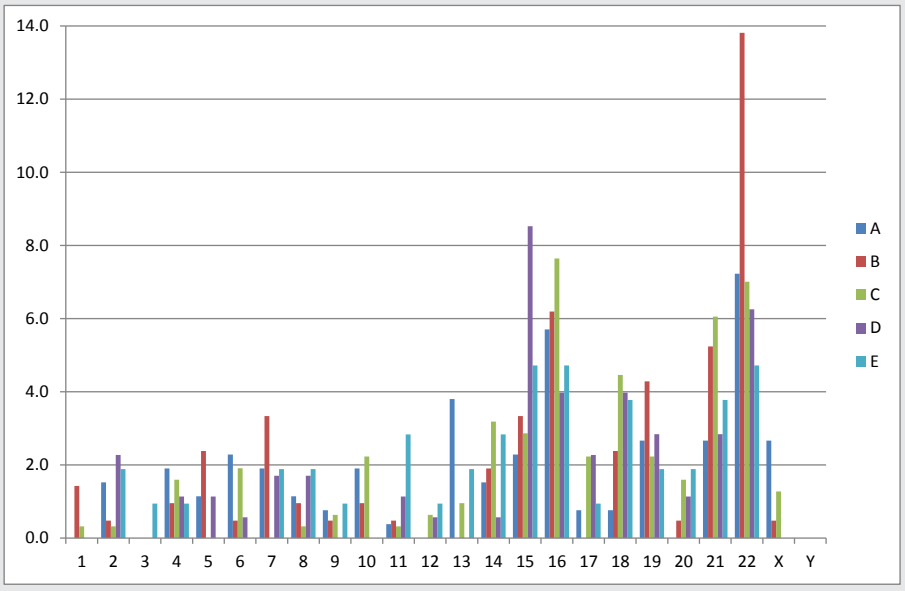
Fifth, cytogenetic analysis from POCs is reliant on viable cells and thus precludes the successful culture of macerated tissues or degraded tissue specimens. This technique is also dependent on a large team of skilled technicians/scientists who must be thoroughly trained in dissection techniques. Maternal cell contamination and overgrowth can give false-normal female results in up to 12% of cases (29), and up to 49% of the samples received by laboratories fail to grow in culture (30). We observed that 11.4% of the samples (n = 83) were not able to obtain a conclusive result, which was reflected in previous reports. Analyzing POC specimens has traditionally been challenging as a result of selective overgrowth of maternally derived cells, which prevents reporting true fetal karyotypes with certainty. Accurate cytogenetic analysis of pregnancy loss tissue provides medically and psychologically important information to the patient and clinician; however, this information has the potential to be misleading, particularly when a diagnosis of "46XX" is reported. Such risk of contamination has been reported as ranging from 5% to 29% (31), more recently determined to be 22% through single-nucleotide polymorphism chromosomal array analysis (32). In general, factors that lead to an erroneous karyotype include the amount of contaminating maternal tissue, the quantity and preservation of villi, and perhaps the genetic makeup and growth rate of the conceptus. It is also possible that some genetically abnormal conceptuses would have a significant growth disadvantage compared with normal maternal cells as a result of genetically lethal chromosomal abnormalities. The advantage of this approach lies in the concurrent availability of genotype information that allows simultaneous detection of maternal cell contamination, triploidy, and uniparental disomy (31).

This study highlights the efficacy of former and current genomic technologies in identifying abnormal embryos that otherwise could appear to be morphologically normal. Preimplantation genetic screening assists the decision process before embryo transfer by detecting for any NCA, which potentially avoids early pregnancy loss. Judicious use of embryonic screening can maximize implantation and delivery rates and minimize the incidence of miscarriages related to chromosomal abnormalities (33, 34). Conversely, for patient seeking to conceive, understanding the etiology of reproductive loss can in the same manner be beneficial to their mental health and can assuage feelings of guilt or irresponsibility (35). Furthermore, this investigation demonstrates that a vast majority of aneuploid human embryos survive preimplantation development and that current and future genetic technologies could help physicians and patients by enhancing embryo selection before transfer. Nevertheless, further studies may provide guidance into optimizing or even improving oocyte quality, such as pronuclear transfer, maternal spindle transfer, or nuclear genome transfer, and into noninvasive ways to identify reproductively competent embryos to improve clinical outcome (36).

REFERENCES

1. Lejeune J, Gautier M, Turpin R. [Study of somatic chromosomes from 9 mongoloid children]. C R Hebd Seances Acad Sci 1959;248:1721–2.
2. Jacobs PA, Baikie AG, Court Brown WM, Strong JA. The somatic chromosomes in mongolism. Lancet 1959;1:710.
3. Lamb NE, Sherman SL, Hassold TJ. Effect of meiotic recombination on the production of aneuploid gametes in humans. Cytogenet Genome Res 2005;111:250–5.
4. Hassold T, Hunt P. Maternal age and chromosomally abnormal pregnancies: what we know and what we wish we knew. Curr Opin Pediatr 2009;21:703–8.
5. Nagaoka SI, Hassold TJ. Hunt PA Human aneuploidy: mechanisms and new insights into an age-old problem. Nat Rev Genet 2012;13:493–504.
6. Angell RR, Aitken RJ, van Look PF, Lumsden MA, Templeton AA. Chromosome abnormalities in human embryos after in vitro fertilization. Nature 1983;303:336–8.
7. Angell R. First meiotic division non-disjunction in human oocytes. Am J Hum Genet 1997;61:23–32.
8. Pellestor F, Andreo B, Anahory T, Hamamah S. The occurrence of aneuploidy in human: lessons from the cytogenetic studies of human oocytes. Eur J Med Genet 2006;49:103–16.
9. Gutierrez-Mateo C, Colls P, Sanchez-Garcia J, Escudero T, Prates R, Ketterson K, et al. Validation of microarray comparative genomic hybridization for comprehensive chromosome analysis of embryos. Fertil Steril 2011; 95:953–8.
10. Treff NR, Tao X, Ferry KM, Su J, Taylor D, Scott RT Jr. Development and validation of an accurate quantitative real-time polymerase chain reaction-based assay for human blastocyst comprehensive chromosomal aneuploidy screening. Fertil Steril 2012;97:819–24.
11. McFadden D, Friedman JM. Chromosome abnormalities in human beings. Mutat Res 1997;396:129–40.
12. Nussbaum RL, McInnes RR, Willard HF. Chapter 5: Principles of clinical cytogenetics. In: Thompson and Thompson Genetics in Medicine. Philladelphia: Saunders Elsevier; 2007:65.
13. Schrijver I, Zehnder JL, Cherry AM. Chromosomal translocations, deletions, and inversions. Available at: http://www.uptodate.com/contents/chromosomal-translocations-deletions-and-inversions. Accessed September 18, 2014.
14. Hassold T, Chen N, Funkhouser J, Jooss T, Manuel B, Matsuura J, et al. A cytogenetic study of 100 spontaneous abortions. Ann Hum Genet 1980;44: 151–78.
15. Werner M, Reh A, Grifo J, Perle MA. Characteristics of chromosomal abnormalities diagnosed after spontaneous abortions in an infertile population. J Assist Reprod Genet 2012;29:817–20.
16. Bettio D, Venci A, Levi Setti PE. Chromosomal abnormalities in miscarriages after different assisted reproduction procedures. Placenta 2008;29(Suppl B): 126–8.
17. Franasiak J, Forman E, Hong K, Werner M, Upham K, Treff N, et al. The nature of aneuploidy with increasing age of the female partner: a review of 15,169 consecutive trophectoderm biopsies evaluated with comprehensive chromosomal screening. Fertil Steril 2014;101:656–63.
18. Schoolcraft WB, Treff NR, Stevens JM, Ferry K, Katz-Jaffe M, Scott RT Jr. Live birth outcome with trophectoderm biopsy, blastocyst vitrification, and single-nucleotide polymorphism microarray-based comprehensive chromosome screening in infertile patients. Fertil Steril 2011;96:638–40.
19. Carr DH. Chromosome studies in selected spontaneous abortions. 3. Early pregnancy loss. Obstet Gynecol 1971;37:570–4.
20. Creasy MR, Crolla JA, Alberman ED. A cytogenetic study of human spontaneous abortions using banding techniques. Hum Genet 1976;31:177–96.
21. Fragouli E, Alfarawati S, Spath K, Jaroudi S, Sarasa J, Enciso M, et al. The origin and impact of embryonic aneuploidy. Hum Genet 2013;132: 1001–13.
22. Hassold T, Hunt P. To err (meiotically) is human: the genesis of human aneuploidy. Nat Rev Genet 2001;2:280–91.
23. Hassol T, Hall H, Hunt P. The origin of human aneuploidy: where we have been, where we are going. Hum Mol Genet 2007;16:R203–8.
24. Hall H, Surti U, Hoffner L, Shirley S, Feingold E, Hassold T. The origin of trisomy 22: evidence for acrocentric: chromosome-specific patterns of nondisjunction. Am J Med Genet 2007;143A:2249–55.
25. McWeeney D, Munné S, Miller R, Cekleniak N, Contag S, Wax J, et al. Pregnancy complicated by triploidy: a comparison of the three karyotypes. Am J Perinatol 2009;26:641–5.
26. Sundvall L, Lund H, Niemann I, Jensen UB, Bolund L, Sunde L. Tetraploidy in hydatidiform moles. Hum Reprod 2013;28:2010–20.
27. Moutou C, Goossens V, Coonen E, De Rycke M, Kokkali G, Renwick P, et al. ESHRE PGD Consortium data collection XII: cycles from January to December 2009 with pregnancy follow-up to October 2010. Hum Reprod 2014;29: 880–903.
28. Alfarawati S, Fragouli E, Colls P, Wells D. First births after preimplantation genetic diagnosis of structural chromosome abnormalities using comparative genomic hybridization and microarray analysis. Hum Reprod 2011;26: 1560–74.
29. Rodgers CS, Creasy MR, Fitchett M, Maliszewska CT, Pratt NR, Waters JJ. Solid tissue culture for cytogenetic analysis: a collaborative survey for the Association of Clinical Cytogenetics. J Clin Pathol 1996;49:638–41.
30. Zhang YX, Zhang YP, Gu Y, Guan FJ, Li SL, Xie JS, et al. Genetic analysis of first trimester miscarriages with a combination of cytogenetic karyotyping, microsatellite genotyping and array CGH. Clin Genet 2009;75:133–40.
31. Bell KA, Van Deerlin PG, Haddad BR, Feinberg RF. Cytogenetic diagnosis of "normal 46,XX" karyotypes in spontaneous abortions frequently may be misleading. Fertil Steril 1999;71:334–41.
32. Levy B, Sigurjonsson S, Pettersen B, Maisenbacher MK, Hall MP, Demko Z, et al. Genomic imbalance in products of conception: single-nucleotide polymorphism chromosomal microarray analysis. Obstet Gynecol 2014;124(2 Pt 1):202–9.
33. Scott RT, Upham KM, Forman EJ, Hong KH, Scott KL, Taylor D, et al. Blastocyst biopsy with comprehensive chromosome screening and fresh embryo transfer significantly increases in vitro fertilization implantation and delivery rates: a randomized controlled trial. Fertil Steril 2013;100: 697–703.
34. Lee E, Illingworth P, Wilton L, Chambers GM. The clinical effectiveness of preimplantation genetic diagnosis for aneuploidy in all 24 chromosomes (PGD-A): systematic review. Hum Reprod 2015;30:473–83.
35. Kong GW, Chung TK, Lok IH. The impact of supportive counselling on women's psychological wellbeing after miscarriage—a randomised controlled trial. BJOG 2014;121:1253–62.
36. Botros L, Sakkas D, Seli E. Metabolomics and its application for non-invasive embryo assessment in IVF. Mol Hum Reprod 2008;14:679–90.

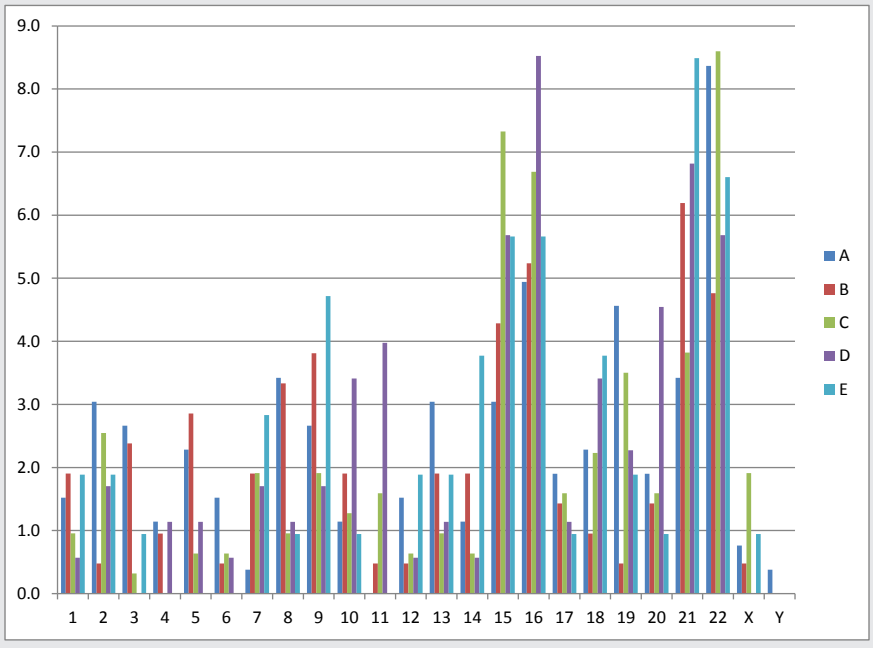
SUPPLEMENTAL FIGURE 1



Correlation of trisomies per age group after PGS.

Rodriguez-Purata. Chromosomal abnormalities with PGS & D&C. Fertil Steril 2015.

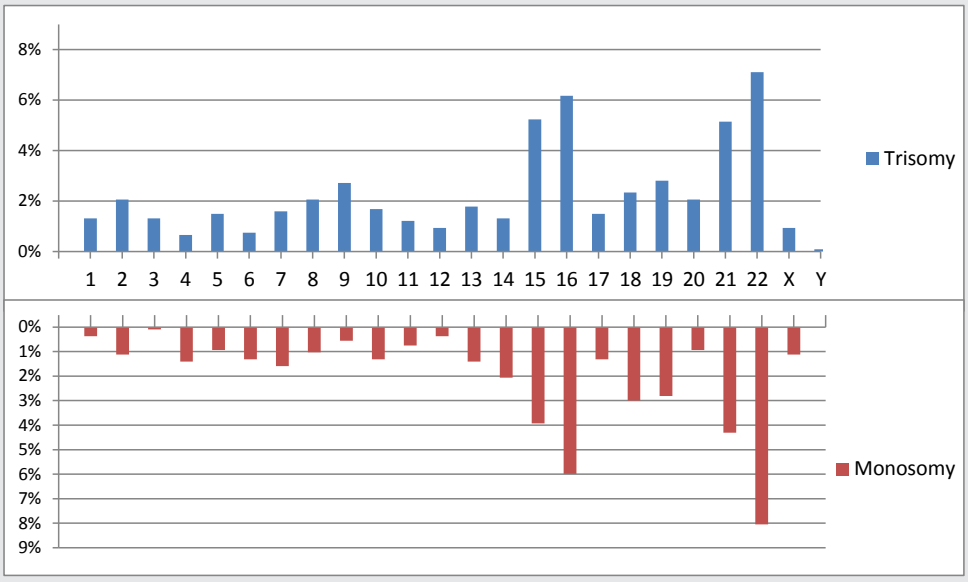
SUPPLEMENTAL FIGURE 2



Correlation of monosomies per age group after PGS.

Rodriguez-Purata. Chromosomal abnormalities with PGS & D&C. Fertil Steril 2015.

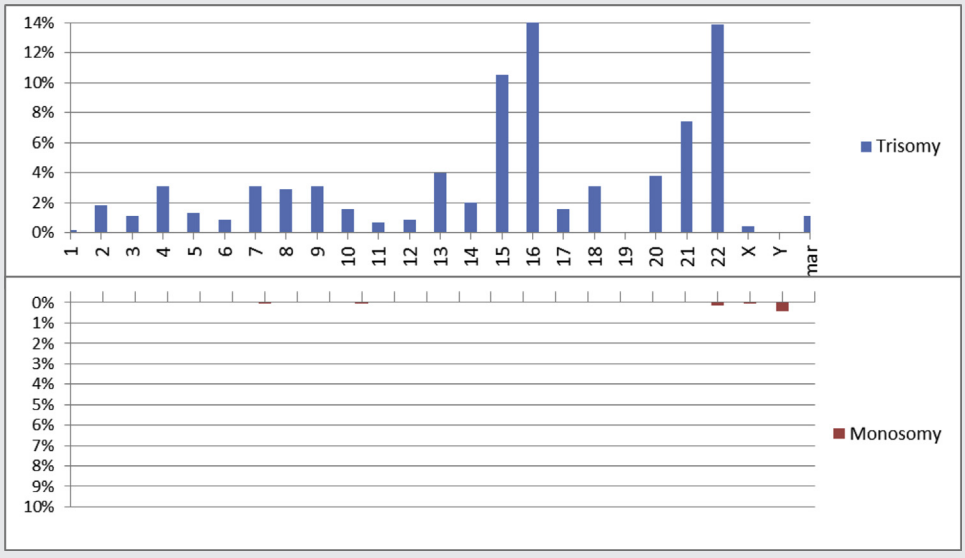
SUPPLEMENTAL FIGURE 3



Comparison of monosomies and trisomies after PGS. Contrasting frequencies of monosomies and trisomies per affected chromosome.

Rodriguez-Purata. Chromosomal abnormalities with PGS & D&C. Fertil Steril 2015.

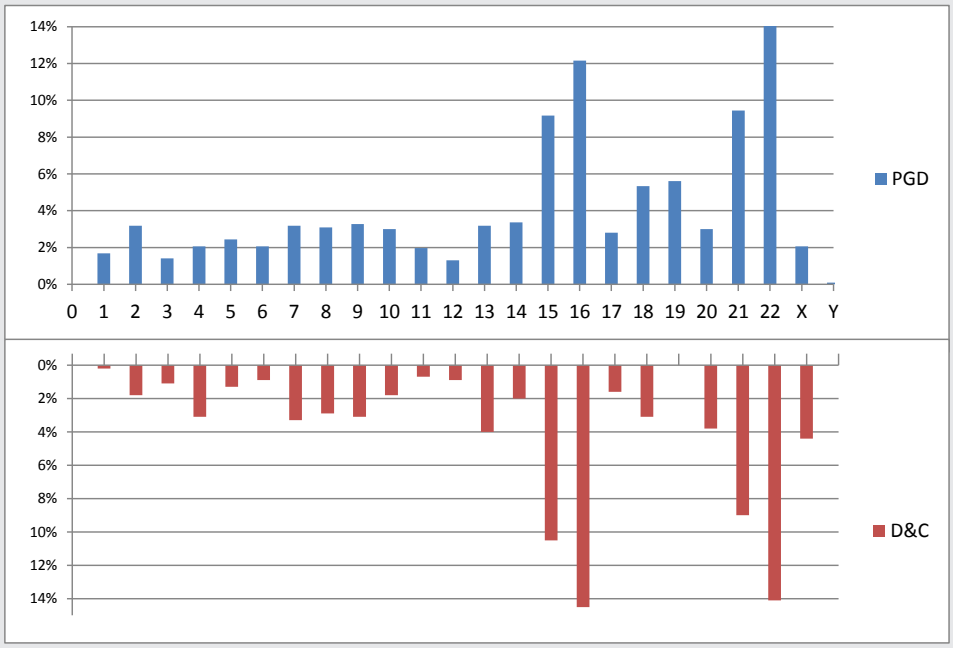
SUPPLEMENTAL FIGURE 4



Comparison of monosomies and trisomies after D&C. Contrasting frequencies of monosomies and trisomies per affected chromosome.

Rodriguez-Purata. Chromosomal abnormalities with PGS & D&C. Fertil Steril 2015.

SUPPLEMENTAL FIGURE 5



Abnormal chromosome frequencies after PGS vs. D&C. Contrasting frequencies of monosomies and trisomies together per affected chromosome per technique.

Rodriguez-Purata. Chromosomal abnormalities with PGS & D&C. Fertil Steril 2015.

SUPPLEMENTAL TABLE 1

Number of embryos biopsied per patient.

No. of embryos biopsied (n = 2,054)	No. of patients (n = 557)
1	129
2	106
3	86
4	70
5	51
6	47
7	22
8	11
9	9
10	7
11	6
12	5
13	3
14	1
15	1
16	1
17	1
18	1

Rodriguez-Purata. Chromosomal abnormalities with PGS & D&C. Fertil Steril 2015.

SUPPLEMENTAL TABLE 2

Cytogenetic (normal/abnormal/inconclusive) result per age group in D&C and PGS.

	Normal		Abnormal		Inconclusive	
Group	**D&C**	**PGS**	**D&C**	**PGS**	**D&C**	**PGS**
A	49.6 (114)	70.3 (612)	38.3 (88)	27.6 (236)	12.2 (28)	2.2 (19)
B	42.7 (64)	62.2 (309)	47.3 (71)	35.7 (177)	10.0 (15)	2.0 (10)
C	31.9 (52)	45.8 (211)	57.7 (94)	50.7 (232)	10.4 (17)	3.5 (16)
D	12.9 (8)	21.3 (32)	75.8 (47)	72.9 (109)	11.3 (7)	5.8 (9)
E	19.3 (17)	17.2 (14)	62.5 (55)	78.1 (64)	18.2 (16)	4.7 (4)
Total	36.8 (255)	57.0 (1,178)	51.2 (355)	40.2 (818)	12.0 (83)	2.8 (58)

Note: Values are expressed as percentage (number).

Rodriguez-Purata. Chromosomal abnormalities with PGS & D&C. Fertil Steril 2015.

SUPPLEMENTAL TABLE 3

Correlation of monosomies/trisomies in age group A.

	D&C		PGS	
Group A	**Monosomies**	**Trisomies**	**Monosomies**	**Trisomies**
1	0	1.1	0	1.5
2	0	1.1	1.5	3.0
3	0	3.2	0	2.7
4	0	5.4	1.9	1.1
5	0	2.2	1.1	2.3
6	0	1.1	2.3	1.5
7	0	3.2	1.9	0.4
8	0	2.2	1.1	3.4
9	0	2.2	0.8	2.7
10	0	0	1.9	1.1
11	0	0	0.4	0
12	0	1.1	0	1.5
13	0	2.2	3.8	3.0
14	0	0	1.5	1.1
15	0	7.5	2.3	3.0
16	0	15.1	5.7	4.9
17	0	0	0.8	1.9
18	0	3.2	0.8	2.3
19	0	0	2.7	4.6
20	0	1.1	0	1.9
21	0	6.5	2.7	3.4
22	0	15.1	7.2	8.4
X	7.5	0	2.7	0.8
Y	0	0	0	0.4
Triploid	9.7		0	
Tetraploid	7.6		0	
mar	2.2		0	

Note: Values are percentages.

Rodriguez-Purata. Chromosomal abnormalities with PGS & D&C. Fertil Steril 2015.

SUPPLEMENTAL TABLE 4

Correlation of monosomies/trisomies in age group B.

	D&C		PGS	
Group B	**Monosomies**	**Trisomies**	**Monosomies**	**Trisomies**
1	0.0	0.0	1.4	1.9
2	0.0	1.5	0.5	0.5
3	0.0	0.0	0.0	2.4
4	0.0	4.6	1.0	1.0
5	0.0	0.0	2.4	2.9
6	0.0	0.0	0.5	0.5
7	0.0	1.5	3.3	1.9
8	0.0	3	1.0	3.3
9	0.0	3	0.5	3.8
10	0.0	4.6	1.0	1.9
11	0.0	0.0	0.5	0.5
12	0.0	0.0	0.0	0.5
13	0.0	3	0.0	1.9
14	0.0	1.5	1.9	1.9
15	0.0	9.2	3.3	4.3
16	0.0	24.6	6.2	5.2
17	0.0	3	0.0	1.4
18	0.0	0.0	2.4	1.0
19	0.0	0.0	4.3	0.5
20	0.0	3	0.5	1.4
21	0.0	7.7	5.2	6.2
22	0.0	12.3	13.8	4.8
X	6.2	0.0	0.5	0.5
Triploid	4.6		0.0	
Tetraploid	4.6		0.0	
mar	1.5		0.0	

Note: Values are percentages.

Rodriguez-Purata. Chromosomal abnormalities with PGS & D&C. Fertil Steril 2015.

SUPPLEMENTAL TABLE 5

Correlation of monosomies/trisomies in age group C.

	D&C		PGS	
Group C	**Monosomies**	**Trisomies**	**Monosomies**	**Trisomies**
1	0	0	0.3	1.0
2	0	2.9	0.3	2.5
3	0	0	0	0.3
4	0	1.0	1.6	0
5	0	1.9	0	0.6
6	0	1.9	1.9	0.6
7	1.0	2.9	0	1.9
8	0	0	0.3	1.0
9	1.0	2.9	0.6	1.9
10	0	0	2.2	1.3
11	0	1.0	0.3	1.6
12	0	0	0.6	0.6
13	0	7.7	1.0	1.0
14	0	5.8	3.2	0.6
15	0	12.5	2.9	7.3
16	0	19.2	7.6	6.7
17	0	1.0	2.2	1.6
18	0	1.9	4.5	2.2
19	0	0	2.2	3.5
20	0	4.8	1.6	1.6
21	1.0	2.9	6.1	3.8
22	0	15.4	7.0	8.6
X	0	0	1.3	1.9
Y	1.9	0	0	0
Triploid	4.8		0	
Tetraploid	3.8		0	
mar	1.0		0	

Note: Values are percentages.

Rodriguez-Purata. Chromosomal abnormalities with PGS & D&C. Fertil Steril 2015.

SUPPLEMENTAL TABLE 6

Correlation of monosomies/trisomies in age group D.

	D&C		PGS	
Group D	**Monosomies**	**Trisomies**	**Monosomies**	**Trisomies**
1	0	1.8	0	0.6
2	0	1.8	2.3	1.7
3	0	0	0	0
4	0	3.6	1.1	1.1
5	0	1.8	1.1	1.1
6	0	0	0.6	0.6
7	0	1.8	1.7	1.7
8	0	1.8	1.7	1.1
9	0	3.6	0	1.7
10	0	5.4	0	3.4
11	0	0	1.1	4.0
12	0	1.8	0.6	0.6
13	0	5.4	0	1.1
14	0	0	0.6	0.6
15	0	14.3	8.5	5.7
16	0	10.7	4.0	8.5
17	0	3.6	2.3	1.1
18	0	3.6	4.0	3.4
19	0	0	2.8	2.3
20	0	3.6	1.1	4.5
21	3.6	16.0	2.8	6.8
22	0	10.7	6.3	5.7
X	1.8	0	0	0
Y	0	0	0	0
Triploid	3.6		0	
Tetraploid	0		0	
mar	0		0	

Note: Values are percentages.

Rodriguez-Purata. Chromosomal abnormalities with PGS & D&C. Fertil Steril 2015.

SUPPLEMENTAL TABLE 7

Correlation of monosomies/trisomies in age group E.

	D&C		PGS	
Group E	**Monosomies**	**Trisomies**	**Monosomies**	**Trisomies**
1	0		0	1.9
2	0	1.5	1.9	1.9
3	0	1.5	0.9	0.9
4	0	2.9	0.9	0
5	0	1.5	0	0
6	0	0	0	0
7	0	0	1.9	2.8
8	0	2.9	1.9	0.9
9	0	4.4	0.9	4.7
10	0	2.9	0	0.9
11	0	1.5	2.8	0
12	0	1.5	0.9	1.9
13	0	2.9	1.9	1.9
14	0	2.9	2.8	3.8
15	0	10	4.7	5.7
16	0	4.4	4.7	5.7
17	0	2.9	0.9	0.9
18	0	5.9	3.8	3.8
19	0	0	1.9	1.9
20	0	10.3	1.9	0.9
21	1.5	8.8	3.8	8.5
22	0	16.2	4.7	6.6
X	1.5	0	0	0.9
Y	0	0	0	0
Triploid	2.9		0	
Tetraploid	4.4		0	
mar	0		0	

Note: Values are percentages.

Rodriguez-Purata. Chromosomal abnormalities with PGS & D&C. Fertil Steril 2015.