ORIGINAL ARTICLE: ASSISTED REPRODUCTION

Effects of maternal age on euploidy rates in a large cohort of embryos analyzed with 24-chromosome single-nucleotide polymorphism–based preimplantation genetic screening

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Objective: To determine the effect of maternal age on the average number of euploid embryos retrieved during oocyte harvest as part of an in vitro fertilization (IVF) cycle, including the probability of retrieving at least one euploid embryo in a cohort (PrE).
Design: Retrospective study.
Setting: Preimplantation genetic screening (PGS) laboratory.
Patient(s): Women aged 18 to 48 years undergoing IVF treatment.
Intervention(s): Use of 24-chromosome single-nucleotide polymorphism (SNP)-based PGS of day-3 and day-5 embryo biopsies.
Main Outcome Measure(s): Relationships between maternal age and the rate of embryos that tested as euploid (hereafter referred to as "euploid embryos"), the average number and proportion of euploid embryos per IVF cycle, and PrE.
Result(s): We analyzed 22,599 day-3 embryos and 15,112 day-5 embryos. In women aged 27 to 35 years, the median proportion of euploid embryos in each cycle remained constant at ~35% in day-3 biopsies and ~55% in day-5 biopsies, but it decreased rapidly after age 35. On average, women in their late 20s had four euploid embryos (day 3 or day 5) per cycle, but this number decreased linearly ($R^2 \geq 0.983$) after 35 years of age. The effect of maternal age on PrE was similar, with a rapid exponential decline ($R^2 = 0.986$). Across all maternal ages, the euploid proportion and number of embryos per cycle were counterbalanced, so the number of euploid embryos per cycle was the same for day-3 and day-5 biopsies. This suggests that the loss of embryos from day 3 to day 5 was primarily due to aneuploidy.
Conclusion(s): Our results confirm the known inverse relationship between advanced maternal age (>35 years) and embryo euploidy, demonstrating that equal numbers of euploid embryos are available at day 3 and day 5. (Fertil Steril® 2016;■:■–■.

Key Words: Aneuploidy, fertility, in vitro fertilization, maternal age, preimplantation genetic screening

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Abnormal chromosome number, or aneuploidy, is common in human embryos (1–3). It is responsible for more than half of all missed abortions and miscarriages (4–6), and it is the leading cause of congenital birth defects (2, 3, 7, 8). Embryo-wide whole chromosomal aneuploidy results mainly from meiotic errors during oocyte generation (2, 7–9), which become increasingly common as women age (1, 3, 7, 10, 11). For example, trisomies occur in nearly 35% of all clinically recognized pregnancies in women over 40 years old, but in only 2% to 3% of all clinically recognized pregnancies in women in their mid-20s (2).

Because of the clinical significance of aneuploidy, it is the primary reason people choose to use preimplantation genetic screening (PGS), a technique that can detect aneuploidy in a single blastomere from day-3 cleavage-stage embryos or from a small number of trophectoderm cells from day-5 blastocyst-stage embryos (10). Preimplantation genetic screening is based on the hypothesis that selective transfer of euploid embryos can improve clinical outcomes for patients undergoing in vitro fertilization (IVF), particularly those at risk for fetal aneuploidy or who have experienced recurrent pregnancy loss (12). Early efforts to realize this goal were hampered by the use of fluorescence in situ hybridization (FISH) analysis, which identifies only ~25% of all chromosomal abnormalities (1, 13, 14) and led to disappointing results in randomized controlled trials (15, 16). More recent studies using array-based PGS have demonstrated major improvements in clinical pregnancy rates (17–20).

Despite the fact that a high percentage of embryonic cells are aneuploid (14) and many day-3 embryos are mosaic (i.e., they include both euploid and aneuploid cells) (21, 22), PGS has until recently typically been performed on day-3 biopsy samples due to its ease and reliability. Advances in embryology and cryopreservation techniques (1, 23) have, however, improved IVF success rates for day-5 embryos. In addition, day-5 embryos, by virtue of the attrition of aneuploid cells that occurs during development, have considerably lower frequencies of mosaicism (22) and aneuploidy (24).

As a result, PGS has shifted toward 24-chromosome comprehensive chromosome screening of day-5 embryos, which can help women with fertility issues, such as advanced maternal age (>35 years old), have a successful pregnancy (12). Two common methods of comprehensive chromosome screening are array comparative genomic hybridization and single-nucleotide polymorphism (SNP) arrays. Although both of these techniques can detect chromosomal copy-number abnormalities, SNP arrays provide additional information about other genetic features of the embryo, such as the parental origin of chromosomes, the mitotic/meiotic origin of aneuploidies (11), and whether disomic chromosomes are uniparental (i.e., from only a single parent) (13).

Previously, we developed an informatics-based SNP array PGS technology called Parental Support, which is as accurate as metaphase karyotyping, the gold standard technique for chromosome analysis (22). Parental Support uses parental genetic information to improve the determination of embryonic chromosomal copy numbers. Importantly, this algorithm computes the statistical confidence for each copy-number call, which allows embryo quality to be assessed quantitatively rather than by qualitative methods based on embryo morphology that may not be associated with chromosome abnormalities (25).

Between 2009 and 2014, more than 46,000 embryos were analyzed using this SNP-based PGS test in our Clinical Laboratory Improvement Amendments (CLIA)–approved laboratory. For both day-3 and day-5 biopsies, the number of embryos submitted per cycle gradually decreased with increasing maternal age, and the proportion of embryos that tested as euploid decreased rapidly after age 35 (Fig. 1) (26). Here, we report the number and proportion of embryos that were determined to be euploid (hereafter referred to as euploid embryos) per IVF cycle and the probability of retrieving at least one euploid embryo per cohort (PrE) as functions of maternal age. We also compare trends between day-3 and day-5 biopsies as well as those between embryos from egg donors and from women who used their own eggs (hereafter referred to as nondonors).

MATERIALS AND METHODS

Samples

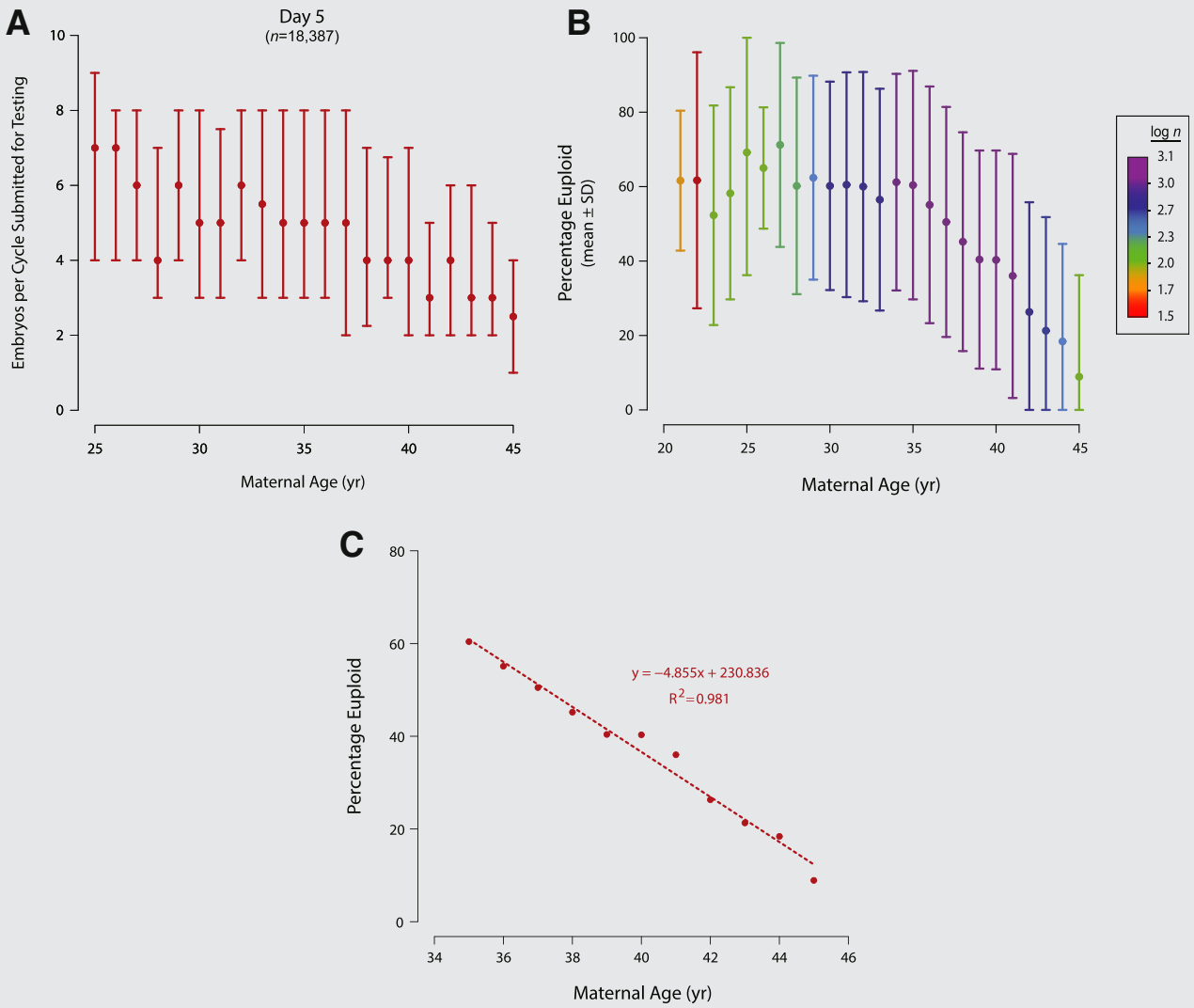
Between February 2009 and March 2014, 181 IVF centers submitted 46,439 embryo biopsies from 6,365 IVF cycles to Natera, Inc. (San Carlos, CA) for 24-chromosome PGS. Indications for PGS included history of aneuploid pregnancy or IVF failure, recurrent pregnancy loss, advanced maternal age, and gender selection. Each IVF center cultured embryos and biopsied cells according to their standard operating procedures and then shipped the isolated cells overnight. Due to the retrospective nature of this study, Ethical & Independent Review Services (Corte Madera, CA) exempted it from institution review board approval (E&I Exemption Number 10806-05).

SNP-based PGS

We performed DNA amplification, SNP genotyping, and copy-number determination as described elsewhere (22). Briefly, single cells were washed, lysed, and amplified using either whole-genome amplification or multiple displacement amplification. Then the amplified DNA was genotyped using Illumina SNP microarrays.

Microarray data were processed with the Parental Support algorithm to infer copy numbers and calculate the confidence of each copy-number call. This algorithm takes advantage of the genetic relationships between parental and embryonic DNA to enhance copy-number calls made from potentially error-prone single-cell microarray data (22). Specifically, parental genotypes and crossover frequency data are used to calculate expected allele distributions for thousands of heterozygous SNPs in the embryonic genome (27). At each SNP, the likelihoods of different copy-number hypotheses (e.g., amplification or deletion) are determined, and then the joint likelihood across all SNPs on a chromosome is calculated. The hypothesis with the maximum likelihood is called as the chromosome copy number, and its statistical confidence is computed by comparing the likelihoods of all hypotheses. On the basis of these results for all chromosomes, embryos were classified as euploid or aneuploid.

FIGURE 1



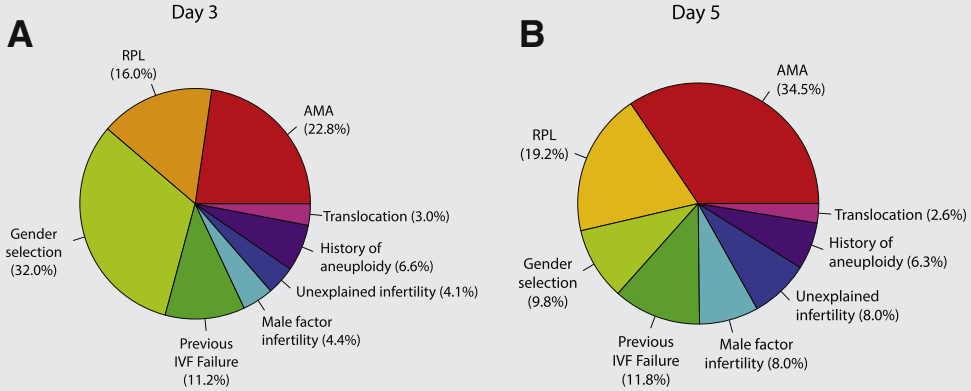
Maternal age relationships in more than 18,000 day-5 preimplantation embryos tested with 24-chromosome single-nucleotide polymorphism (SNP)-based preimplantation genetic screening (PGS). (**A**) The number of embryos per in vitro fertilization (IVF) cycle submitted for testing gradually decreases as maternal age increases from 25 to 45 years old. Data points indicate the 50th percentile, and whiskers above and below these points correspond to the 75th and 25th percentiles, respectively. Age groups with less than 45 cycles are not shown. (**B**) On the basis of PGS results, embryos from both egg donors and nondonors were classified as either euploid or aneuploid. Women 24 to 35 years old have the highest percentage of euploid embryos. Data points and whiskers show the mean and one standard deviation (SD), respectively. The logarithm of the number of embryos in each age group is mapped onto a color gradient from *red* (small *n*) to *purple* (large *n*). Age groups with less than 10 cycles are not shown. (**C**) After age 35, mean euploidy rates decline with increasing maternal age, as shown by the close fit between the data points and the dashed regression line. The strong linear correlation ($R^2 = 0.981$) indicates that the equation for this line can be used to accurately calculate (interpolate) average euploidy rates in women 35 to 45 years old. Adapted from Figures 2 and 3 in McCoy et al., 2015 (26).

Demko. Maternal age effects on embryo euploidy. Fertil Steril 2016.

Data Analyses

Data were analyzed with R (version 3.1.2; www.r-project.org). We stratified data by maternal age and analyzed distributions with quartiles. In addition, for each year of age, we calculated the average number of euploid embryos, proportion of euploid embryos, and PrE. The probability of not having any euploid embryos was defined as the number of cycles without any euploid embryos divided by the total number of cycles. Subtracting this probability from 1 gives the probability of retrieving at least one euploid embryo (PrE). These outcome measures were compared for day-3 and day-5 biopsies, and for embryos from egg donors and nondonors. To quantitatively describe the relationship between these measures and advanced maternal age, we used linear and nonlinear regression analyses, and we measured the strength of the correlation between observed and predicted values with the regression R^2 values.

FIGURE 2



Reported reasons for testing day-3 (**A**) and day-5 (**B**) biopsies with single-nucleotide polymorphism (SNP)–based preimplantation genetic screening (PGS). The percentages of IVF cycles that submitted biopsies for eight different reasons are shown. It should be noted that reasons were not reported in 51.1% of cycles that submitted day-3 biopsies and 23.0% of cycles that submitted day-5 biopsies. AMA = advanced maternal age; RPL = recurrent pregnancy loss.

Demko. Maternal age effects on embryo euploidy. Fertil Steril 2016.

RESULTS

A total of 46,439 embryo biopsies (6,325 cycles) from women 18 to 48 years old were submitted for PGS analysis. Overall, more day-3 biopsies were submitted than day-5 biopsies (28,052 vs. 18,387, respectively). For both day-3 and day-5 biopsies, the most common reasons for performing this test were advanced maternal age, recurrent pregnancy loss, and gender selection (Fig. 2). Because we aimed to determine the effects of maternal age on embryo euploidy, we omitted 5,005 samples with unknown maternal age. In addition, we excluded 3,723 samples that did not have sufficient information to determine embryo ploidy. The exclusion criteria were low-confidence copy-number calls [<80% confidence (22)] for five or more chromosomes or embryos called nullisomic for all chromosomes. A total of 37,711 embryos from 5,821 cycles remained for analysis. The day-3 biopsies comprised 22,599 embryos from 2,652 cycles and day-5 biopsies included 15,112 embryos from 3,169 cycles. There were 3,679 embryos (9.8%; 391 cycles) from egg donors and 34,032 embryos (90.2%; 5,430 cycles) from nondonors.

Proportion of Euploid Embryos per Cycle

The median proportion of euploid embryos in each cycle remained relatively steady at ~35% in day-3 biopsies and ~55% in day-5 biopsies in women 27 to 35 years old, and then rapidly declined to 0 by age 44 (data not shown). These results imply that the effect of maternal age on the proportion of euploid embryos in each cycle is independent of biopsy timing. Although the interquartile range (difference between the 25th and 75th percentiles) of the euploid proportion in day-5 embryos was higher than that in day-3 embryos for nearly all maternal ages, this most likely reflects the smaller number of day-5 embryos compared with day-3 embryos in all age groups. For embryos from egg donors and nondonors younger than 33 years old, the euploid proportion of day-3 and day-5 embryos in each cycle were similar.

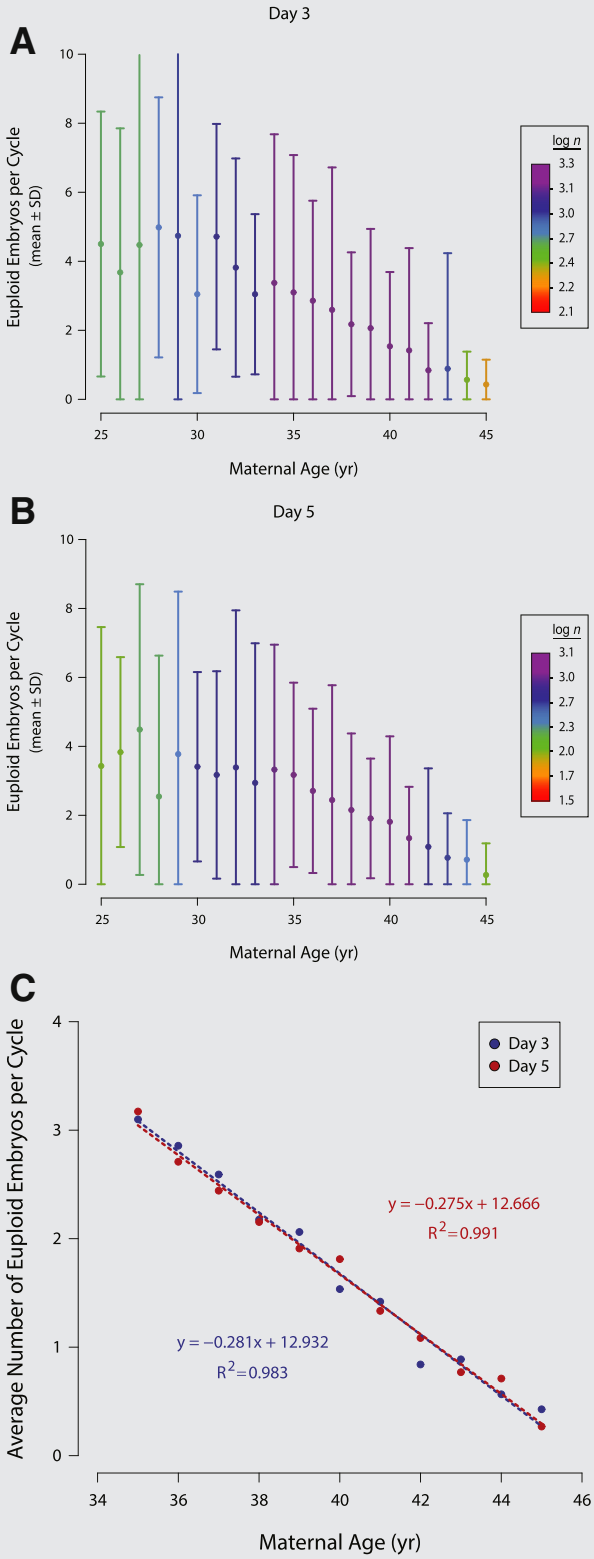
Number of Euploid Embryos per Cycle

For both day-3 and day-5 embryos, the average number of euploid embryos per cycle declined from ~4 euploid embryos/cycle for women in their late 20s to <1 euploid embryo/cycle after age 42 (Fig. 3A and 3B). After age 35, the average number of euploid embryos decreased sharply, with a strong inverse linear relationship with age in both day-3 and day-5 embryos (R^2 = 0.983 and 0.991, respectively; Fig. 3C). The lack of a difference in these age-related declines between day-3 and day-5 biopsies indicates that embryo attrition during this period can be attributed to embryo aneuploidy. For embryos from egg donors and nondonors under 33 years old, both day-3 and day-5 biopsies had similar average numbers of euploid embryos. Specifically, egg donors between 25 and 32 years old averaged 4.6 euploid embryos /cycle on day 3 and 3.2 euploid embryos/cycle on day 5, whereas nondonors in the same age range averaged 4.1 euploid embryos/cycle on day 3 and 3.4 euploid embryos/cycle on day 5. These results suggest that the number of euploid embryos per cycle does not depend on egg donor/nondonor status.

Probability of Retrieving at least One Euploid Embryo in a Cohort

An important metric for women undergoing IVF is the likelihood of having at least one euploid embryo in a fertility cycle (i.e., PrE). Consistent with the observed effect of maternal age on the number and proportion of euploid embryos, PrE was high before age 35 years, and dropped quickly thereafter in both day-3 and day-5 embryos (Fig. 4). Specifically, at age 35, at least one euploid embryo can be expected in approximately 85% of all cycles; this percentage drops to approximately 75% at age 40, and to approximately 45% by age 44. For women over 32, PrE is approximated by a Weibull probability density function (R^2 = 0.986), which is an exponential model of age-related failure rates (28, 29) that is

FIGURE 3



Distribution of the number of euploid embryos per IVF cycle from women 25 to 45 years old. (**A, B**) The average number of euploid embryos per cycle at (**A**) day 3 and (**B**) day 5 tends to decline with increasing maternal age. Data points and whiskers show the mean and one SD, respectively. In (**A**), the upper whiskers for ages 27 and 29 extend to 12.7 and 14.6 euploid embryos per cycle, respectively. (**C**) After age 35, the average number of euploid embryos per cycle decreases with increasing maternal age, as shown by the strong correlation between the data points and the dashed regression line. Colors represent the number of embryos in each age group, as in Figure 1B. Age groups with less than 45 cycles are not shown.

Demko. Maternal age effects on embryo euploidy. Fertil Steril 2016.

consistent with the acceleration in the age-related decrease in PrE with increasing maternal age we observed here. For embryos from egg donors and nondonors younger than 33 years old, PrE was consistently high in both day-3 and day-5 biopsies. Thus, PrE appears to be independent of biopsy timing and not dependent on egg source.

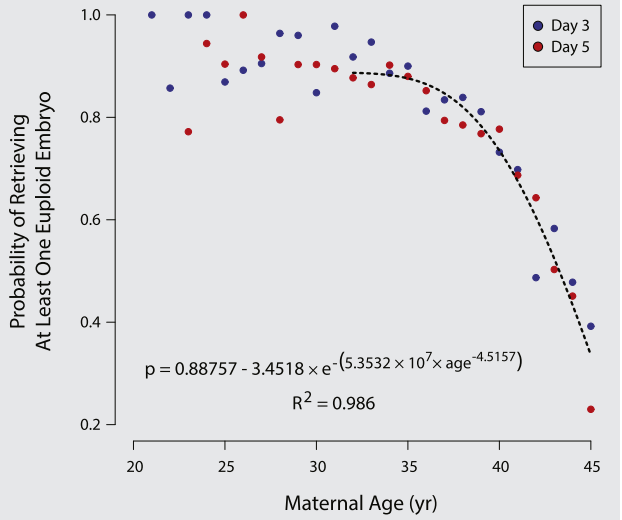
DISCUSSION

This is the largest known study of the effects of maternal age on euploidy rates in preimplantation embryos analyzed with 24-chromosome PGS. Our results corroborate previously reported euploidy rates as well as known relationships between embryo euploidy and maternal age, biopsy timing, and egg donor status (6, 12, 24, 30–33). Specifically, our finding that only approximately one-third to one-half of all preimplantation embryos (day 3 and day 5) from women 18 to 48 years old test as euploid is consistent with previous reports that 30% to 60% of preimplantation embryos screened with comparative genomic hybridization or SNP arrays test as aneuploid (7). Our data also confirm that euploidy rates in both day-3 and day-5 embryos remain roughly constant between 24 and 35 years of age, with a striking decrease thereafter. These trends agree well with a recent study of 15,169 day-5 embryos analyzed with SNP arrays that showed that embryo aneuploidy rates and probabilities of there being no euploid embryos in a cohort are lowest in women 26 to 30 years old, and markedly increased in women over 35 (30).

Egg donors, who are required to be within the age range associated with peak euploid proportion and PrE (30), can help women of advanced maternal age overcome age-related decreases in embryo euploidy and achieve a successful pregnancy (9). Because the proportion of euploid embryos from egg donors and nondonors under 33 was not statistically significantly different, the lower overall euploidy rate in embryos from nondonors was consistent with a reduction in euploid embryos as women age beyond 33 years. This finding underscores the importance of considering maternal age when predicting the likelihood of successful IVF outcomes, particularly at advanced maternal ages. In addition, it suggests that aneuploidy is not the primary cause of failed IVF cycles in younger patients.

The finding that the number of euploid embryos per cycle is essentially the same for day-3 and day-5 biopsies is in agreement with a recent comparison of day-3 and day-5 embryos using 24-chromosome array comparative genomic hybridization analysis (34). Likewise, consistent with other reports (6, 14, 24, 35, 36), we also observed that the proportion of euploid embryos at day 5 tends to be higher

FIGURE 4



Probability of retrieving at least one euploid embryo in a cohort of embryos from women aged 21 to 45 years old. For both day-3 and day-5 embryos, the probability of there being at least one euploid embryo was relatively high in women <35 years old, but it decreased rapidly in older women. After the age of 32, this decline is modeled by an exponential curve (R^2 = 0.986; dotted black curve). Note that this curve was fit to data from both day-3 and day-5 embryos.

Demko. Maternal age effects on embryo euploidy. Fertil Steril 2016.

than at day 3 across all maternal ages, but that the total number of embryos at day 5 was less than that at day 3. The precise counterbalance of these two metrics suggests that the attrition of embryos from day 3 to day 5 is due largely to aneuploidy; however, there are other possible explanations, including attrition of euploid embryos combined with trisomy rescue, and mosaicism.

The main strength and distinctive feature of our study is the large number of preimplantation embryos that we analyzed, which allowed us to characterize maternal age relationships in unprecedented detail and with accurate mathematic models. These data should help fertility specialists make more age-specific predictions of success for women undergoing IVF with or without PGS.

Due to the retrospective nature of our study, our results may have been limited by selection or information biases. However, because of our large sample size, selection bias resulting from samples that are not representative of the population is likely to be minimal. In addition, information bias due to misclassification of embryo ploidy is not likely to be significant because the false-detection rate of SNP-based PGS is as low as that of metaphase karyotyping (<4%), the gold standard for chromosome analysis (22). Furthermore, the consistency of our findings with known maternal age relationships suggests that these common limitations of retrospective studies were not significant in our study.

It should be noted that although PGS is expected to increase implantation and pregnancy rates by selectively eliminating aneuploid embryos, there are many other factors that may affect clinical outcomes. For example, chromosome number is only one determinant of embryo health. Additionally, technical aspects of IVF procedures such as the timing of embryo biopsy, vitrification, and transfer can affect pregnancy outcomes. Finally, mosaicism in preimplantation embryos, which is common at day 3 (21, 22), may lead to an incorrect assessment of the potential of an embryo to develop into a healthy euploid baby (37).

Nevertheless, because providing meaningful counseling to patients undergoing IVF requires being able to compare patients with peers of the same age and egg donor status, we hope that our findings will help physicians, reproductive specialists, and genetic counselors optimize IVF treatment. Specifically, age- and donor-specific euploid proportions and PrE values can be used to predict the likelihood of a successful IVF outcome, help patients set realistic expectations during treatment cycles, and facilitate the transition to subsequent cycles in the event of a failed cycle.

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