Check for updates

Euploidy rates within blastocysts obtained from differently sized cohorts and age groups approximate a binomial distribution

Thomas T. F. Huang, Ph.D.,[a,b] Sheela Ali, Ph.D.,[c] Kyle M. Ishikawa, Ph.D.,[d] Hyeong Jun Ahn, Ph.D.,[d] Ammon H. Bayles, Ph.D.,[e] Shawn E. Gurtcheff, M.D.,[e] Nabil Arrach, Ph.D.,[f] and Santiago Munne, Ph.D.[f]

[a] Department of Obstetrics and Gynecology and Women's Health, University of Hawaii John A Burns School of Medicine, Honolulu, Hawaii; [b] Pacific IVF Institute, Kapiolani Medical Center for Women and Children, Honolulu, Hawaii; [c] Scientific Director Services, Frisco, Texas; [d] Department of Quantitative Health Science, John A Burns School of Medicine, Honolulu, Hawaii; and [e] Utah Fertility Center, Pleasant Grove, Utah; and [f] Progenesis USA, La Jolla, California

Objective: To investigate whether the distribution of euploid blastocysts within cohorts can be predicted.
Design: Retrospective observational study.
Subjects: A total of 1,719 autologous egg retrieval cycles for in vitro fertilization from a single center that resulted in blastocysts biopsied for preimplantation genetic testing for aneuploidy.
Exposure: Trophectoderm biopsy.
Main Outcome Measures: Analysis of the statistical distribution structure of blastocyst euploidy within cohorts as functions of patient age and discrete cohort size.
Results: A total of 9,385 blastocyst biopsy results were analyzed. Results were first categorized into the following genotype categories: euploid (61.1%); uniform whole chromosome aneuploid (32.9%); mosaic and/or segmental (3.3%); and inconclusive results (2.7%). Cohorts were then stratified by both age group (<30, 30–34, 35–37, 38–40, 41–42, and ≥43 years) and by cohort size from 1 to 10 blastocysts. Within age groups, the probability that a blastocyst was euploid progressively decreased with increasing age from 74.5% (<30 years) to 19.2% (≥43 years). Within each of the age groups, the probability for euploidy increased as the biopsied cohort size increased from 1 to 10 (odds ratio, 1.03 for each unit increase). Biopsies from younger age groups of <40 years showed predictably smooth increases in the number of e+/cohort as cohort size increased. Notably, the observed distribution of euploids/cohort showed no statistical differences from those expected from theoretical binomial calculations in 52 (91.2%) of 57 discrete subgroups identified by age and biopsied cohort size.
Conclusion: The probability distribution structure of euploids within discrete cohort sizes is binomial within all age groups and nearly all cohort sizes. The probability distribution ranges identified represent a more accurate frame of reference that describes and predicts the number of euploid blastocysts most likely to be present within an individual cohort before biopsy compared with estimates from simple age group averages. These results offer new approaches for counseling and clinical decision-making. (F S Rep® 2025;6:478–86. ©2025 by American Society for Reproductive Medicine.)

Key Words: In vitro fertilization, blastocyst biopsy, preimplantation genetic screening for aneuploidy, binomial distribution

Since the introduction of preimplantation genetic testing for aneuploidy (PGT-A) (1) using blastocyst trophectoderm (TE) biopsy and comprehensive genetic screening, multiple randomized clinical trials have shown that PGT-A, as originally designed, can improve the ongoing pregnancy rates per transfer in women aged ≥35 years (2–7). The unassailable rationale for such screening was to avoid the transfer of embryos with meiotically derived, whole chromosome imbalances that

Received May 22, 2025; revised September 5, 2025; accepted September 9, 2025.
Supported by intramural funding from the Pacific IVF Institute and the Department of Obstetrics and Gynecology of the University of Hawaii John A. Burns School of Medicine and approved as exempted research by its Institutional Review Board (application CHS#23407).
Preliminary results from portions of this work have been presented at the Annual American Society for Reproductive Medicine meeting (Denver, CO, 2024).
Correspondence: Thomas T. F. Huang, Ph.D., Department of Obstetrics and Gynecology and Women's Health, University of Hawaii John A. Burns School of Medicine, Kapiolani Medical Center, Room 829, 1319 Punahou Street, Honolulu, Hawaii 96826 (E-mail: huangt@hawaii.edu).

F S Rep® Vol. 6, No. 4, December 2025 2666-3341
© 2025 The Authors. Published by Elsevier Inc. on behalf of American Society for Reproductive Medicine. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
https://doi.org/10.1016/j.xfre.2025.09.004

would affect all cells of the early developing embryo. Blastocysts with such meiotic aneuploidies seldom form genetically normal live births; however, they account for many spontaneous pregnancy losses in patients of all ages, despite from embryos that have relatively normal morphology (8–11). In addition, successive transfers of single euploids, when sufficiently available, have yielded compelling predictive information related to reproductive certainty (12–15).

Although PGT-A was originally aimed at women of advancing maternal age, who produce relatively high rates of aneuploid embryos, it has also been applied to younger patients, where the PGT-A results are less compelling because they produce fewer aneuploid embryos. Some randomized clinical trials in young patients show no improvement of results (5, 16, 17), whereas others do (18, 19). Those that do not have been attributed to the fact that, at the time of the study, mosaic embryos were not being replaced; this lowered the implantation potential of the test arm (5, 17) along with possible damage from the embryo biopsy techniques (16). In a recent analysis of the latest Society for Assisted Reproductive Technology data (SART 2022), Munné and Griffin (20) have reported that PGT-A results are associated with higher ongoing pregnancy rates per transfer and reduced miscarriage rates in all female age categories. As an embryo selection technique, PGT-A cannot improve results when there are only one or few embryos to select from, and it cannot improve cumulative pregnancy rates where there is no selection because all are replaced in the control arm.

Other issues still remain for ongoing investigation relating to the viability and risk of replacing intermediate copy number (putative mosaic) embryos (21–23) although several studies suggest that low-level mosaics can implant as well as euploids (24–26). In an effort to more systematically clarify these risks and related efficacy concerns among a more widely diverse patient population, the purpose of this study was to describe more detailed statistical distributions of normal euploid blastocysts from the perspectives of both discrete blastocyst cohort size and maternal age. Historically, Ata et al. (27) used an array comparative genomic hybridization platform to analyze 1,349 blastocyst biopsies obtained from over 70 different referring programs from patient ages ranging from <35 to ≥ 43 years. As expected, they reported that increasing maternal age reduced the proportion of euploids/cohort; however, they found no discernable effect of cohort size on the probability that a blastocyst is euploid (P|e+) *within* any of 5 stratified age groups studied. In a more recent study, Huang et al. (28) examined similar correlations using a next-generation sequencing platform reporting intermediate copy number results. Although they also reported the expected age effect on the probability of euploidy, they did observe an increase in this probability as cohort sizes increased within each of only 2 stratified age groups (<37 and ≥ 37 years). More significantly, this study also applied a novel binomial analysis that found a strong concordance between the observed and expected distributions of euploids/cohort among both intermediate and larger cohort sizes.

Using this same frame of analysis, we extend these studies here by describing the statistical distribution of euploid blastocysts from both a different and larger sample population of 9,385 blastocysts biopsies from a single program using a single molecular genetics platform. A binomial analysis of the distribution of euploid embryos was performed across 10 discrete blastocyst cohort size groups and 6 stratified maternal age groups ranging from <30 to >43 years using empirically observed P|e+ averages representative of each of the stratified groups. These results confirm a remarkable concordance between the binomially predicted and observed distributions of euploids/cohort across each of the discrete cohort sizes. These observations support consideration of a paradigm shift useful for clinical decision-making away from simply considering general age group averages for a blastocyst's euploidy to a specific cohort's "probability distribution of euploidy" on the basis of blastocyst cohort size and patient age. This different frame of reference may be more useful to accurately weigh and measure a given patient's pregnancy risks and benefits from a TE biopsy.

MATERIALS AND METHODS

This study analyzed 9,385 blastocyst biopsies from 1,719 infertility patient cycles (Table 1) from a single program (Utah Fertility Center, Pleasant Grove, UT) over a 4 year period from January 21, 2019, to December 23, 2022. There were no specific inclusion or exclusion criteria other than a patient's autonomous choice to perform biopsy. All biopsies were from day 5 and 6 blastocyst-stage embryos that were group-cultured in a single-step culture medium. Morphological assessments of blastocysts were performed by trained embryologists using the Gardner grading system (29). Biopsies were prepared and loaded into polymerase chain reaction tubes and transported to Progenesis for testing via commercial overnight courier service. Results for all study group patients were reported as 1 of 4 categories: euploids (61.1%); aneuploids (involving whole chromosomes, presumably meiotic in origin, 32.9%); mosaic and/or with subchromosomal segmental abnormalities (3.3%); and those with inconclusive biopsy results (2.7%; Supplemental Fig. 1, available online). With Progenesis' platform, the threshold for mosaics is 20%–40% with higher values interpreted as aneuploid.

Statistical methods and data analysis

Frequencies and percentages of biopsy results were calculated for each age group as shown in Table 1. Six age groups were stratified into six age groups. These age groupings were determined by adding both one younger (<30 years) and one older (≥ 43 years) age groups to the four age groups (<35, 35–37, 38–40, and >40 years) reported standardly in the United States to the Society for Assisted Reproductive Technology. For binomial-based analyses, genotypes from discrete cohort sizes of 1–10 were classified as either euploid (e+) or not euploid (e−). Binomially predicted values for e+ were calculated with the combinatoric equations defined by the binomial theorem (28, 30) using the observed probability

TABLE 1

The number of study patients and euploid blastocysts stratified by patient age in years and biopsied cohort sizes from 1 to 10.

Age (y)	Patients	Blastocysts	Euploids	All	Euploidy by cohort size									
					1	2	3	4	5	6	7	8	9	10
All	1,719	9,385	5,731 (61.1%)	0.555	0.472	0.457	0.484	0.555	0.544	0.576	0.608	0.613	0.656	0.700
<30	349	2,392	1,781 (74.5%)	0.727	0.657	0.688	0.714	0.735	0.697	0.732	0.753	0.685	0.778	0.771
30–34	496	3,020	2,059 (68.2%)	0.658	0.578	0.609	0.578	0.663	0.674	0.643	0.719	0.693	0.696	0.730
35–37	369	2,010	1,153 (57.4%)	0.569	0.578	0.568	0.556	0.595	0.533	0.568	0.599	0.534	0.563	0.586
38–40	291	1,309	583 (44.5%)	0.422	0.478	0.359	0.347	0.417	0.417	0.444	0.398	0.509	0.491	0.550
4142	147	503	126 (25.0%)	0.262	0.324	0.237	0.281	0.221	0.200	0.200	0.229	0.333	0.444	0.300
≥43	67	151	29 (19.2%)	0.124	0.100	0.105	0.185	0.167	0.133	0.500	0.143			

Huang. Binomial euploid blastocyst distribution. F S Rep 2025.

values for euploidy shown in Table 1. The goodness-of-fit between the observed and binomially expected euploid distributions was assessed with the Pearson chi-square test using a significance level of .05 as interpreted with consideration of group size (31).

A multivariable mixed-effects logistic regression model was also developed to assess the relationship between age group, cohort size, and euploidy while taking into account that euploidy and cohort size vary within patients. Each unit of observation was a blastocyst status (euploid vs. non-euploid) accompanied by the patient's age, cohort size, and patient identification to keep track of repeated measurements for each patient. The model included patients with cohort sizes greater than 10. A separate model was created to test the significance of interaction terms between age groups and cohort size. Statistical significance was set at .05. All analyses were performed in R version 4.3.2. Odds ratios were interpreted with respect to the reference group of patients aged <30 years in this study (Supplemental Fig. 2). The odds ratio of cohort size was interpreted while holding age constant.

This work was approved by the Institutional Review Boards the University of Hawaii John A. Burns School of Medicine as exempted (application CHS#23407).

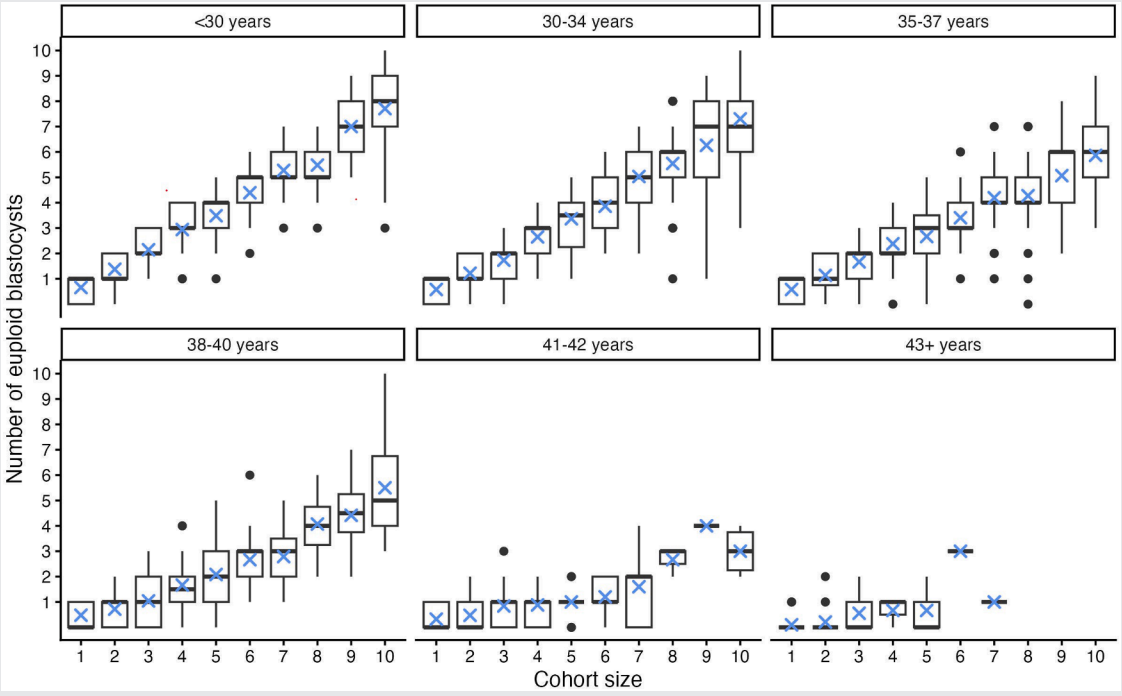
RESULTS

The probability that a blastocyst is euploid decreases with maternal age but increases by cohort size within six stratified age groups

A summary of total study euploid blastocyst count and probabilities within each of the stratified age groups and cohort sizes is shown in Table 1. All age groups except the oldest had some cohorts with 10 blastocysts available for biopsy. The overall study population's probability of euploid (P|e+) was 0.611 when including all cohort sizes. This value was age dependent and decreased from a maximum of 0.745 in the youngest to 0.192 in the oldest of the stratified age groups. The P|e+ also increased from 0.472 to 0.700 in the study group of discrete cohort sizes from 1 to 10. The cohort size–based increases in P|e+ shown in Table 1 were found to be statistically significant (<.001) when analyzed as odds ratios (Supplemental Fig. 2). In the overall study group, the odds for euploidy increased by 4% (e.g., 1.04) for each unit increase in cohort size across all age groups. When holding cohort size constant, this age effect was relatively greater in the youngest age group than in the oldest group. When interaction terms between age group and cohort size were added to the model, only the oldest age group (≥43 years) yielded a statistically significant interaction term. However, this result should be interpreted with caution because it may reflect the influence of outliers or unstable estimates because of the small sample size in this subgroup.

Figure 1 describes the distribution of the number of e+/cohort as quartiles as functions of age and cohort size. In such box and whisker plots, box boundaries represent the range of e+/cohort found in the second and third cohort quartiles. The whiskers represent quartiles containing either fewer (first quartile) or more (fourth quartile) e+/cohort than their adjacent quartiles. Outliers are shown as single

FIGURE 1



Box and whisker plots of data quartiles describing the progressive, age-related decline in the distribution of euploids/cohort for the study's age groups discrete biopsied cohort sizes from 1 to 10. As further described in Table, 2, plot boxes contain cohort quartiles 2 + 3. Whiskers represent upper quartile 1 and lower quartile 4 below boxes, respectively. Outliers are represented as single dots.

Huang. Binomial euploid blastocyst distribution. F S Rep 2025.

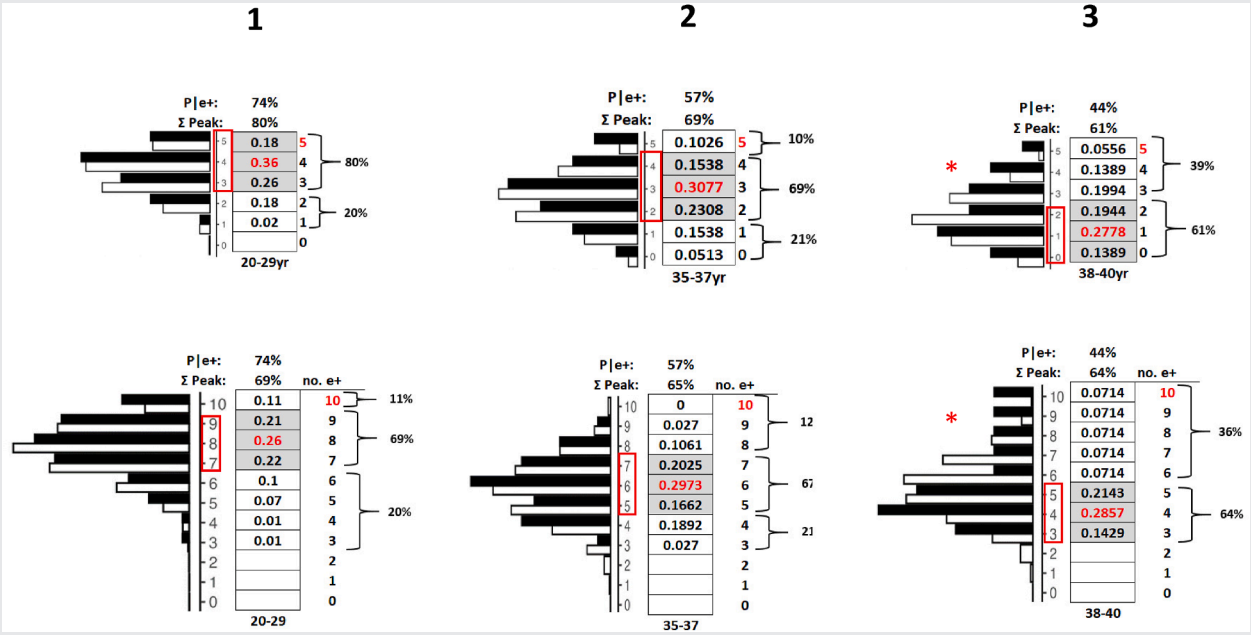
cohorts that fell outside of statistically defined limits of the quartiles.

In the three youngest age groups <38 years (Fig. 1 and Supplemental Fig. 1), there was a smooth and progressive increase in all four cohort size quartiles together, even beginning with the initial cohort size of 2. As shown by the positions of lower whiskers and boxes, the absence of even a single euploid among smaller cohorts was uncommon in these three youngest age groups. In contrast, with the age transition to 38–40 years, there first appeared an increasing persistence of either no or very few e+/cohort within the smaller cohort sizes of 1–4 blastocysts (Fig. 1). In patients aged >40 years, such persistence increased, and multiple euploids were observe in only a minority of the few larger biopsied cohort sizes in the older age groups >40 years (Supplemental Fig. 3).

Within all age groups, the observed distributions of euploids within all cohort sizes show strong congruence with simple, theoretical distributions predicted from the binomial theorem

The histograms shown in Figure 2 clearly show that the probability distribution of euploids within cohorts is not uniform, which is implied from age group euploidy averages. The histogram architectures suggest a more formal underlying statistical structure. Using the P|e+ values shown in Table 1, the observed distributions were found to closely match those theoretically expected using the binomial theorem (28, 30). A comparison of such expected and observed distributions are shown in Figure 2 for 2 of the larger cohort sizes of 5 and 10 for the age groups of 20–29, 35–37, and 38–40 years. In these larger cohort sizes, this statistical architecture becomes clearer. In each of the 6 panels, the most commonly found number of e+/cohort and their probability values are highlighted in red and correspond to the observed histogram peaks. The observed peaks in these panels were nearly always identical to those peaks expected from theoretical binomial calculations. A further goodness-of-fit chi-square analysis showed that these observed distributions were significantly different from the expected in only 5 (8.8%) of the 57 total group panels (Supplemental Fig. 3). The 2 panels describing the 38–40-year age group for both cohort sizes 5 and 10 (asterisks) represent 2 of these exceptional distributions. In the panel describing cohort size 5, the peak number of e+/cohort observed was actually smaller than the peak expected (e.g., 1 vs. 2). In contrast, in the largest cohort size of 10 in the 38–40-year age group, both the observed and expected peaks were the same; however, the observed number of cohorts with 9–10 e+/cohort was

FIGURE 2



Examples comparing the number of binomially predicted (gray) with the number of observed (black) distributions of euploid blastocysts from cohort sizes of 5 (**A**) and 10 (**B**) for 3 of the stratified age groups (20–29, 35–37, and 38–40 years). The probability of the most frequently observed distribution peak value is identified in red. The sum of this value with each of its nearest neighbor (highlighted) values are shown at the top of each cell columns as the "peak Σ" probability as well as the overall average group's P|e+. Examples of distributions that significantly differed from those binomially expected using the chi-square are indicated by asterisks (*) for panels A3 (P=.034) and B3 (P<.001).

Huang. Binomial euploid blastocyst distribution. F S Rep 2025.

actually higher than would be expected from binomial calculations. Despite these statistical differences, the probability masses for euploidy (the $\sum$ peak values) were still quite similar and found to include the second and third most prevalently found numbers of e+/cohort—a hallmark feature expected of binomial distributions (30) (Supplemental Figs. 4–6). Thus, from the practical perspective predictions related to clinical decision-making, both distributions are effectively congruent.

DISCUSSION

Although results from TE biopsies have shown that the number of euploid blastocysts within cohorts can vary widely, there have been no studies evaluating whether there is actually any underlying statistical structure to their distributions. The data described here confirm a recent report (28) from a smaller data set that these distribution structures are binomial. A binomial distribution describes the whole number (e.g., "discrete") distribution of successes in a fixed number of trials that have one of two possible outcomes (e.g., euploid vs. not euploid). Such probability mass distributions generally appear skewed, depending on the probability for a given outcome that is used. The combinatorial equation that is used to describe such a distribution is as follows, where k is the discrete sampling size (e.g., cohort size), n is the total number of blastocysts in the population being sampled that have that discrete cohort size, and p is that population's overall P|e+ (28) (a specific example is shown in Supplemental Fig. 7):

$$f(k,n,p) = \Pr(X=k) = \binom{n}{k} p^k (1-p)^{n-k} \quad (1)$$

In contrast, normal distributions are symmetrically bell shaped and describe "continuous" variables (e.g., height and weight). The derived probability density curves for normal distributions can also be mathematically described using the sample's mean values (μ) and variances (σ) as follows:

$$f(x) = \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\frac{(x-\mu)^2}{2\sigma^2}} \quad (2)$$

What is common to both binomial and normal distributions is that their expected peaks and associated probabilities surrounding those peaks (e.g., "$\sum$ peak" indicated in Fig. 2) are both defined and predictable. In this context, data described in this manuscript show that the observed distribution of euploids within discrete cohort sizes, with few exceptions using chi-square analysis, closely matched with the values expected from the binomial calculations. These findings support the hypothesis that the distributions of euploids within cohorts can be predicted even before biopsy given a program's database of observed P|e+ values. As a corollary,

TABLE 2

Summary statistics of Figure 1 showing the number of euploid blastocysts representing the mean, median, minimum, maximum, interquartile range values (as defined by the boxes in Figure 1), standard deviations, and 95% confidence intervals.

		Cohort size								
Age group	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
<30 y	Mean: 0.66	1.38	2.14	2.94	3.49	4.39	5.27	5.48	7.00	7.71
	Median: 1	1	2	3	4	5	5	5	7	8
	IQ values: 0–1	1–2	2–3	3–4	3–4	4–5	5–6	5–6	6–8	7–9
	Min: 0	0	1	1	1	2	3	3	5	3
	Max: 1	2	3	4	5	6	7	7	9	10
30–34 y	Mean: 0.58	1.22	1.73	2.65	3.37	3.86	5.03	5.55	6.26	7.30
	Median: 1	1	2	3	3.5	4	5	6	7	7
	IQ values: 0–1	1–2	1–2	2–3	2.25–4	3–5	4–6	5–6	5–8	6–8
	Min: 0	0	0	1	1	2	2	1	1	3
	Max: 1	2	3	4	5	6	7	8	9	10
35–37 y	Mean: 0.58	1.14	1.67	2.38	2.67	3.41	4.19	4.28	5.07	5.86
	Median: 1	1	2	2	3	3	4	4	6	6
	IQ values: 0–1	0.75–2	1–2	2–3	2–3.5	3–4	4–5	4–5	4–6	5–7
	Min: 0	0	0	0	0	1	1	0	2	3
	Max: 1	2	3	4	5	6	7	7	8	9
38–40 y	Mean: 0.48	0.72	1.04	1.67	2.08	2.67	2.79	4.07	4.42	5.50
	Median: 0	1	1	1.5	2	3	3	4	4.5	5
	IQ values: 0–1	0–1	0–2	1–2	1–3	2–3	2–3.5	3.25–4.75	3.75–5.25	4–6.75
	Min: 0	0	0	0	0	1	1	2	2	3
	Max: 1	2	3	4	5	6	5	6	7	10
41–42 y	Mean: 0.32	0.47	0.84	0.88	1.00	1.20	1.60	2.67	4.00	3.00
	Median: 0	0	1	1	1	1	2	3	4	3
	IQ values: 0–1	0–1	0–1	0–1	1–1	1–2	0–2	2.5–3	4–4	2.25–3.75
	Min: 0	0	0	0	0	0	0	2	4	2
	Max: 1	2	3	2	2	2	4	3	4	4
≥43 y	Mean: 0.10	0.21	0.56	0.67	0.67	3.00	1.00	*NA*	*NA*	*NA*
	Median: 0	0	0	1	0	3	1			
	IQ values: 0–0	0–0	0–1	0.5–1	0–1	3–3	1–1			
	Min: 0	0	0	0	0	3	1			
	Max: 1	2	2	1	2	3	1			

Note: IQ = interquartile; Max = maximum; Min = minimum.

Huang. Binomial euploid blastocyst distribution. F S Rep 2025.

these predictive distributions should be similar among programs using the same genetics platform for analysis of blastocysts selected for biopsy using similar criteria.

From a statistical perspective, such a high concordance was actually unexpected given the assumptions required for binomial distributions. Binomial distributions assume that trial samples derive from the allocation of randomly drawn choices from large sample sizes into discretely sized groups (e.g., cohorts). However, clinical cohorts of blastocysts actually represent nonrandomly chosen samples that already exist as "presorted," patient-defined cohorts. This preclustering represents a bias that could explain both some of the statistical outliers and the limited discordant results from chi-square analysis seen within several of the cohort sizes. Of notable clinical significance, however, these few discordances did not appear to affect the larger cumulative probability masses of euploids identified as the $\sum$ of peaks identified in those specific cases (Fig. 2).

The binomial distribution of e+/cohort also highlights where there are accuracy limitations in predicting the likely number of euploids within any given cohort when using only general age group's average P|e+. For example, for the 38–40-year age group, the overall group's population average P|e+ was 0.445 (Table 1). If euploids were distributed "uniformly" within all cohort sizes with this average, the theoretical probability of randomly selecting two euploid unbiopsied blastocysts from any cohort would be $0.445^2 = 19.8\%$. For the smaller cohort size of 2 blastocysts, the overall P|e+ observed was actually lower than the total group's (e.g., 0.359 vs. 0.445). Although this reduced the theoretical 19.8% probability alluded to above to 12.9%, this was still concordant with the 12.8% that was actually observed (Fig. 2); however, a binomial distribution analysis revealed that 41% of these cohorts had 0 e+/cohort (Table 2). In contrast, for the larger cohort size of 10 in the same age group, the observed P|e+ was higher than the overall age group average (e.g., 0.55 vs. 0.445). Were euploids uniformly distributed within all cohorts with this average, the theoretical probability of randomly selecting two euploid blastocysts from these larger cohorts would be $0.55^2 = 0.303$. This is nearly double that expected from the smaller cohort sizes of 2 in the same age group and would likely exceed the twin risk protection intended from professional guidelines for this age group. Thus, the binomial distributions show that this age group's average probability of 0.303 can actually vary from 0.02 to 1.0 within any given individual

cohort having a size of 10 (Fig. 3). Of further clinical significance, very few or no cohorts were either binomially predicted or observed to have fewer than 3 e+/cohort in this good responder group aged 38–40 years (Fig. 2); thus, any initial pregnancy failure after the transfer of either single or double unbiopsied blastocysts from such cohorts is unlikely to be due to a significant absence of euploids in the cohort. Such uncertainty can lead to thawing and biopsy to resolve such concerns.

In contrast to the youngest patient age groups, the low overall P|e+ associated with patients aged >40 years (0.25–0.12; Table 1) arguably presents greater predictive variability than seen in the younger age group cohorts. Binomial analysis shows that 0 or 1 represents the median values from these older patients having biopsied cohort sizes ≤4 (Fig. 1 and Table 2). Moreover, very few cohorts will have more than 1 e+/cohort until a cohort size of 5 is reached. These results suggest that these smaller cohort sizes from older patient groups are relatively more vulnerable to biopsy accuracy risk, particularly when mosaicism is also present; such results are more clinically impactful because no embryo transfer may occur. This suggests a need for additional retrospective (28) or nonselection study designs than enroll from such patient groups. These concerns may not be trivial because data from several studies suggests that there is a decrease in the expected pregnancy rates when measured on an intention to treat basis (5, 32). Although these concerns clearly need to be weighed against risks of miscarriage and other meiotically based aneuploidy risks that incontrovertibly increase with maternal age, such biopsy accuracy risk is less consequential from larger cohort sizes in the younger patient groups because the binomial analysis described here shows that multiple euploids will nearly always be identified for transfers (Fig. 1 and Table 2). Multiple euploids within younger cohorts act to offset any potential biopsy inaccuracy/no result risks that would result in failure to transfer a potentially totipotent blastocyst from cohorts from older age groups.

Taken together, the data presented here act to reframe a given patient's blastocyst cohort size from a probability "average" of euploidy to a probability "distribution" of euploidy. By themselves, however, such distributions do not identify where any given patient's cohort specifically lies within that distribution. As shown in Figure 2, a 35–37-year-old patient with five biopsiable blastocysts had a peak Σ of 69% of having from two to four euploids in their cohort; however, there is also a 21% probability that only 0–1 euploid exists as well as a 10% chance that all five blastocysts are euploid. Thus, it remains to see to what degree additional noninvasive assessments either from subjective standard grading morphology alone (29) or from more quantitative noninvasive approaches such as time-lapse imaging are able to better identify where a blastocyst cohort more likely lies within a particular area of the binomial distribution. To this end, the distributions described in this study can now be used to better predict if they are associated in distribution areas with a higher or lower number of euploids. The statistical distribution template described in this study should also be particularly relevant to emerging artificial intelligence platforms (33–35) for validation testing of accuracy over a more complete spectrum of blastocyst cohort sizes and maternal age groups.

As a practical matter, the binomial analysis described here offers a challenging amount of statistical information to search for any given patient. As a result, the database is currently being developed as both web-based and mobile applications (Supplemental Fig. 8). Such tools would be quite helpful for real-time decision-making for individual patients because such applications require entering only two parameters of age group and blastocyst cohort size to retrieve both pertinent and robust statistical distribution data. This enables flexibility to either confirm or change any treatment plan to biopsy while the preimplantation period is in progress.

Finally, the study design notes several limitations that may impact on reproducibility. This includes correlations between biopsy results with confounders from clinical factors (body mass index, ovarian reserve, stimulation protocols, and sperm quality), laboratory variables (selection criteria of blastocysts for biopsy), or the molecular genetics platforms used for biopsy analysis. A previous studies investigating the effect of cohort size within the same age group found no effect on aneuploidy rates (27). That study was multicentric, with some centers producing larger cohorts of embryos, at the same age, than others. Differences between centers in selecting which embryos to biopsy also varied. In this study, by using data from a single center, this variability is eliminated; however, it remains to be seen if it is exportable to other centers.

CONCLUSION

The probability distribution structure of euploids within discrete cohort sizes is binomial within all age groups and nearly all cohort sizes. The probability distribution ranges identified represent a more accurate frame of reference that describes and predicts the number of euploid blastocysts most likely to be present within an individual cohort before biopsy compared with estimates from simple age group averages. These results offer new approaches for counseling and clinical decision-making.

Acknowledgment

The authors acknowledge the administrative assistance of Ms. Jordan McGuire of Progenesis in the preparation of the data set for this manuscript.

CRediT Authorship Contribution Statement

Thomas T.F. Huang: Writing – original draft, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Sheela Ali:** Writing – review & editing, Project administration, Data curation. **Kyle M. Ishikawa:** Writing – review & editing, Formal analysis, Data curation. **Hyeong Jun Ahn:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Ammon H. Bayles:** Writing – review & editing, Investigation. **Shawn E. Gurtcheff:** Writing – review & editing, Investigation. **Nabil Arrach:** Writing – review & editing, Resources. **Santiago Munne:** Writing – review & editing, Resources, Investigation.

Declaration of Interests

T.T.F.H. reports travel support from Pacific IVF Institute. S.A. reports that Progenesis provided retrospective study data, and that no funding or other study materials were provided. K.M.I. reports funding from the National Institute on Minority Health and Health Disparities (Grant Number U54MD007601) and National Institute of General Medical Sciences, National Institutes of Health (Grant Number U54GM138062) outside the submitted work. H.J.A. reports funding from PIKO, U54GM138062 (the biostatistician [H.J.A.] is partially supported by the grant), and Ola Hawaii, U54MD007601 (the biostatistician [H.J.A.] is partially supported by the grant). A.H.B. has nothing to disclose. S.E.G. has nothing to disclose. N.A. has nothing to disclose. S.M. has nothing to disclose.

SUPPLEMENTAL MATERIAL

Supplemental data for this article can be found online at https://doi.org/10.1016/j.xfre.2025.09.004.

REFERENCES

1. Munné S, Lee A, Rosenwaks Z, Grifo J, Cohen J. Diagnosis of major chromosome aneuploidies in human preimplantation embryos. Hum Reprod 1993;8:2185–91.
2. Scott RT Jr, 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.
3. Forman EJ, Hong KH, Ferry KM, Tao X, Taylor D, Levy B, et al. In vitro fertilization with single euploid blastocyst transfer: a randomized controlled trial. Fertil Steril 2013;100:100–7.e1.
4. Rubio C, Bellver J, Rodrigo L, Castillón G, Guillén A, Vidal C, et al. In vitro fertilization with preimplantation genetic diagnosis for aneuploidies in advanced maternal age: a randomized, controlled study. Fertil Steril 2017;107:1122–9.
5. Munné S, Kaplan B, Frattarelli JL, Child T, Nakhuda G, Shamma FN, et al. STAR Study Group. Preimplantation genetic testing for aneuploidy versus morphology as selection criteria for single frozen-thawed embryo transfer in good-prognosis patients: a multicenter randomized clinical trial. Fertil Steril 2019;112:1071–9.e7.
6. Sui YL, Lei CX, Ye JF, Fu J, Zhang S, Li L, et al. In vitro fertilization with single-nucleotide polymorphism microarray-based preimplantation genetic testing for aneuploidy significantly improves clinical outcomes in infertile women with recurrent pregnancy loss: a randomized controlled trial. Reprod Dev Med 2020;4:32–41.
7. Sun Q, Xu J, Yao Y, Huang X, Zhao D, Lu S, et al. Efficacy of non-invasive chromosome screening, preimplantation genetic testing for aneuploidy, and morphological grading in selecting embryos of patients with advanced maternal age: a three-armed prospective cohort study. BMC Pregnancy Childbirth 2024;24:545.
8. Capalbo A, Rienzi L, Cimadomo D, Maggiulli R, Elliott T, Wright G, et al. Correlation between standard blastocyst morphology, euploidy and implantation: an observational study in two centers involving 956 screened blastocysts. Hum Reprod 2014;29:1173–81.
9. Fragouli E, Alfarawati S, Spath K, Wells D. Morphological and cytogenetic assessment of cleavage and blastocyst stage embryos. Mol Hum Reprod 2014;20:117–26.
10. Cimadomo D, Fabozzi G, Vaiarelli A, Ubaldi N, Ubaldi FM, Rienzi L. Impact of maternal age on oocyte and embryo competence. Front Endocrinol (Lausanne) 2018;9:327.
11. Fragouli E, Munne S, Wells D. The cytogenetic constitution of human blastocysts: insights from comprehensive chromosome screening strategies. Hum Reprod Update 2019;25:15–33.
12. Tiegs AW, Tao X, Zhan Y, Whitehead C, Kim J, Hanson B, et al. A multicenter, prospective, blinded, nonselection study evaluating the predictive value of an aneuploid diagnosis using a targeted next-generation sequencing-based preimplantation genetic testing for aneuploidy assay and impact of biopsy. Fertil Steril 2020;115:627–37.
13. Pirtea P, De Ziegler D, Tao X, Sun L, Zhan Y, Ayoubi JM, et al. Rate of true recurrent implantation failure is low: results of three successive frozen euploid single embryo transfers. Fertil Steril 2021;115:45–53.
14. Bamford T, Smith R, Young S, Evans A, Lockwood M, Easter C, et al. A comparison of morphokinetic models and morphological selection for prioritizing euploid embryos: a multicentre cohort study. Hum Reprod 2024;39:53–61.
15. Gill P, Ata B, Arnanz A, Cimadomo D, Vaiarelli A, Fatemi HM, et al. Does recurrent implantation failure exist? Prevalence and outcomes of five consecutive euploid blastocyst transfers in 123 987 patients. Hum Reprod 2024;39:974–80.
16. Ozgur K, Berkkanoglu M, Bulut H, Yoruk GDA, Candurmaz NN, Coetzee K. Single best euploid versus single best unknown-ploidy blastocyst frozen embryo transfers: a randomized controlled trial. J Assist Reprod Genet 2019;36:629–36.
17. Yan J, Qin Y, Zhao H, Sun Y, Gong F, Li R, et al. Live birth with or without preimplantation genetic testing for aneuploidy. N Engl J Med 2021;385:2047–58.
18. Katz-Jaffe M, Gassen C, Makloski R, Reed L, Schoolcraft WB. Impact of aneuploidy on reproductive success in young infertile women: prospective analysis. Reprod Biomed Online 2024;49:103858.
19. Yang Z, Liu J, Collins GS, Salem SA, Liu X, Lyle SS, et al. Selection of single blastocysts for fresh transfer via standard morphology assessment alone and with array CGH for good prognosis IVF patients: results from a randomized pilot study. Mol Cytogenet 2012;5:24.
20. Munné S, Griffin DK. Opinion: contemporary insights into the efficacy of preimplantation genetic testing for aneuploidy (PGT-A) by mining the Society for Assisted Reproductive Technology (SART) database. BMJCCGG 2024;1:e000003.
21. Munné S, Spinella F, Grifo J, Zhang J, Beltran MP, Fragouli E, et al. Clinical outcomes after the transfer of blastocysts characterized as mosaic by high resolution next generation sequencing- further insights. Eur J Med Genet 2020;63:103741.
22. Viotti M, Victor AR, Barnes FL, Zouves CG, Besser AG, Grifo JA, et al. Using outcome data from one thousand mosaic embryo transfers to formulate an embryo ranking system for clinical use. Fertil Steril 2021;115:1212–24.
23. Viotti M, Greco E, Grifo JA, Madjunkov M, Librach C, Cetinkaya M, et al. Chromosomal, gestational, and neonatal outcomes of embryos classified as a mosaic by preimplantation genetic testing for aneuploidy. Fertil Steril 2023;120:957–66.
24. Dahdouh EM. Preimplantation genetic testing for aneuploidy: a review of the evidence. Obstet Gynecol 2021;137:528–34.
25. Capalbo A, Poli M, Rienzi L, Girardi L, Patassini C, Fabiani M, et al. Mosaic human preimplantation embryos and their developmental potential in a prospective, non-selection clinical trial. Am J Hum Genet 2021;108:2238–47.
26. Popovic M, Borot L, Lorenzon AR, Lopes ALRC, Sakkas D, Lledó B, et al. Implicit bias in diagnosing mosaicism amongst preimplantation genetic testing providers: results from a multicenter study of 36 395 blastocysts. Hum Reprod 2024;39:258–74.
27. Ata B, Kaplan B, Danzer H, Glassner M, Opsahl M, Tan SL, et al. Array CGH analysis shows that aneuploidy is not related to the number of embryos generated. Reprod Biomed Online 2012;24:614–20.

28. Huang TTF, Walker B, Ahn HJ, Ishikawa KM, Arnett C, Huang CTF. Predicting frequency distributions of blastocyst biopsy genotypes by their discrete cohort size using the binomial theorem. Reprod Biomed Online 2025;50:104428.
29. Gardner DK, Lane M, Stevens J, Schlenker T, Schoolcraft WB. Blastocyst score affects implantation and pregnancy outcome: towards a single blastocyst transfer. Fertil Steril 2000;73:1155–8.
30. Drager K, von Collani E. Binomial distribution handbook for scientists and engineers. New York: Springer-Verlag; 2012.
31. Bergh D. Chi-squared test of fit and sample size-a comparison between a random sample approach and a chi-square value adjustment method. J Appl Meas 2015;16:204–17.
32. Huang TTF, Kosasa T, Walker B, Ohta A, Arnett C, Huang CTF, et al. Selection of blastocysts for transfer using a quantitative bioassay of blastocoel expansion rather than standard morphology grading. JSM Invitro Fertil 2023;4:1025–35.
33. Jiang VS, Bormann CL. Artificial intelligence in the in vitro fertilization laboratory: a review of advancements over the last decade. Fertil Steril 2023; 120:228–34.
34. Barnes J, Brendel M, Gao VR, Rajendran S, Kim J, Li Q, et al. A non-invasive artificial intelligence approach for the prediction of human blastocyst ploidy: a retrospective model development and validation study. Lancet Digit Health 2023;5:e28–40.
35. Diakiw SM, Hall JMM, VerMilyea MD, Amin J, Aizpurua J, Giardini L, et al. Development of an artificial intelligence model for predicting the likelihood of human embryo euploidy based on blastocyst images from multiple imaging systems during IVF. Hum Reprod 2022;37:1746–59.