# Minimum number of mature oocytes needed to obtain at least one euploid blastocyst according to female age in in vitro fertilization treatment cycles

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**Objective:** To find a useful tool for estimating the minimum number of metaphase II (MII) oocytes needed to obtain at least one euploid blastocyst according to female age.
**Design:** Retrospective analysis of in vitro fertilization (IVF) treatment cycles with preimplantational genetic testing for aneuploidies (PGT-A) performed over 5 years in IVIRMA Valencia (Spain), January 2017–March 2022. Approval from the Institutional Review Board of IVI Valencia (2204-VLC-040-CR).
**Setting:** Private infertility clinic in Spain.
**Patients:** Eligible patients were undergoing their first IVF-PGT-A treatment cycle, in which at least one MII oocyte was obtained, regardless of oocyte and semen origin. Oocyte donation cycles were included in the donor group ($\leq$34 years old). Treatment cycles from women with their own oocytes were selected only when the oocytes were aged $\geq$35 years (patient group). Only trophoectoderm biopsies performed on days 5 or 6 of development and analyzed using next-generation sequencing were included. Preimplantational genetic testing for aneuploidy cycles because of a known abnormal karyotype were excluded.
**Intervention:** Not applicable.
**Main Outcome Measures:** Number of MII oocytes needed to obtain one euploid blastocyst according to female age.
**Results:** A total of 2,660 IVF-PGT-A treatment cycles were performed in the study period in the eligible population (patients group = 2,462; donors group =198). The mean number of MII oocytes needed to obtain one euploid blastocyst increased with age, as did the number of treatment cycles that did not get at least one euploid blastocyst. An adjusted multivariate binary regression model was designed using 80% of the patient group sample (n = 2,462; training set). A calculator for the probability of obtaining at least one euploid blastocyst was created using this model. The validation of this model in the remaining 20% of the patient group sample (n = 493; validation set) showed that it could estimate the event of having at least one euploid blastocyst with an accuracy of 72.0%.
**Conclusions:** Our results show a preliminary model capable of predicting the number of MII oocytes needed to obtain at least one euploid blastocyst according to female age, calculated with the largest database of IVF-PGT-A treatment cycles ever used for this purpose, including only treatment cycles using next-generation sequencing on trophoectoderm biopsies. Once this model has been properly validated, it could help with decision-making for both clinicians and patients coming to an infertility clinic. (Fertil Steril® 2024;122:658–66. ©2024 by American Society for Reproductive Medicine.)
**El resumen está disponible en Español al final del artículo.**

**Key Words:** Oocyte, euploid blastocyst, PGT-A, female age, NGS

Female age is significantly and directly related to embryo aneuploidy rates (1). Indeed, these rates rise from approximately 40% in women aged >35 years to approximately 70%–80% in women aged >40 years and 90% in women aged >45 years (2).

The current delay in motherhood has led to a large proportion of women of advanced maternal age seeking infertility treatment (3), thus presenting significantly higher embryo aneuploidy rates. Consequently, these patients are characterized by lower chances of success in in vitro fertilization (IVF) treatments with their own

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oocytes (4), and many of them are finally encouraged to enter the oocyte donation program.

In the assumption of the statement "the older the patient, the lower the number of euploid blastocysts," there is a frequently asked question in the day-to-day operations of an infertility clinic: how many oocytes each of our patients' needs, according to female age, to have the highest chances of obtaining at least one euploid blastocyst in their IVF treatment cycles?

The answer to this question would constitute useful information for both the clinician and the patient. On the one hand, the clinician may be able to better assess each patient's possibilities and the feasibility of their treatment cycle because it will be easier to explain the patient's options. On the other hand, the patient will understand this information more easily, helping her to cope emotionally with treatment. This is nowadays feasible because predicting ovarian response with high precision before starting treatment has become possible with the use of novel biomarkers, such as antimüllerian hormone levels and/or antral follicle count (5).

This issue has been recently addressed by other investigators. The Patient-Oriented Strategies Encompassing Individualized Oocyte Number group published a novel prediction model in 1,296 trophoectoderm (TE) biopsies in 2019 that estimated the probability of blastocyst euploidy and the number of blastocysts required to obtain at least one euploid embryo for transfer on the basis of female age (6). The same group, also in 2019, published a similar model, but in this case to estimate the number of mature oocytes required to obtain at least one euploid blastocyst for transfer, using data from 347 patients (7). They designed a computerized tool to assist in clinical counseling and individualized treatment planning, which was subsequently validated in a multicenter study with a total of 1,464 patients (8).

The aim of the present study was to design a similar tool to determine the number of metaphase II (MII) oocytes needed to obtain at least one euploid blastocyst regarding female age in IVF treatment cycles, considering our own data from the last 5 years using next-generation sequencing (NGS) on TE biopsies. This information will help to decide the best strategy for each patient and her individual situation.

## MATERIALS AND METHODS

### Study design

Retrospective analysis of all IVF treatment cycles with preimplantational genetic testing (PGT) for aneuploidies (PGT-As) meeting the inclusion criteria was performed over 5 years in IVI RMA Valencia (Spain), from January 2017 to March 2022. The study was approved by the Institutional Review Board of IVI RMA Valencia (study code: 2204-VLC-040-CR).

### Study population

Eligible patients were aged 18–50 years, without any body mass index (BMI) or antimüllerian hormone level limitation. In vitro fertilization–PGT-A treatment cycles included those in which at least one mature oocyte was obtained, regardless of oocyte and semen origin. Only TE biopsies performed on days 5 or 6 of development and analyzed using NGS were included.

Preimplantation genetic testing for aneuploidy was used for reasons such as implantation failure or recurrent miscarriage (n = 206 in the patient group and n = 43 in the donor group), male factor (usually recommended when sperm concentration <1 million/mL; n = 84 in the patient group and n = 95 in the donor group), or previous chromosomopathy (n = 36 in the patients group and n = 44 in the donor group). When there was no additional indication, PGT-A was performed because of advanced maternal age (n = 2,136 in the patient group). Preimplantation genetic testing for aneuploidy indication was not available in 16 cases of the donor group. Preimplantation genetic testing for aneuploidy treatment cycles because of a known abnormal karyotype was excluded. These account for an abnormal fluorescent in situ hybridization of spermatozoa, as well as any numeric and/or structural karyotype alteration in any of the progenitors that requires PGT-A analysis.

The study population was subdivided into two categories:

- Donors group: IVF-PGT-A treatment cycles using donated oocytes (aged between 18 and 34 years).
- Patients group: IVF-PGT-A treatment cycles from women with their own oocytes in which the oocyte age was ≥35 years, to avoid any bias related to PGT-A indications in younger patients (not included, n = 322). In Spain, PGT-A is not performed in patients aged <35 years unless there is an indication, such as recurrent miscarriage, implantation failure, or severe male factor infertility. This is therefore a group that is not representative of the general population in this age range.

Only the first IVF-PGT-A treatment cycle performed per patient was included to avoid repeated measures in the dataset.

### IVF treatment procedures

Ovarian stimulation was performed after the routine clinical practice in IVIRMA Valencia, as described elsewhere (9, 10). The initial dose of gonadotropins was determined on the basis of the patient's age, BMI, and ovarian reserve test values. Administered gonadotropins included recombinant follicle-stimulating hormones: Gonal-F (Merck, Darmstadt, Germany), Puregon (MSD, Kenilworth, NJ), or Bemfola (Gedeon Richter, Budapest, Hungary), highly purified human menopausal gonadotropin (Menopur, Ferring Pharmaceuticals, Copenhagen, Denmark), or a combination of both. Ovulation induction was triggered using the gonadotropin-releasing hormone agonist (Decapeptyl, Ipsen Pharma, France), the human chorionic gonadotroin (Ovitrelle, Merck & Co., Inc., Rahway, NJ), or the combined action of both.

Oocyte retrieval, denudation, and intracytoplasmic sperm injection treatment were performed according to routine clinical practice at IVI Valencia, as described elsewhere (11). Fresh oocytes were fertilized approximately 4 hours after oocyte retrieval, although vitrified oocytes were warmed approximately 2 hours before fertilization using the Kitazato method (12, 13).

Fertilization was checked 16–20 hours after intracytoplasmic sperm injection, and it was considered successful when 2 pronuclear structures and 2 polar bodies were observed. Fertilized oocytes were incubated in Gems culture medium (Genea Biomedx, Australia) under mineral oil at 37 $^{o}$C, 6% $CO_2$, and 5% $O_2$ up to the blastocyst stage.

On day 3 of development, assisted hatching was performed on embryos using laser technology (Fertilase, Octax, Sweden). Afterward, the embryos were placed in fresh medium and were cultured until days 5 or 6. Trophoectoderm biopsies were performed on days 5 or 6, according to embryo stage of development.

Embryo quality was classified according to the Spanish Association for the Study of Biology of Reproduction classification (14). This classification assigns each blastocyst to a category from A–D on the basis of the TE and the inner cell mass morphology, with A being the best and D the worst quality. Only embryos graded A–C were considered for biopsy.

Blastocyst biopsy consisted of the isolation of approximately five TE cells using the pulling method, as described elsewhere (15). Blastocysts were vitrified by the Kitazato method (12, 13), awaiting the genetic results. Preimplantation genetic testing for aneuploidy analysis was performed using NGS (iGenomix; Paterna, Valencia, Spain); or Juno Genetics; Oxford, United Kingdom).

Euploid blastocysts were warmed using the Kitazato method (12, 13) and transferred in the context of a modified natural or artificial endometrial preparation cycle, as described elsewhere (16) (Labarta E, Rodríguez-Varela C, Vidal C, Doblinger J, Alamá P, Marzal A, et al., unpublished data). Gestational results are not the objective of this study.

## Statistical analysis

A descriptive analysis was performed for patient demographics and cycle characteristics. Categorical data are presented as the number of cases (percentage). Continuous values are summarized according to the mean, standard deviation, and confidence interval (CI). A homogeneity analysis has been performed to identify variables that are distributed differently between groups. Comparisons among continuous variables were made using the analysis of variance test. Categorical variables are shown using proportion and were compared using the $\chi^2$ test.

Oocyte age refers to the age of the patient or donor at the time oocytes were obtained, in the own and oocyte donation cycles, respectively. The minimum number of MII oocytes needed to obtain at least one biopsied and euploid blastocyst was calculated for the patients' and for the donors' groups. It was calculated taking into account all treatment cycles performed—treatment cycles in which at least one euploid blastocyst was or was not achieved. The patient group was displayed year by year. The donor group was displayed as a whole rather than year by year, because the number of MII needed to obtain at least one euploid blastocyst (mean 4.26; $P = .927$) was comparable regardless of oocyte age in this group.

In the patient group, a multivariate binary logistic regression model was performed for the assessment of having at least one euploid blastocyst (dependent variable) depending on the number of mature oocytes retrieved (independent variable). The model was adjusted for the following variables: oocye age, patients' BMI, oocyte status (fresh, vitrified, or mixed), semen origin (partner or donor), semen collection (ejaculated or testicular biopsy), and reason for PGT (only advanced maternal age, or any other additional indication: male factor infertility, recurrent miscarriage, implantation failure, or previous chromosomopathy). Preimplantation genetic testing indications of recurrent miscarriage and implantation failure were grouped in the same category to ease the statistical analysis.

This model was created using 80% of the patient group sample (the training set) and later validated using the remaining 20% (the validation set). The validation process consisted of assessing the success rate of the model, whose response would be binomial (euploid blastocyst yes/no). The binary logistic model was right when predicting that at least one euploid blastocyst would be achieved (probability of at least one euploid blastocyst $>50\%$), and indeed at least one euploid blastocyst was obtained ($\geq 1$ euploid blastocyst). Likewise, the binary logistic model was right when predicting that no euploid blastocyst (probability $\leq 50\%$) would be achieved, and indeed no euploid blastocyst was obtained (0 euploid blastocysts).

A receiver operating characteristic curve has been performed to test the predictive value of the model, obtaining an area under the curve value from the graphic representation of the sensitivity over the specificity. In addition, a calculator for the probability of obtaining at least one euploid blastocyst has been developed using this model. All statistical analyses were performed using R software version 4.3.0. For all tests, a significance level of 5% has been used.

# RESULTS

## Descriptive analysis

A total of 2,660 IVF-PGT-A treatment cycles were performed in the study period in the eligible population. Of these, 2,462 (92.6%) were treatment cycles from women with their own oocytes aged $\geq 35$ years (the patient group), and 198 (7.4%) were treatment cycles with donated oocytes (the donor group).

The main indication for PGT-A in the patient group was advanced maternal age (86.8%), followed by recurrent miscarriage (4.3%), implantation failure (4.1%), male factor infertility (3.4%), and a previous chromosomopathy (1.5%). In the donor group, the main reason for PGT-A was male factor infertility (48.0%), followed by implantation failure (15.7%), previous chromosomopathy (10.6%), and recurrent miscarriage (6.1%).

A descriptive analysis of the treatment cycles included is shown in Table 1. Treatment cycle outcomes and their comparison between the patient and donor groups are shown in Supplemental Table 1 (available online). Embryo descriptive statistics according to oocyte age are shown in Table 2.

## The number of MII oocytes needed

The mean number of MII oocytes needed to obtain at least one euploid blastocyst and a blastocyst suitable for biopsy

## TABLE 1

**Main characteristics of the included IVF–PGT-A treatment cycles; overall and in each group (patients and donors).**

**Characteristics of the IVF-PGT-A treatment cycles**

| Characteristics | Overall (n = 2,660) | Patients group (n = 2,462) | Donors group (n = 198) |
|---|---|---|---|
| Oocyte age (y ± SD) | 40.1 ± 2.7 | 40.0 ± 2.5 | 25.0 ± 4.3 |
| BMI (kg/m$^2$ ± SD) | 23.6 ± 4.1 | 23.7 ± 4.1 | 23.0 ± 3.4 |
| AMH level (ng/mL ±SD) | 1.8 ± 1.6 | 1.8 ± 1.6 | 5.1 ± 3.4 |
| Oocyte status, n (%) | | | |
| Fresh | 2,442 (91.8) | 2,331 (94.7) | 111 (56.1) |
| Vitrified | 128 (4.8) | 52 (2.1) | 76 (38.4) |
| Fresh and vitrified | 90 (3.4) | 79 (3.2) | 11 (5.6) |
| Semen origin, n (%) | | | |
| Own | 2,191 (82.4) | 2,050 (83.3) | 141 (71.2) |
| Donated | 469 (17.6) | 412 (16.7) | 57 (28.8) |
| Semen collection, n (%) | | | |
| Ejaculated | 2,589 (97.3) | 2,400 (97.5) | 189 (95.5) |
| Testicle biopsy | 71 (2.7) | 62 (2.5) | 9 (4.5) |

*Note:* Continuous variables are shown by mean ± standard deviation or by proportion (95% confidence interval).
AMH = antimüllerian hormone; BMI = body mass index; IVF = in vitro fertilization; PGT-A = preimplantation genetic testing for aneuploidy; SD = standard deviation.

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according to oocyte age are shown in Figure 1A and B, respectively. Results in the reference group of donors (n = 198 treatment cycles) are shown on the left side of the plot, although the right side refers to the patient group (n = 2,462 treatment cycles) displayed year by year.

## A Binary logistic regression model for the number of MII oocytes needed to obtain at least one euploid blastocyst in patient group

**Model design.** In the patient group (n = 2,462 treatment cycles), a binary logistic regression model for the number of MII oocytes needed to obtain at least one euploid blastocyst was designed in 80% of the sample (the training set; n = 1,969 treatment cycles). Sample size distribution according to oocyte age in this training set was: 35 years, n = 73; 36 years, n = 83; 37 years, n = 162; 38 years, n = 258; 39 years, n = 296; 40 years, n = 304; 41 years, n = 301; 42 years, n = 224; 43 years, n = 114; 44 years, n = 76; and ≥44 years, n = 78.

The univariate analysis showed how the number of MII oocytes was significantly and positively related to the event of having at least one euploid blastocyst (odds ratio [OR], 1.20; 95% CI, 1.18–1.23; $P<.001$). After adjusting for potential confounding factors, multivariate analysis showed similar results (adjusted OR, 1.19; 95% CI, 1.16–1.21; $P<.001$). From the potential confounding factors, the only variable that exerted a significant impact on the event of having at least one euploid blastocyst was oocyte age (adjusted OR, 0.68; 95% CI, 0.64–0.72; $P<.001$).

A mathematical formula using this model was defined, and its application is graphically represented in Figure 2. The receiver operating characteristic curve showed a significant predictive value of the number of MII oocytes (area under

## TABLE 2

**Embryo descriptive statistics of the included IVF–PGT-A treatment cycles according to oocyte age.**

| Oocyte age, y | Cycles with 0 biopsied blastocysts (n, %) | Cycles with 0 euploid blastocysts (n, %) | Number of euploid blastocysts (mean, 95% CI) |
|---|---|---|---|
| Donors group (n = 198) | 9 (4.55) | 17 (8.59) | 3.8 (3.4–4.1) |
| 35 (n = 92) | 13 (14.13) | 20 (21.74) | 2.57 (2.07–3.06) |
| 36 (n = 106) | 16 (15.10) | 33 (31.13) | 1.73 (1.38–2.07) |
| 37 (n = 191) | 26 (13.61) | 62 (32.46) | 1.47 (1.24–1.70) |
| 38 (n = 313) | 37 (11.82) | 114 (36.42) | 1.35 (1.17–1.53) |
| 39 (n = 363) | 53 (14.60) | 153 (42.15) | 1.07 (0.93–1.21) |
| 40 (n = 395) | 78 (19.75) | 231 (58.48) | 0.67 (0.57–0.77) |
| 41 (n = 370) | 83 (22.43) | 242 (65.41) | 0.52 (0.43–0.62) |
| 42 (n = 285) | 73 (25.61) | 208 (72.98) | 0.33 (0.26–0.40) |
| 43 (n = 155) | 63 (40.65) | 134 (85.45) | 0.17 (0.10–0.25) |
| 44 (n = 98) | 52 (53.10) | 90 (91.84) | 0.08 (0.03–0.14) |
| ≥45 (n = 94) | 68 (72.34) | 91 (96.81) | 0.03 (0.0–0.7) |
| Total in patients group (n = 2,462) | 562 (22.82) | 1,378 (55.97) | 0.85 (0.80–0.91) |

*Note:* CI = confidence interval; IVF = in vitro fertilization; PGT-A = preimplantation genetic testing for aneuploidy.

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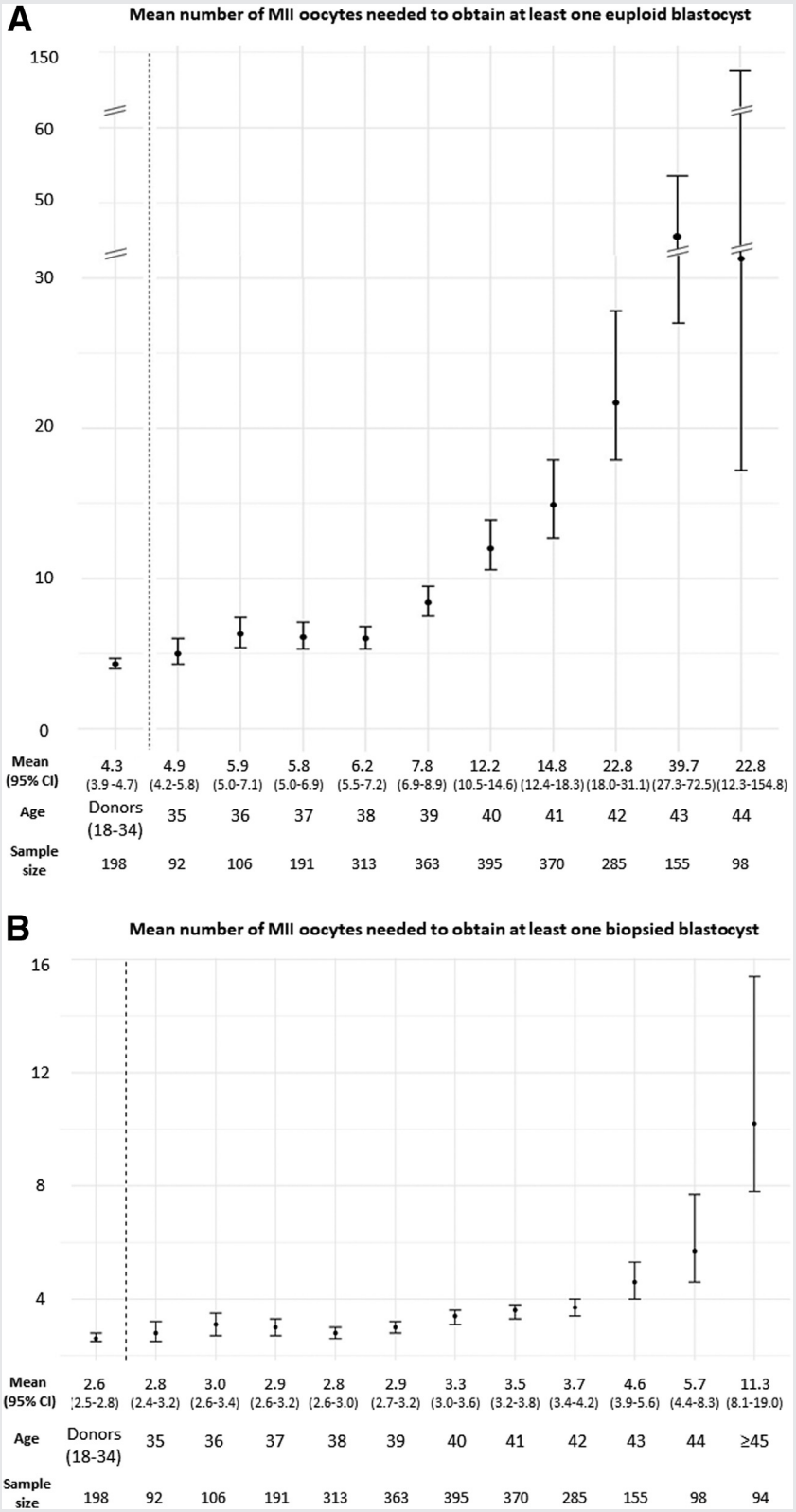
## FIGURE 1




Mean number of metaphase II (MII) oocytes needed to obtain at least one euploid blastocyst (**A**) and one biopsied blastocyst (**B**), according to oocyte age in the donors group (left side) and in the patients group (right side). *Note:* Data are shown as mean (95% confidence interval [CI]).

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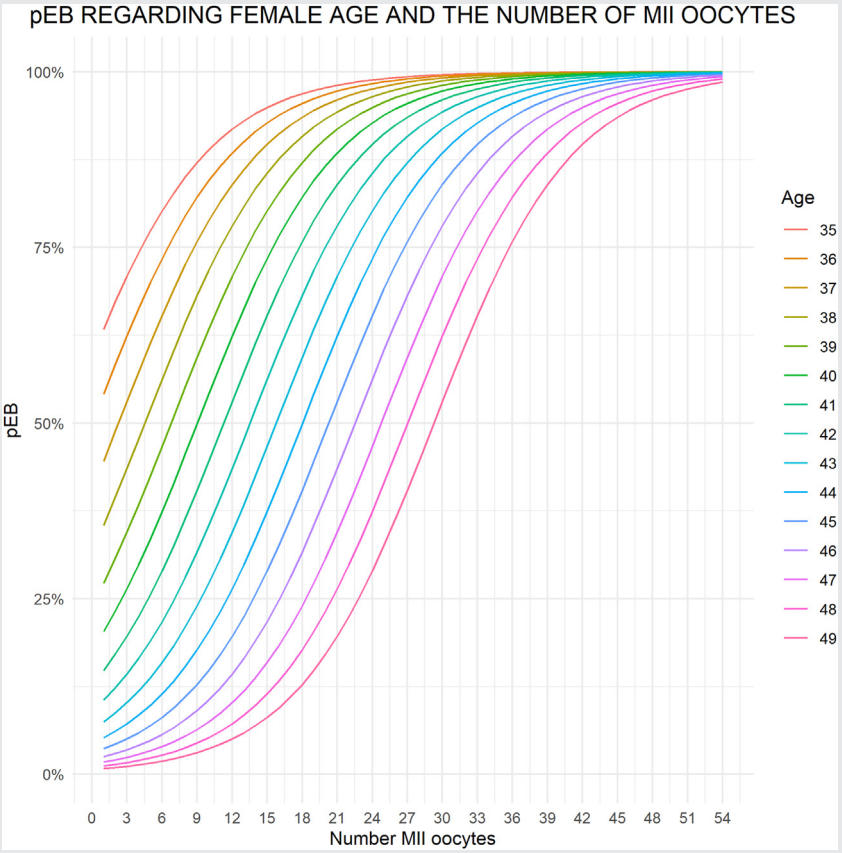
## FIGURE 2




Graphic representation of the model. The x axis shows the number of metaphase II (MII) oocytes and the y axis the probability of having at least one euploid blastocyst (pEB). Each line represents oocyte age in years. Sample size distribution according to oocyte age refers to the one described for the training set.

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the curve value, 0.80 [0.78–0.81]) for this model (Supplemental Fig. 1, available online).

**Model validation.** This model was subsequently validated in the remaining 20% of the sample (the validation set; n = 493 treatment cycles), showing that it could estimate the event of having at least one euploid blastocyst with an accuracy of 72.0%.

On the one hand, the model prediction for the event of having at least one euploid blastocyst was right in 116 cases, whereas it was wrong in 60 cases that did not have at least one euploid blastocyst. On the other hand, the model prediction for the event of no euploid blastocysts was right in 239 cases, whereas it was wrong in 78 cases that did obtain at least one euploid blastocyst. Therefore, the total success rate of the model was 72.0% (116 plus 239 = 355 rights/493 cycles).

## DISCUSSION

To our knowledge, this is the largest database retrospectively analyzing the association between female age and aneuploidy rates in IVF-PGT-A treatment cycles. We have included 2,660 treatment cycles with accessible and well-documented PGT-A information, numbers that drastically contrast with previous studies with the same objective (7). All these procedures have been performed homogeneously in the same IVF treatment unit, without any potential clinic- or laboratory-dependent bias.

Moreover, only IVF-PGT-A treatment cycles using NGS on TE biopsies on days 5 or 6 of development were included, which constitutes the most reliable and precise genetic technique available nowadays. In contrast, many studies found in the literature use fluorescence in situ hybridization or arrays for comparative genomic hybridization (17, 18), either using NGS but with a much smaller sample size (7).

This study included treatment cycles with both own and donated oocytes, in contrast to many of the already published studies in this field, which did not include treatment cycles from the oocyte donation program (7). In our case, only patients aged ≥35 years using their own oocytes were included in the model, to avoid any bias related to the PGT indication. The group of donors has been proposed as a reference group when looking at the mean number of MII needed to get at least one euploid blastocyst, which can be also useful in clinical practice to show patients what their chances are in case they move into the oocyte donation program. However,

IVF-PGT-A treatment cycles in oocyte donation treatment cycles may be biased, because this analysis would be done because of a clinical indication not present in most fertile populations at this age.

On this basis, our data clearly show how the mean number of euploid blastocysts decreases as maternal age increases, here presented as oocyte age. Indeed, the frequency of patients with at least one euploid blastocyst decreases as we move up in oocyte age. Likewise, the minimum number of MII oocytes needed to biopsy at least one blastocyst and to obtain at least one euploid blastocyst increases with oocyte age, keeping these numbers low in the donors' group in both cases.

These findings are in line with all the previous data found in the literature associating female age with aneuploidy rates (17–19). All these investigators, and us among them, have only clinically demonstrated the association between maternal age and impaired oocyte meiotic progression, already proven in many molecular studies. With age, the oocyte loses the capacity for optimal spindle assembly, chromosome segregation, and its mitochondrial function aggravates, among others, leading to a suboptimal oocyte meiotic progression (4, 20).

Indeed, this association was further confirmed with an adjusted multivariate regression model for the number of MII oocytes needed to obtain at least one euploid blastocyst, developed using 80% of the Patients Group sample (n = 1,969, the training set). Using this model, we have created a calculator that estimates the probability of obtaining at least one euploid blastocyst regarding oocyte age and the number of MII oocytes. The graphical representation of this model clearly shows how, for the same initial number of MII oocytes, the probability of obtaining at least one euploid blastocyst significantly decreases with advancing oocyte age. Hence, the older the oocyte the higher the number of MII oocytes needed to have enough chances of obtaining at least one euploid blastocyst.

A similar tool was published previously by the Patient-Oriented Strategies Encompassing Individualized Oocyte Number group in 2019, with the main difference being that the semen source was also included in the model (7). However, our calculator was designed with a sample size 5 times larger (1,969 vs. 347 cycles), thus increasing the expected predictive power of the model. Despite this, although Esteves' calculator has been subsequently externally validated in a multicenter study including 1,464 IVF-PGT-A treatment cycles (8), ours has been internally validated using remaining 20% of the sample included (n = 493; validation set).

The external validation of the assisted reproductive technology (ART) calculator provides the percentage of patients with at least one euploid blastocyst, only in those cases in which the minimum number of MII oocytes calculated using the ART calculator was obtained, according to 3 different probabilities of success (70%, 80%, and 90%). These percentages were 84.8%, 87.5%, and 90.0%, respectively (8), high numbers in comparison to the accuracy obtained after the internal validation of our model. However, our model did not define probabilities of success, so validation is performed taking into account a probability of success of 100%, higher than the ones calculated for the ART calculator. In addition, we did not make any distinction according to whether the minimum number of MII oocytes had been reached or not, as opposed to Esteves et al. (8), which could have increased our level of accuracy, bringing us closer to the numbers obtained in the validation of the ART calculator. This places particular emphasis on the need for a more robust, larger sample size, and multicentric validation of the model designed in the current study. This external validation will probably be the focus of the next publication on this model.

Nevertheless, despite the fact that this analysis was performed in IVF treatment cycles with PGT-A, we hypothesized that our results could be extrapolated to any patient coming to our clinic to perform an IVF treatment cycle. Once our model has been properly validated, clinicians may use this information as part of their routine clinical practice even when their patients do not decide to perform a PGT-A analysis.

One of the strategies that can be recommended on the basis of this information is oocyte or embryo accumulation before an IVF–PGT-A treatment cycle to increase the cohort of available blastocysts for TE biopsy, thus augmenting the possibilities of obtaining the euploid embryo. To note, there is not a "magical number" of oocytes that will ensure finding the chromosomally normal embryo, and it would not be ethical to encourage the patient to accumulate an extreme number of oocytes because the psychological burden can be very high when failing to achieve the objective after several treatment cycles. Having said this, the information given here offers an accurate estimation of the mean number of oocytes needed to have an acceptable probability of obtaining a normal embryo, and this informs both the clinician and the patient about how to proceed.

This study has many limitations that should be addressed. First, the model should be optimized because the 72% accuracy should be increased to make it a trustworthy clinical tool. Second, an internal prospective validation of the model is imperative, as is a multicenter external validation once its robustness has been increased sufficiently. Finally, despite the main limitation of this study being its retrospective design, which makes it more difficult to control for other confounding factors, the large sample size and the multivariate logistic regression performed allow for a good control of the potential confounders. Moreover, only real-world evidence can address this particular issue.

Indeed, although oocyte age is a determining factor in the estimation of the number of oocytes needed, it is important to consider the whole context of the patient (ovarian reserve, ovarian stimulation response, history of poor oocyte quality, sperm quality, and others). Clinicians could first estimate the minimum number of oocytes needed to obtain at least one euploid blastocyst regarding the patient's oocyte age, and then expect this number to be higher or lower, taking into consideration these additional variables.

## CONCLUSIONS

The minimum number of MII oocytes needed to have high chances of obtaining at least one euploid blastocyst increases with increasing maternal age. Our model estimates with an accuracy of 74% the probability of having at least one euploid blastocyst, considering oocyte age and the number of MII

oocytes. This model has been created with the largest database of IVF-PGT-A treatment cycles ever used for this purpose, including only PGT-A treatment cycles using NGS on TE biopsies. Once this model has been validated prospectively and in multicenter studies, it may be useful for both the clinician and the patient coming to an infertility clinic, whether or not a PGT-A analysis is performed. The clinician may use this data to propose the best strategy for each patient, whereas the patient may use this information to better understand the likelihood of obtaining an euploid blastocyst, helping her to cope emotionally with IVF treatment. Nevertheless, to this day, our model has limited clinical value. It should be further validated and optimized to use it as a clinical support tool, in our own clinic and in many others.

## CRediT Authorship Contribution Statement

**Cristina Rodríguez-Varela:** Writing – original draft, Investigation, Formal analysis, Data curation. **Juan Manuel Mascarós:** Methodology, Data curation. **Elena Labarta:** Writing – review & editing, Supervision, Resources, Conceptualization. **Noelia Silla:** Investigation, Conceptualization. **Ernesto Bosch:** Writing – review & editing, Supervision, Resources, Conceptualization.

## Declaration of Interests

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**Número mínimo de ovocitos maduros necesarios para obtener al menos un blastocisto euploide según la edad de la mujer en ciclos de tratamiento de fecundación in vitro**

**Objetivo:** Encontrar una herramienta útil para estimar el número mínimo de ovocitos en metafase II (MII) necesarios para obtener al menos un blastocisto euploide según la edad de la mujer.

**Diseño:** Análisis retrospectivo de los ciclos de tratamiento de fecundación in vitro (FIV) con estudio genético preimplantacional de aneuploidías (PGT-A) realizados durante 5 años en IVIRMA Valencia (España), enero de 2017-marzo de 2022. Aprobación del Comité de Revisión Institucional de IVI Valencia (2204-VLC-040-CR).

**Entorno:** Clínica privada de infertilidad en España.

**Paciente(s):** Las pacientes elegibles estaban en su primer ciclo de tratamiento de FIV-PGT-A, en el que se había obtenido al menos un ovocito MII, independientemente del origen del ovocito y del semen. Los ciclos de donación de ovocitos se incluyeron en el grupo de donantes ($\leq$34 años). Los ciclos de tratamiento de mujeres con sus propios ovocitos se seleccionaron solo cuando los ovocitos tenían una edad de $\geq$35 años (grupo de pacientes). Solo se incluyeron biopsias de trofoectodermo realizadas en los días 5 o 6 de desarrollo y analizadas mediante secuenciación de nueva generación. Se excluyeron los ciclos para las pruebas genéticas preimplantacionales de aneuploidía por un cariotipo anormal conocido.

**Intervención(es):** No aplicable.

**Principales medidas de resultados:** Número de ovocitos MII necesarios para obtener un blastocisto euploide según la edad de la mujer.

**Resultados:** Se realizaron un total de 2.660 ciclos de tratamiento FIV-PGT-A en el periodo de estudio en la población elegible (grupo de pacientes=2.462; grupo de donantes=198). El número medio de ovocitos MII necesarios para obtener un blastocisto euploide aumentó con la edad, al igual que el número de ciclos de tratamiento que no obtuvieron al menos un blastocisto euploide. Se diseñó un modelo de regresión binaria multivariante ajustado utilizando el 80% de la muestra del grupo de pacientes (n= 2.462; conjunto de adiestramiento). Se creó una calculadora para la probabilidad de obtener al menos un blastocisto euploide utilizando este modelo. La validación de este modelo en el 20% restante de la muestra del grupo de pacientes (n= 493; conjunto de validación) mostró que podía estimar el evento de tener al menos un blastocisto euploide con una precisión del 72,0%.

**Conclusión(es):** Nuestros resultados muestran un modelo preliminar capaz de predecir el número de ovocitos MII necesarios para obtener al menos un blastocisto euploide en función de la edad de la mujer, calculado con la mayor base de datos de ciclos de tratamiento FIV-PGT-A jamás utilizada para este fin, incluyendo únicamente ciclos de tratamiento mediante secuenciación de última generación sobre biopsias de trofoectodermo. Una vez que este modelo haya sido validado adecuadamente, podría ayudar en la toma de decisiones tanto de los médicos como de las pacientes que acuden a una clínica de infertilidad.