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Automated Quantification of the Meiotic Spindle–Polar Body Angle and Its Association with Embryo Euploidy

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Abstract

Non-invasive assessment of embryo euploidy from oocyte-derived parameters remains a major challenge in assisted reproduction. Oocyte meiotic competence is closely associated with embryo ploidy. Our proof–of-concept study establishes a methodology for identifying a laboratory-specific meiotic spindle–polar body (MS–PB) angle range in which embryo euploidy is more frequent than outside this range. MS–PB angles were automatically quantified from polarized light microscopy images using a U-Net–based deep learning model. The analysis included 81 metaphase II oocytes imaged ~ 40 h after trigger administration. Embryo ploidy was assessed by preimplantation genetic testing for aneuploidy (PGT-A). Embryos derived from oocytes within the calibrated MS–PB angle range (8–32°) exhibited significantly higher euploidy rates than those outside this interval (72.2% vs. 31.1%; p = 0.000236). Patient-clustered binary logistic regression showed 5.76-fold higher odds of euploidy within the calibrated range (OR = 5.76, 95% CI 2.03–16.31; p = 0.00098). Modeling the absolute deviation from the empirically identified optimum (18.4°) yielded an AUC of 0.725, supporting a non-linear association between MS–PB angle and embryo euploidy. The MS–PB angle may therefore provide complementary information for future embryo euploidy prediction models integrating oocyte morphology, embryo time-lapse, and clinical data.

1. Introduction

Reliable non-invasive estimation of embryo euploidy remains an important challenge for in vitro fertilization (IVF). Current assessment is largely based on embryo morphology and development, while definitive determination of ploidy requires invasive PGT-A[1,2]. Because embryo ploidy is strongly associated with oocyte quality and maternal meiotic competence, oocyte-derived parameters may provide early information on embryo euploidy before fertilization[3,4]. Among the available non-invasive biomarkers, the meiotic spindle has attracted considerable attention[5–7] and has been associated with fertilization success and subsequent embryo development [8–10], [6,11–16].

In our previous studies[16–18], we demonstrated that adjusting the timing of intracytoplasmic sperm injection (ICSI) according to meiotic spindle status improved fertilization outcomes, indicating that spindle assessment provides clinically relevant information about oocyte developmental status. Because most embryonic aneuploidies originate from maternal meiotic errors, reliable non-invasive assessment of oocyte quality could improve embryo selection and provide complementary information for embryo evaluation [19–21].

Besides spindle morphology, the spatial relationship between the meiotic spindle and the first polar body (MS–PB angle) has attracted increasing attention as a potential marker of oocyte quality. Previous studies have generally [17,19,22,23] suggested that relatively small MS–PB angles are associated with improved developmental outcomes; however, there is theoretical concern that in some contexts (aging or cortical disorganization), tight MS-PB positions might not reflect optimal oocyte function. The optimal angular range has not yet been established, and accurate quantitative assessment remains technically

challenging. Manual measurement by embryologists[24,25] is labor-intensive and subject to inter-observer variability, limiting its clinical applicability [7,8,26–30].

To overcome the limitations of manual assessment, we developed a deep learning–based approach for automated identification[23] of the meiotic spindle and polar body from polarized light microscopy images and video recordings, enabling objective and reproducible measurement of the MS–PB angle. The aim of this prospective study was to establish a reproducible quantitative methodology for identifying a laboratory-specific MS–PB angle range in which embryo euploidy is more frequent than outside this range [31,32]. The optimal interval may depend on laboratory-specific conditions, including the timing of image acquisition, as the MS–PB angle changes during oocyte maturation[17,18], as well as trigger type and other clinical protocols [17,19,22,23]. Thus, our proof-of-concept demonstrates a procedure for identifying a locally relevant interval rather than a universal cut-off. In future, the MS–PB angle may provide complementary pre-fertilization information that can be integrated with embryo time-lapse analysis[33] and clinical parameters to improve automated embryo euploidy prediction models.

2. Results

2.1. Localization of oocyte structures from microscope images and video recordings using deep learning

To objectively quantify the meiotic spindle–polar body (MS–PB) angle, we developed a deep learning framework for automatic localization of three key oocyte structures: the cytoplasm (CYT), meiotic spindle (MS), and first polar body (PB) – **Fig. 1** [31,32]. The proposed framework achieved high localization accuracy for all three structures from microscopy images and video recordings. More than 90% of predicted structure centers were localized within a few pixels of the expert annotations, and more than 70% of predicted MS–PB angles differed from the reference measurements by less than 2°. Cytoplasm localization showed the highest accuracy, followed by the meiotic spindle, whereas polar body localization exhibited greater variability because of lower image contrast and occasional partial occlusion. The model also predicts the presence or absence of individual structures, although this task proved less reliable for the polar body, reflecting class imbalance and its lower visibility in some images. Pretraining substantially improved convergence and model stability, resulting in good generalization without noticeable overfitting. Our results demonstrate that the proposed framework provides sufficiently accurate and robust localization for reliable automated MS–PB angle measurement in human oocytes.

2.2. Association Between the Meiotic Spindle–Polar Body Angle and Embryo Euploidy

In this prospective study, we analyzed 81 oocytes from 55 patients undergoing 55 IVF cycles with both a visible MS and first PB that developed into embryos successfully evaluated by PGT-A.

The search for the MS–PB angle interval associated with higher embryo euploidy was guided by our previous observations on meiotic spindle maturation and optimized ICSI timing[17,18].

To identify and assess the robustness of a laboratory-specific MS–PB angle range, we performed a sensitivity analysis by systematically varying the lower and upper boundaries defining category A. For each candidate interval, the difference in embryo euploidy rates between oocytes inside and outside the interval was calculated (**Tab. S1**). The resulting heatmap (**Fig. S1**) demonstrated a broad region of increased separation in euploidy rates rather than an isolated optimum.

The 8°–32° interval was selected as the primary laboratory-specific interval, balancing separation in euploidy rates, sample size, and robustness to small changes in the interval boundaries. The 8°–32° interval was retained for the primary analysis and produced a 41.1% difference in euploidy rates. Several narrower candidate intervals produced larger apparent differences but contained fewer oocytes, illustrating the trade-off between effect size and sample size.

Overall, 40 of 81 embryos (49%) were euploid. Of the 81 oocytes, 36 (44%) were classified as Category A (MS–PB angle 8°–32°) and 45 (56%) as Category B (MS–PB angle < 8° or > 32°). (Fig. 2, Table 1). The observed distribution of MS–PB angles was consistent with distributions reported in previous studies using similar imaging conditions [6,7,18,19,22].

The proportion of euploid embryos was significantly higher in category A than in category B oocytes (72.2%, 26/36 vs. 31.1%, 14/45). The distribution of embryos according to oocyte category and ploidy status is summarized in the contingency table (Table 1). Percentages in parentheses represent the proportion of embryos in each cell relative to the total number of embryos (81). Pearson's chi-square test demonstrated a statistically significant association between oocyte category and embryo euploidy (χ^2 = 13.52, df = 1, p = 0.000236). Thus, under the specific IVF and imaging conditions of the present study, the locally calibrated 8°–32° interval was associated with a higher probability of embryo euploidy.

Binary logistic regression with patient-level cluster-robust standard errors demonstrated that oocytes within the 8°–32° angle range had substantially higher odds of producing euploid embryos than oocytes outside this interval (OR = 5.76, 95% CI 2.03–16.31, p = 0.00098).

Receiver operating characteristic (ROC) analysis was performed as a complementary analysis using the absolute deviation of the measured MS–PB angle from the empirically identified optimal angle (~ 18.4°). This analysis yielded an AUC of 0.725, supporting a non-linear association between MS–PB angle and embryo euploidy (Fig. 3).

Patient-level bootstrap analysis supported the robustness of the selected MS–PB angle interval. In 10,000 bootstrap resamples, in which patients and all their associated oocytes were resampled together, the median difference in euploidy rates between oocytes within and outside the 8°–32° interval was 41.2 percentage points (95% bootstrap CI: 18.9–61.7). The corresponding median odds ratio was 5.93 (95% bootstrap CI: 2.16–19.72), and a higher euploidy rate within the interval was observed in 99.96% of

bootstrap samples. Re-optimization of the interval boundaries demonstrated greater variability in the exact cut-off values, whereas the region associated with higher euploidy remained stable.

Table 1
Contingency table showing the relationship between oocyte categories and embryo PGT-A outcomes. Absolute counts and percentages (in parentheses) of euploid and aneuploid embryos are presented for each oocyte category (A and B). Percentages are calculated relative to the total study population (n = 81).

Category	Euploidy		
	Euploid	Aneuploid	Total
A	26 (32%)	10 (12%)	36 (44%)
B	14 (17%)	31 (38%)	45 (56%)
Total	40 (49%)	41 (51%)	81 (100%)

The average number of previous OPU was 2.3 per patient, the average number of oocytes per cycle was 14.2, and the average number of MII oocytes per cycle was 10.7. Average number of pronuclear (2PN) embryos per cycle was 8.4, average utilization rate (the number of high-quality embryos divided by the number of oocytes with a detected PB) was 0.47.

The quality of sperm was also monitored so that correlation between sperm quality and utilization and pregnancy rates could be evaluated. There was no relevant correlation between sperm quality and embryo ploidy probability ($p > 0.05$), between sperm quality and MS/polar body angle ($p > 0.05$).

Workflow of automated MS–PB angle analysis and its association with embryo euploidy – see Fig. 4.

4. Discussion

The present prospective study introduces a reproducible oocyte assessment method for identifying a laboratory-specific MS–PB angle range within which the probability of subsequent embryo euploidy is higher than outside this range. As embryo ploidy is closely linked to oocyte quality and maternal meiotic competence, oocyte-derived parameters may provide an early indication of embryo euploidy before fertilization. Unlike previous studies [2,34]. using manual measurements, we developed a fully automated deep learning pipeline for objective MS–PB orientation assessment from polarized light microscopy images and videos. The developed framework enabled reliable and reproducible MS–PB angle measurement, with high localization accuracy and low angle measurement error, supporting its application in large-scale IVF studies.

The association between the locally calibrated MS–PB angle range and embryo euploidy was supported by complementary statistical analyses. Embryos derived from oocytes within the MS–PB angle range 8–

32° exhibited significantly higher euploidy rates than those outside this interval (72.2% vs. 31.1%; p = 0.000236). Patient-clustered logistic regression demonstrated a 5.76-fold increase in the odds of euploidy within the calibrated range. ROC analysis based on deviation from the intermediate optimum of approximately 18.4° yielded an AUC of 0.725, further supporting a non-linear association between MS–PB angle and embryo euploidy. The relationship between MS–PB angle and embryo euploidy was non-linear and consistent with an inverted U-shaped pattern. Bootstrap analysis further supported the robustness of the fixed 8°–32° interval.

A possible biological explanation for the observed association is that the MS–PB angle reflects the spatial organization of the meiotic apparatus during the final stages of oocyte maturation. The observed non-linear relationship between the MS–PB angle and embryo euploidy suggests that intermediate spindle orientation may be associated with more favorable oocyte developmental competence, whereas deviations in either direction are associated with a reduced probability of embryo euploidy. Such a relationship is common in biological systems, where physiological function is optimized within an intermediate range rather than at extreme values. Small MS–PB angles may reflect incomplete spindle migration or insufficient separation of the spindle from the first polar body, whereas large angles may indicate excessive spindle displacement or disturbed cytoskeletal organization. Both configurations could represent suboptimal spindle positioning and consequently increase the likelihood of chromosome segregation errors during meiosis.

Our study has several limitations. The study was conducted prospectively in a single IVF center with a relatively limited number of oocytes. The identified 8°–32° interval should therefore be regarded as a laboratory-specific range rather than a universal clinical cut-off. The optimal MS–PB angle interval may depend on laboratory-specific conditions, including the timing of image acquisition, as the MS–PB angle changes during oocyte maturation, as well as trigger type and other clinical protocols. Further multicenter studies are needed to determine whether laboratory-specific calibration provides an advantage over a common MS–PB angle range.

Taken together, this proof-of-concept study demonstrates that automated MS–PB angle measurement can identify a laboratory-specific angular range associated with a higher probability of embryo euploidy than outside this range. As an oocyte-derived parameter, the MS–PB angle may provide information complementary to subsequent embryo morphokinetics. Its integration with time-lapse and clinical data in future AI models may improve embryo euploidy prediction. In routine clinical practice, the MS–PB angle could potentially serve as an early non-invasive marker of oocyte competence and contribute to prioritizing embryos for PGT-A.

5. Methodology

5.1. Patients

A total of 81 oocytes from 55 patients undergoing 55 IVF cycles were evaluated in this prospective study. Patients were 30–44 years old (mean age, 37.18 years; **Fig. S2**) and were treated at the Centre of Assisted Reproduction (CAR), Department of Gynaecology, Obstetrics and Neonatology, First Faculty of Medicine, General University Hospital in Prague (VFN), between 2024 and 2025. All the experimental protocols were approved by a Department of Gynaecology, Obstetrics and Neonatology of the First Faculty of Medicine, General University Hospital, Apolinarska 18, 128 51 Prague 2, Czech Republic and by Ethics Committee of the General University Hospital, Prague (the list is enclosed). The study protocol was approved by the Ethics Committee of the General University Hospital in Prague (approval no. 1821/20 S-IV), and all procedures were performed in accordance with the Declaration of Helsinki and applicable institutional and national regulations. Written informed consent was obtained from all participants before inclusion in the study. Key criteria for inclusion were planned PGT, a spermiogram containing at least 1 mil/ml of progressively motile spermatozoa measured during previous spermiogram evaluation.

5.2. Oocytes treatment

Intracytoplasmic sperm injection (ICSI) was performed according to standard protocols using ICSI and holding micropipettes (Microtech IVF, Czech Republic), polyvinylpyrrolidone (ICSI™, Vitrolife, Sweden) and an Eppendorf micromanipulation system (Hamburg, Germany) equipped with a thermoplate (Tokai Hit, Japan). Following oocyte pick-up (OPU), cumulus cells were removed using HYASE-11X™ (Vitrolife, Sweden), and the maturation stage of each oocyte was assessed. Oocytes at the germinal vesicle (GV) stage were excluded from further analysis. All oocytes were cultured in 4-well plates (NUNC) using Sage Fertilization Medium™ and Sage 1-Step™ (Origio, Denmark), under paraffin oil (OVOIL™, Vitrolife, Sweden) at 37.2°C in an atmosphere of 6% CO_2 and 5% O_2. Oocyte pick-up (OPU) was performed 37 h after trigger administration. Polarized light microscopy images were acquired ~ 40 h after trigger administration, and ICSI was performed 2 h later (42 h after trigger administration). Times from trigger administration are reported as mean values with a range of up to 20 minutes. Only oocytes that developed into embryos successfully biopsied and analyzed by PGT-A were included in the present study, as embryo ploidy was the primary outcome measure.

5.3. Meiotic spindles evaluation

Oocytes were photographed or recorded using video camera during IVF process in polarized light ~ 40 hours after trigger application and 2 hours before ICSI. Optical microscope with a Nikon CEE GmbH polarizing filter was used. The relative positions of the polar body and MS were clearly visible in 97% of oocytes. MS photograph/record lasted on average 3–5 minutes. The oocytes which gained the maturation stage/polar body were examined. GV stage oocytes were not included. The study focused on individual oocytes rather than patients, with the objective of relating the developmental status of each oocyte, represented by its MS–PB angle, to the euploidy outcome of the embryo originating from that same oocyte. Within-patient clustering of multiple oocytes was accounted for in the statistical analysis. Oocytes with visible first PB and without a visible MS were excluded from the MS–PB angle analysis, as

our previous studies demonstrated[16–18], that MS visibility itself is associated with embryo euploidy and therefore represents a distinct marker of oocyte developmental competence.

5.3.1. Localization of oocyte structures in microscope images and video records using deep learning

We developed a deep learning framework for automatic localization of CYT, MS, and PB-**Figs, 1, 2**. The code for training and inference, along with the trained weights, is available online https://github.com/barucden/oocyte-angle. The task was formulated as a heatmap-based keypoint detection problem rather than full-image segmentation, because only the centers of these structures are required for angle computation. The model predicts a probability map (heatmap) for each structure, and the final coordinates are obtained from the location of maximum probability. The MS–PB angle is subsequently calculated using the cytoplasm center as the origin.

The proposed framework is based on a U-Net encoder–decoder architecture with a ResNet-18 encoder and skip connections, enabling simultaneous extraction of contextual and spatial information. Images were resized to 448 × 448 pixels before analysis. Heatmap prediction was preferred over direct coordinate regression because it provides greater robustness to image noise, scale variation, and partial occlusion while achieving higher localization accuracy for small biological structures.

The localization model was trained using a dataset of 495 polarized-light microscopy images of human metaphase II oocytes annotated with the positions of the cytoplasm, polar body, and meiotic spindle. We used extensive data augmentation to train the model, including geometric

transformations and intensity variations. The relationship between the MS–PB angle and embryo ploidy was subsequently evaluated in an independent dataset of 81 oocytes for which the ploidy status of the corresponding embryos was known.

For video recordings, the oocyte was gently rotated during image acquisition. The predictor was applied independently to each video frame, generating MS–PB angle estimates together with an associated probability score. Because predictions from poorly oriented or low-quality frames were less reliable, only high-confidence estimates were retained. For each video, the confidence threshold was defined as the 90th percentile of the probability scores across all frames; thus, only predictions within the highest-confidence decile were considered. The video-level MS–PB angle was defined as the maximum predicted angle among these retained frames, corresponding to the imaging plane passing through the centers of both the meiotic spindle and the polar body. This procedure reduces the influence of low-confidence predictions while minimizing projection-related underestimation of the true three-dimensional angle[32].

5.4. Embryo cultivation

Embryos were cultivated before biopsy, usually for 5–6 days. Embryos were cultivated in an ASTEC Time-lapse incubator CCM-iBIS at 37°C and 6% CO_2 and 5% O_2. Trophectoderm biopsy was performed on day 5 or 6 in blastocysts that met the laboratory criteria for biopsy, including adequate blastocoel expansion and sufficient inner cell mass and trophectoderm quality [35]. Embryo ploidy outcomes were recorded for all patients included in the study.

5.5. Preimplantation genetic testing

Preimplantation genetic testing for aneuploidy (PGT-A) was performed during IVF treatment to identify embryos with higher developmental potential for transfer. PGT-A was performed by GENNET s.r.o. as part of routine clinical genetic testing using a next-generation sequencing (NGS)-based method (NGS rapid, SOP PGTL-004). The present study did not analyze sequencing data; only the resulting embryo ploidy classification (euploid, aneuploid, or mosaic) was used as the study outcome.

All the PGT tested embryos were distributed into 3 groups: euploid embryos (can be used), aneuploid embryos (must not be used) and mosaic (may be used with the consent of the geneticist). Preimplantation genetic testing was used in couples with an increased risk of chromosomal abnormalities in the embryo (e.g. advanced maternal age, repeated implantation failures, pregnancy losses). Mosaic embryos were classified as non-euploid and included in the aneuploid group for all statistical analyses.

5.6. Statistical evaluation of the data

Based on the MS–PB angle measured from photographs or video recordings, oocytes were categorized according to their relative MS–PB position, with corresponding embryo ploidy as the study endpoint. A sensitivity analysis was performed by systematically varying the lower and upper boundaries defining Category A (**Fig. S1**). Category A comprised oocytes with MS–PB angles within the laboratory-specific range of 8°–32° identified by the sensitivity analysis, whereas Category B comprised oocytes with angles < 8° or > 32°. Oocytes for which the angle between the MS and PB may be affected by extrinsic factors, such as mechanical manipulation during cumulus cell removal, were excluded from the study[36,32].

Pearson's chi-square test was used to evaluate the association between the MS–PB angle category and embryo euploidy. The null hypothesis assumed that embryo euploidy was independent of the MS–PB angle category, and was tested using the corresponding contingency table. Associations of sperm quality and patient age with embryo ploidy and the MS–PB angle were also evaluated. To evaluate the non-linear association, the absolute deviation from the empirically identified optimal angle was used as a transformed predictor for ROC analysis. Because multiple oocytes originated from some patients, binary logistic regression was performed using patient-level cluster-robust standard errors to estimate the odds ratio and its 95% confidence interval for euploidy within versus outside the 8°–32° interval. Continuous variables are presented as mean ± SD, categorical variables as counts and percentages. Patient-level bootstrap resampling (10,000 iterations), with all oocytes from each selected patient resampled

together, was additionally performed to assess the robustness of the selected MS–PB angle interval and the corresponding effect estimates.

Declarations

Data availability: No DNA sequence data were generated or analyzed as research data in the present study. PGT-A was performed as part of routine clinical genetic testing, and only the resulting embryo ploidy classification was used for the analyses reported here. The individual-level data underlying this study contain potentially identifiable clinical and genetic information and therefore cannot be made publicly available. De-identified data supporting the findings of this study are available from the corresponding author upon reasonable request, subject to applicable ethical and data-protection requirements.

Authorship Contribution Statement:

Irena Kratochvílová– conceptualization, investigation, writing—original draft preparation; validation;

Olga Teplá – methodology, investigation, conceptualization, formal analysis;

Simona Jirsová- methodology, investigation;

Martina Moosová -data acquisition;

Jan Kybic – validation; writing and editing

Denis Baručić – conceptualization, statistical analysis;

Katerina Komrskova – validation, writing and editing

Jaromír Mašata-conceptualization, validation, writing and editing.

All authors have read and agreed to the published version of the manuscript.

Study registration: The present prospective observational study was conducted within the framework of the registered SMARTAI study, “Artificial Intelligence in Assisted Reproductive Techniques to Assess Oocyte Quality and Embryo Ploidy” (ClinicalTrials.gov identifier NCT06539104). The present manuscript reports a specific observational analysis within this broader study.[37]

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Figures

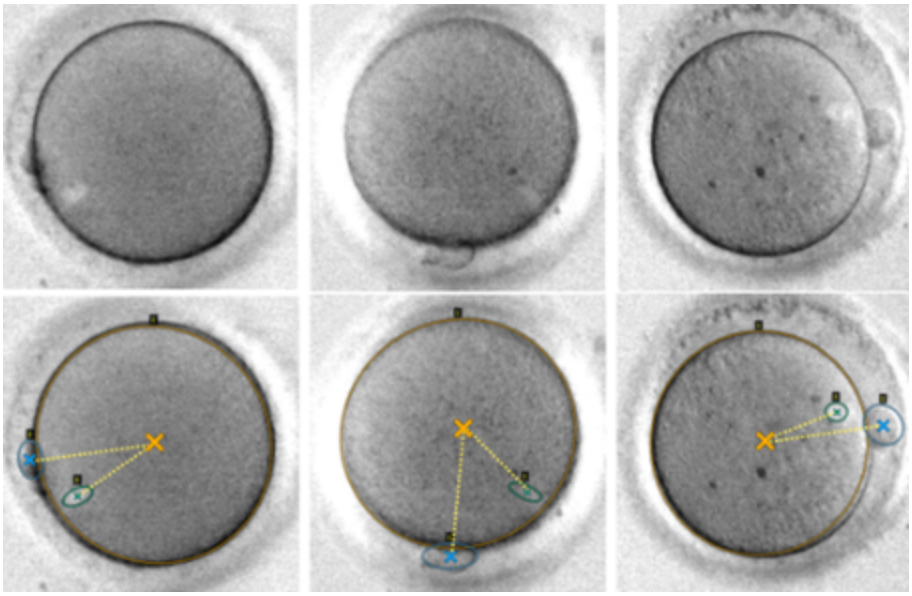


Figure 1

Example images (top) with the annotations (bottom) of the center of cytoplasm, the center of MS, and the center of PB highlighted with crosses and the angle of interest indicated by dotted yellow lines.

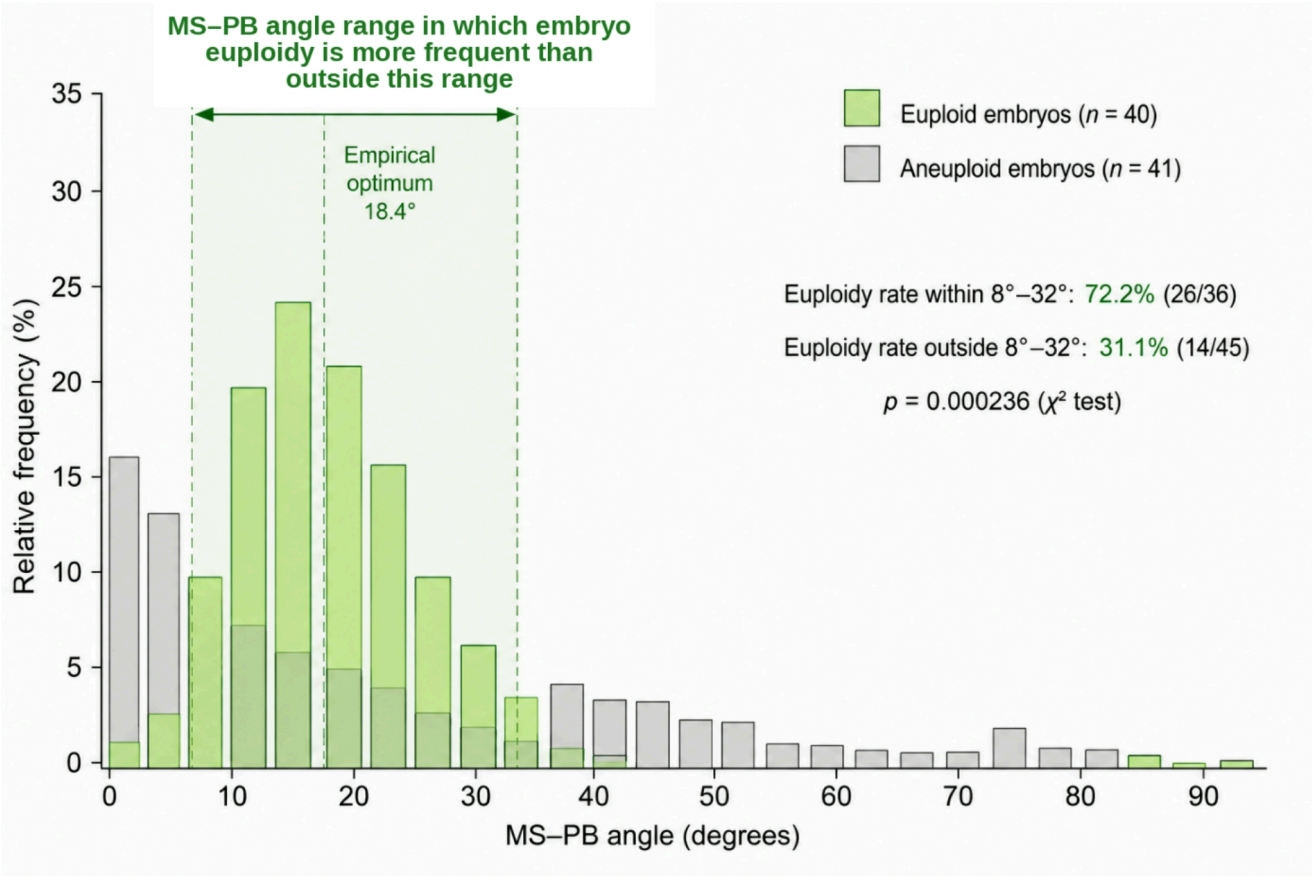


Figure 2

Distribution of MS–PB angles according to embryo ploidy. Relative frequency histograms of meiotic spindle–polar body (MS–PB) angles measured in oocytes that subsequently developed into euploid (green) or aneuploid (gray) embryos. The shaded area denotes the interval (8°–32°), within which embryo euploidy was significantly more frequent than outside this range (72.2% vs. 31.1%; p = 0.000236). The dashed line indicates the empirically identified optimal MS–PB angle (~18.4°).

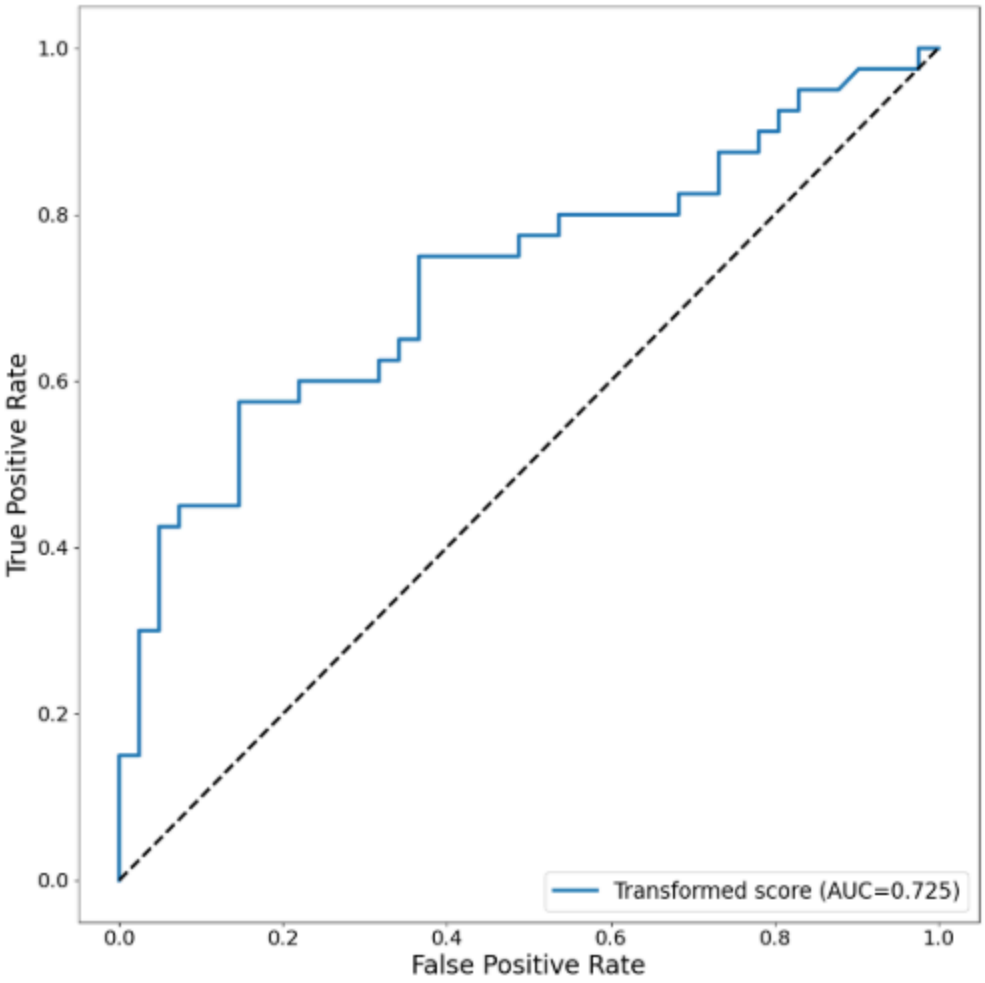


Figure 3

Receiver operating characteristic (ROC) curve based on the absolute deviation of the measured meiotic spindle–polar body (MS–PB) angle from the empirically identified optimal angle of approximately 18.4°. The analysis yielded an area under the ROC curve (AUC) of 0.725, supporting a non-linear association between MS–PB angle and embryo euploidy.

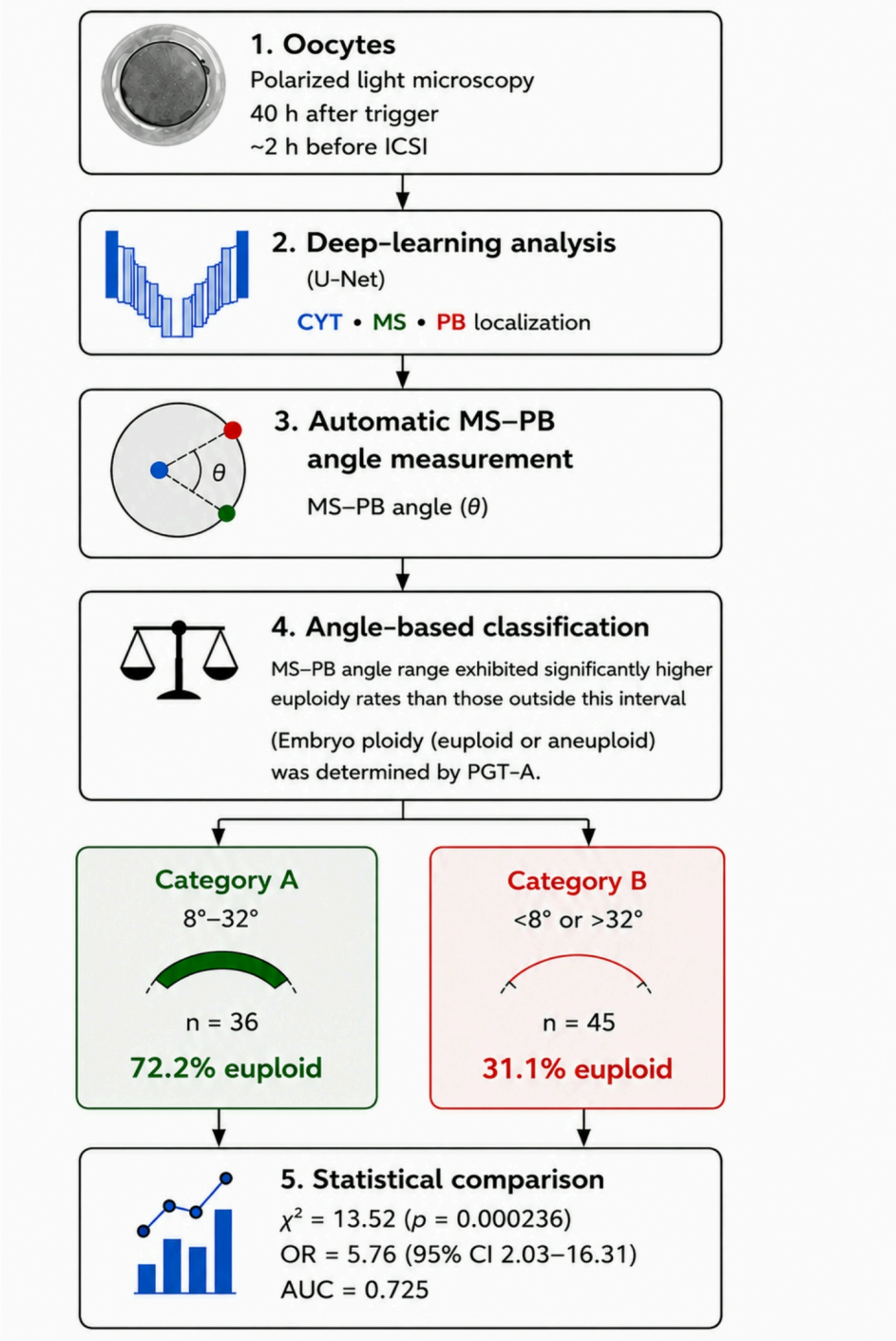


Figure 4

Workflow of automated MS–PB angle analysis and its association with embryo euploidy. Eighty-one metaphase II (MII) oocytes from 55 patients undergoing 55 IVF cycles were examined by polarized light microscopy ~ 40 h after trigger administration and approximately 2 h before ICSI. A U-Net–based deep learning model automatically localized the cytoplasm (CYT), meiotic spindle (MS), and first polar body (PB), enabling objective measurement of the MS–PB angle. Oocytes were automatically classified

according to the measured angle into Category A (8°–32°) and Category B (<8° or >32°). Embryo ploidy was subsequently determined by PGT-A and used as the reference standard. Statistical analysis demonstrated significantly higher euploidy rates in Category A oocytes (72.2% vs. 31.1%; $\chi^2 = 13.52$, $p = 0.000236$), with a patient-clustered odds ratio of 5.76. ROC analysis based on the absolute deviation from the intermediate optimum (~18.4°) yielded an AUC of 0.725, supporting a non-linear association between MS–PB angle and embryo euploidy.

Supplementary Files

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- SupplementaryIK28.8.2026.docx
- floatimage1.png