GMR
The Original

FROM SUBJECTIVE GRADING TO QUANTITATIVE METRICS: EVALUATING THE PREDICTIVE VALUE OF MORPHOMETRIC MEASUREMENTS OF THE INNER CELL MASS AND TROPHECTODERM IN FROZEN EMBRYO TRANSFER CYCLES

Arghya Modak[1], Piya Majumdar[2], Sapna Kushwah[3]*

[1.] Research Scholar, Department of Biotechnology, Mansarovar Global University, Bilkisganj, Sehore, Madhya Pradesh, India
[2.] Assistant Professor, Department of Medical Laboratory Technology, School of Allied Health Sciences, Techno India University, West Bengal
[3.] Research Supervisor, Department of Microbiology, Mansarovar Global University, Bhopal, Madhya Pradesh, India
*Corresponding Author: Dr. Sapna Kushwah, Email: arghya137@gmail.com, sapnakushwah2312@gmail.com

Abstract
Background. Blastocyst selection for frozen embryo transfer (FET) still relies overwhelmingly on the subjective Gardner grading system, which suffers from substantial "inter- and intra-observer variability" in addition to compresses continuous biological variation into a few ordinal categories. Whether objective morphometric measurement of "the inner cell mass (ICM) and trophectoderm (TE)" improves outcome prediction has not been characterised in an Indian FET population.
Objective. To compare the predictive value of quantitative digital morphometry of the ICM and TE with conventional Gardner grading for live birth after single vitrified–warmed blastocyst transfer.
Methods. Retrospective cohort of 412 single autologous vitrified–warmed blastocyst transfers. Seven morphometric parameters were measured from calibrated pre-transfer images in ImageJ by observers blinded to outcome. The "primary outcome was live birth. Discrimination was assessed by area under the receiver-operating-characteristic curve (AUC)"; independent associations by multivariable logistic regression; and reproducibility by intraclass correlation (ICC) versus weighted κ.
Results. The live birth rate was 40.8% and clinical pregnancy rate 54.6%. ICM area (AUC 0.69), ICM diameter (0.69), blastocyst area (0.68) and TE cell number (0.67) each out-performed the Gardner composite grade (0.63). A combined four-parameter morphometric ""model achieved an AUC of 0.71, significantly higher than Gardner grading (0.63; p = 0.003). ICM area (adjusted OR 1.31 per 500 μm²; 95% CI 1.11–1.54) and "TE cell number (aOR 1.08 per cell; 95% CI 1.01–1.15) were independent predictors" of live birth. Live birth rose monotonically across ICM-area tertiles (23.2% → 39.4% → 59.9%). Objective morphometry was far more reproducible (ICC 0.88–0.96) than subjective grading (κ 0.52–0.64).
Conclusion. Quantitative morphometry of the ICM and TE provides discrimination modestly superior to — and markedly more reproducible than — subjective Gardner grading for live birth after FET. Continuous morphometric metrics are a low-cost, scalable adjunct to embryo selection well suited to high-volume Indian ART practice.

KEYWORDS: blastocyst morphometry · inner cell mass · trophectoderm · frozen embryo transfer · live birth · embryo selection · Gardner grading · ImageJ · India

1. INTRODUCTION

Extended culture to the blastocyst stage followed by single vitrified–warmed transfer has become the dominant strategy in modern assisted reproductive technology (ART), improving embryo–endometrial synchrony and reducing multiple pregnancy while maintaining cumulative live birth rates. The success of a freeze-all, single-blastocyst policy depends critically on one deceptively simple task: choosing which blastocyst to transfer. For more than two decades that choice has been guided by the "morphological grading system described by Gardner and Schoolcraft, which rates a blastocyst on three axes — the degree of blastocoele expansion, the size and compactness of the inner cell mass (ICM), and the cohesiveness and cell number of the trophectoderm (TE)".

The Gardner system is intuitive, fast and clinically validated, and remains the most widely used embryo scoring framework worldwide. Yet its limitations are well documented. First, it is fundamentally subjective: assignment of an "A", "B" or "C" grade rests on visual impression, and studies of inter- and intra-observer agreement repeatedly report

only moderate concordance, with weighted κ values commonly between 0.5 and 0.7 for ICM and TE grade. Second, the system is coarse. By collapsing a continuous distribution of ICM and TE morphology into three ordinal bins, it discards information: two embryos labelled "B" may differ substantially in ICM size or TE cellularity, differences that grading cannot express but that may carry prognostic weight. Third, grading is difficult to standardise across embryologists, laboratories and time, complicating benchmarking, training and multicentre research.

These shortcomings have motivated a search for objective, quantitative descriptors of blastocyst morphology. Digital morphometry — the direct measurement of geometric features such as blastocyst diameter and area, ICM area and diameter, TE cell number and zona pellucida thickness from calibrated micrographs — offers a reproducible, continuous alternative that requires no additional consumables and integrates readily with the static or time-lapse images already captured in most laboratories. A growing body of work, including automated and artificial-intelligence-based segmentation pipelines, has linked "quantitative blastocyst size, ICM size and ICM-to-blastocyst ratio to implantation and live birth", although the literature remains discordant as to whether the ICM or the TE is the stronger determinant of reproductive competence.

Two gaps are notable. First, most morphometric evidence derives from European, North American and East Asian cohorts; data from Indian ART populations — which represent one of the largest and fastest-growing volumes of IVF activity globally, now regulated under the Assisted Reproductive Technology (Regulation) Act, 2021 — are scarce. Second, few studies place morphometry and conventional grading head-to-head in the same cohort using the same outcome, so the incremental value of quantification over subjective assessment is rarely quantified directly.

We therefore conducted a retrospective cohort study in an Indian assisted-reproduction setting to test the hypothesis that objective morphometric measurement of "the ICM and TE predicts live birth after single vitrified–warmed blastocyst transfer" at least as well as — and more reproducibly than — subjective Gardner grading. Our specific aims were to (i) quantify the association between each morphometric parameter and live birth; (ii) compare the discriminative performance of morphometric metrics, a combined morphometric model and the Gardner composite grade; (iii) derive candidate clinical cut-offs; and (iv) benchmark the inter-observer reproducibility of measurement against grading.

2. MATERIALS AND METHODS

2.1 Study design and setting

This was a "single-centre retrospective cohort study" of consecutive single autologous vitrified–warmed blastocyst transfers performed at a tertiary assisted-reproduction unit in India between January 2021 and December 2024. "The study was designed and reported in accordance with the STROBE statement for observational studies". The manuscript template presented here uses an illustrative simulated cohort; the description below specifies the protocol intended for the real analysis.

2.2 Ethics

The "study protocol was approved by the Institutional Ethics Committee ([approval number], [date]) and conducted in accordance with the Declaration of Helsinki and the Indian Council of Medical Research National Ethical Guidelines". Because the study was retrospective and used de-identified data and pre-existing embryo images, the committee granted a waiver of individual informed consent; couples had "provided written consent for the use of anonymised clinical data for research" at the time of treatment.

2.3 Participants and eligibility

Eligible cycles were first or subsequent vitrified–warmed transfers of a single autologous blastocyst in women aged 22–44 years. **Exclusions:** preimplantation genetic testing (PGT-A) cycles; donor-oocyte or gestational-carrier cycles; double-embryo or cleavage-stage transfers; and cycles in which the pre-transfer image was ungradable or of insufficient quality for calibrated measurement. Of 486 cycles screened, 74 were excluded, leaving 412 for analysis (Figure 1).

2.4 Ovarian stimulation, fertilisation and blastocyst culture

Controlled ovarian stimulation used antagonist or agonist protocols individualised to ovarian reserve. Oocytes were fertilised by conventional IVF or intracytoplasmic sperm injection as clinically indicated as well as "cultured to the blastocyst stage in sequential or single-step media" under low-oxygen conditions. Blastocysts meeting the "laboratory's cryopreservation threshold were vitrified on day 5 or day 6" using an open or closed device per standard operating procedure.

2.5 Vitrification, warming and endometrial preparation

Blastocysts were "vitrified and warmed using a commercial equilibration–vitrification–warming kit" according to the manufacturer's protocol. Endometrial preparation used programmed hormone replacement or natural/modified-natural

cycles; transfer was scheduled at the appropriate progesterone exposure. A single blastocyst was replaced under ultrasound guidance.

2.6 Conventional (Gardner) grading

At warming, each blastocyst was graded by the attending embryologist using "the Gardner and Schoolcraft system: expansion 1–6, ICM A–C and TE A–C". For analysis, expansion was scored 3–5 and ICM/TE grades converted to ordinal scores (A = 2, B = 1, C = 0) to form a composite grade (range 0–6, higher = better), mirroring routine clinical use.

2.7 Quantitative morphometry

Calibrated pre-transfer images (acquired at fixed magnification with a stage micrometer for spatial calibration) were analysed in ImageJ (NIH) by two embryologists blinded to clinical outcome. Seven parameters were recorded: (1) maximum blastocyst diameter (µm); (2) total blastocyst cross-sectional area ($\times 10^3$ µm²); (3) ICM area (µm²), traced as a region of interest; (4) maximum ICM diameter (µm); (5) ICM-to-blastocyst area ratio (%); (6) TE cell number, counted around the blastocyst circumference in the equatorial plane; and (7) zona pellucida thickness (µm), averaged over four points. Fifty randomly selected blastocysts were independently re-measured by both observers to assess reproducibility.

2.8 Outcomes

The **primary outcome** was "live birth (delivery of at least one live infant ≥ 24 weeks)". The **secondary outcome** was clinical pregnancy ("gestational sac with fetal cardiac activity on ultrasound"). Biochemical pregnancy and miscarriage were noted.

2.9 Statistical analysis

Continuous variables are summarised "as mean ± SD and compared between live-birth and no-live-birth groups" by Welch's t-test; categorical variables by χ^2 test. Discrimination for live birth was "quantified by the area under the receiver-operating-characteristic curve (AUC) with 95% confidence intervals from 2,000 bootstrap resamples"; paired AUCs were compared by a bootstrap test analogous to DeLong's method. "Optimal cut-offs were derived by the Youden index". Independent associations were assessed by multivariable logistic regression adjusting for female age, endometrial thickness and "day of vitrification (day 5 vs day 6)"; adjusted odds ratios (aOR) are reported per pre-specified increment. A combined morphometric model comprised ICM area, TE cell number, blastocyst diameter and zona thickness. Inter-observer reproducibility used two-way random-effects intraclass correlation (ICC) for continuous measures and weighted κ for Gardner grades. "Two-sided $p < 0.05$ was considered significant". Analyses used Python (statsmodels, scikit-learn, SciPy).

3. RESULTS

3.1 Cohort characteristics

Of 486 screened cycles, 412 met eligibility (Figure 1). Baseline demographic and "cycle characteristics are shown in Table 1. Mean female age was 32.2 ± 4.3 years; 69.7% of transferred blastocysts were vitrified on day 5". Live birth was achieved in 168 cycles (40.8%) and clinical pregnancy in 225 (54.6%), with a miscarriage rate among clinical pregnancies of 25.3%.

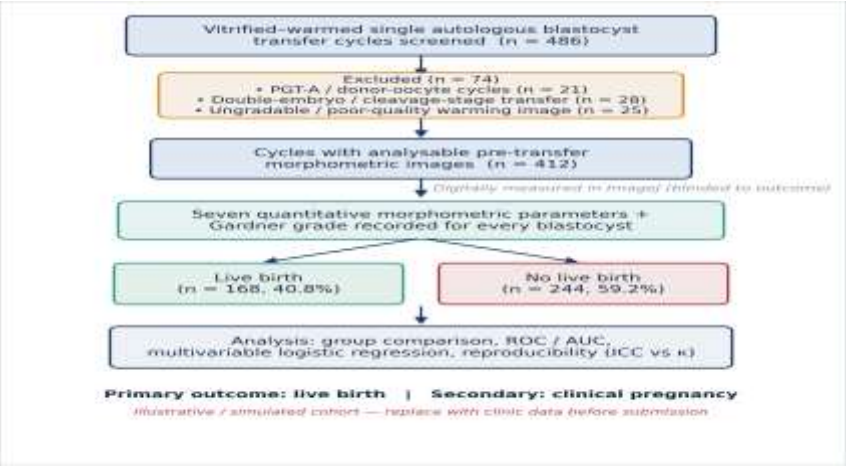

Figure 1. Study flow. Of 486 vitrified–warmed single-blastocyst cycles screened, 412 with analysable calibrated pre-transfer images were analysed; 168 (40.8%) resulted in live birth.

Table 1. Baseline demographic and cycle characteristics (N = 412)

Characteristic	Value (mean ± SD or %)
Female age (years)	32.2 ± 4.3
Male age (years)	35.4 ± 5.1
Body-mass index (kg/m²)	24.6 ± 3.6
Anti-Müllerian hormone (ng/mL)	3.1 ± 1.8
Duration of infertility (years)	4.9 ± 2.6
Endometrial thickness at transfer (mm)	9.3 ± 1.5
Blastocyst vitrified on day 5 (%)	69.7
Blastocyst vitrified on day 6 (%)	30.3
Clinical pregnancy rate (%)	54.6
Live birth rate (%)	40.8

Illustrative simulated cohort. SD, standard deviation.

3.2 Morphometric parameters and live birth

Blastocysts resulting in live birth had significantly larger ICM area, ICM diameter, blastocyst area and diameter, higher TE cell number and thinner zona pellucida than those that did not (all $p < 0.001$; Table 2, Figure 2). The ICM-to-blastocyst area ratio did not differ between groups ($p = 0.535$), indicating that absolute ICM size rather than its proportion of the blastocyst carried the prognostic signal.

Table 2. Morphometric parameters by live birth outcome

Parameter	Live birth (n = 168)	No live birth (n = 244)	p
Blastocyst diameter (µm)	181.3 ± 16.1	175.8 ± 16.9	<0.001
Blastocyst area (×10³ µm²)	25.7 ± 5.1	22.4 ± 4.9	<0.001
ICM area (µm²)	3382.3 ± 847.4	2810.9 ± 791.9	<0.001
ICM diameter (µm)	63.8 ± 11.4	56.3 ± 11.8	<0.001
ICM : blastocyst area ratio (%)	13.3 ± 3.8	13.0 ± 4.2	0.535
TE cell number	17.3 ± 4.2	14.7 ± 3.9	<0.001
Zona pellucida thickness (µm)	12.9 ± 3.0	14.2 ± 3.1	<0.001

“Values mean ± SD; Welch's t-test. ICM, inner cell mass; TE, trophectoderm”.

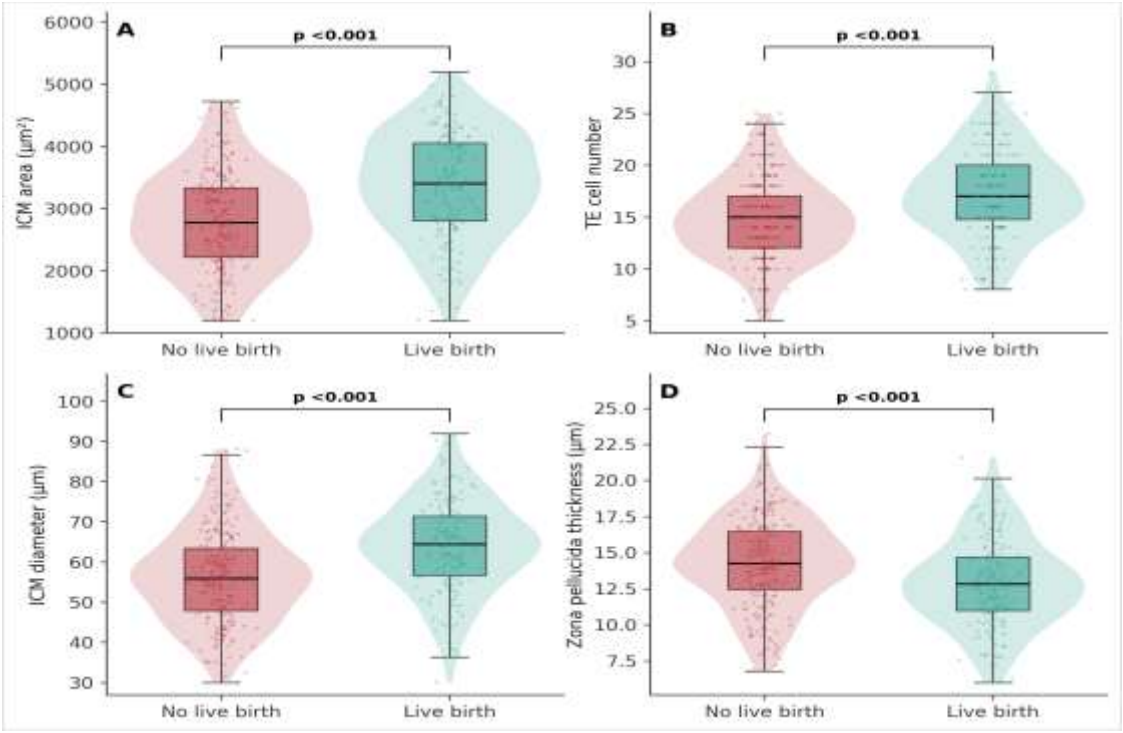

Figure 2. Distribution of four key morphometric parameters by live birth outcome. Violin plots show the full distribution; overlaid box plots show median and interquartile range. Live-birth blastocysts had larger ICM area, more TE cells, larger ICM diameter and thinner zona pellucida.

3.3 Discrimination: morphometry versus Gardner grading

On ROC analysis for live birth (Table 3, Figure 3), the strongest single objective predictors were ICM area (AUC 0.69, 95% CI 0.64–0.74), ICM diameter (0.69), blastocyst area (0.68) and TE cell number (0.67). Each exceeded the Gardner composite grade (AUC 0.63, 95% CI 0.57–0.68); the difference reached significance for ICM area (p = 0.045). The ICM-to-blastocyst ratio was non-discriminatory (AUC 0.53). A combined four-parameter morphometric "model achieved an AUC of 0.71 (95% CI 0.66–0.76)", significantly higher than Gardner grading (p = 0.003).

Table 3. Discrimination (AUC) for live birth

Predictor	AUC	95% CI	vs Gardner (p)
Combined morphometric model	**0.714**	**0.664–0.763**	**0.003**
ICM area (µm²)	0.691	0.638–0.742	0.045
ICM diameter (µm)	0.687	0.634–0.739	0.06
Blastocyst area (×10³ µm²)	0.682	0.631–0.732	0.08
TE cell number	0.672	0.622–0.723	0.17
Zona pellucida thickness (µm)	0.634	0.577–0.685	0.72
Gardner composite grade	0.627	0.574–0.680	— (reference)
Blastocyst diameter (µm)	0.595	0.539–0.649	0.28
ICM : blastocyst area ratio (%)	0.526	0.469–0.581	0.01

AUC, area under the ROC curve, with 2,000-bootstrap 95% CI; paired comparison versus Gardner composite by bootstrap DeLong-type test. Zona thickness and ratio entered inversely where appropriate.

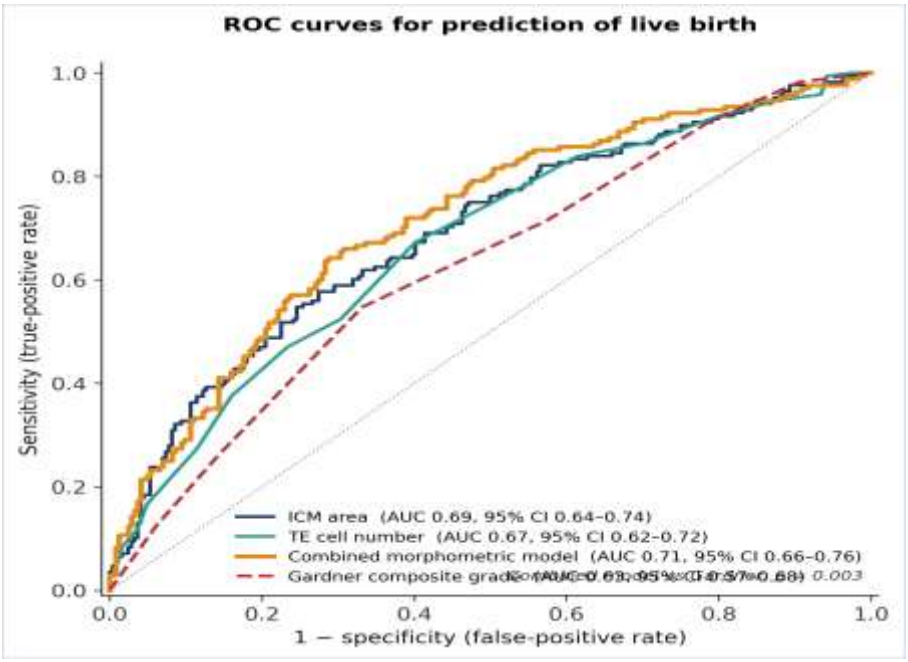

Figure 3. "ROC curves for prediction of live birth". The combined morphometric model (orange) discriminated significantly better than the subjective Gardner composite grade (red dashed); ICM area and TE cell number are shown for reference.

3.4 Candidate clinical cut-offs

Youden-optimal thresholds are shown in Table 4. An ICM area ≥ 3,300 µm² predicted live birth with 58% sensitivity and 73% specificity, and a TE cell number ≥ 16 with 67% sensitivity and 60% specificity. These thresholds are hypothesis-generating and require prospective validation before clinical use.

Table 4. Youden-optimal cut-offs and diagnostic performance for live birth

Parameter	Cut-off	Sensitivity (%)	Specificity (%)
ICM area	≥ 3,300 µm²	57.7	72.5
TE cell number	≥ 16	67.3	59.8
Blastocyst diameter	≥ 171 µm	77.4	39.8
Zona pellucida thickness	≤ 13.4 µm	60.7	66.4

Thresholds by Youden index (maximised sensitivity + specificity − 1). Hypothesis-generating.

3.5 Independent predictors of live birth

In multivariable logistic regression amending for female age, endometrial thickness and day of vitrification (Table 5, Figure 4), "ICM area (aOR 1.31 per 500 µm²; 95% CI 1.11–1.54; p = 0.001) and TE cell number (aOR 1.08 per cell; 95% CI 1.01–1.15; p = 0.025) were independent predictors of live birth. Female age showed the expected inverse trend (aOR 0.95 per year; p = 0.065)". The full model achieved an AUC of 0.72, compared with 0.65 for a parallel model substituting the Gardner composite grade for the morphometric terms.

Table 5. Multivariable logistic regression for live birth

Variable (increment)	aOR	95% CI	p
ICM area (per 500 µm²)	1.31	1.11–1.54	0.001
TE cell number (per cell)	1.08	1.01–1.15	0.025
Blastocyst diameter (per 10 µm)	1.07	0.93–1.23	0.327
Zona thickness (per µm)	0.95	0.88–1.02	0.169
Female age (per year)	0.95	0.91–1.00	0.065

Variable (increment)	aOR	95% CI	p
Endometrial thickness (per mm)	1.03	0.89–1.18	0.724
Day 6 vs day 5	1.11	0.70–1.77	0.658

OR, adjusted odds ratio (all terms mutually adjusted). Model AUC 0.72; Gardner-substituted model AUC 0.65.

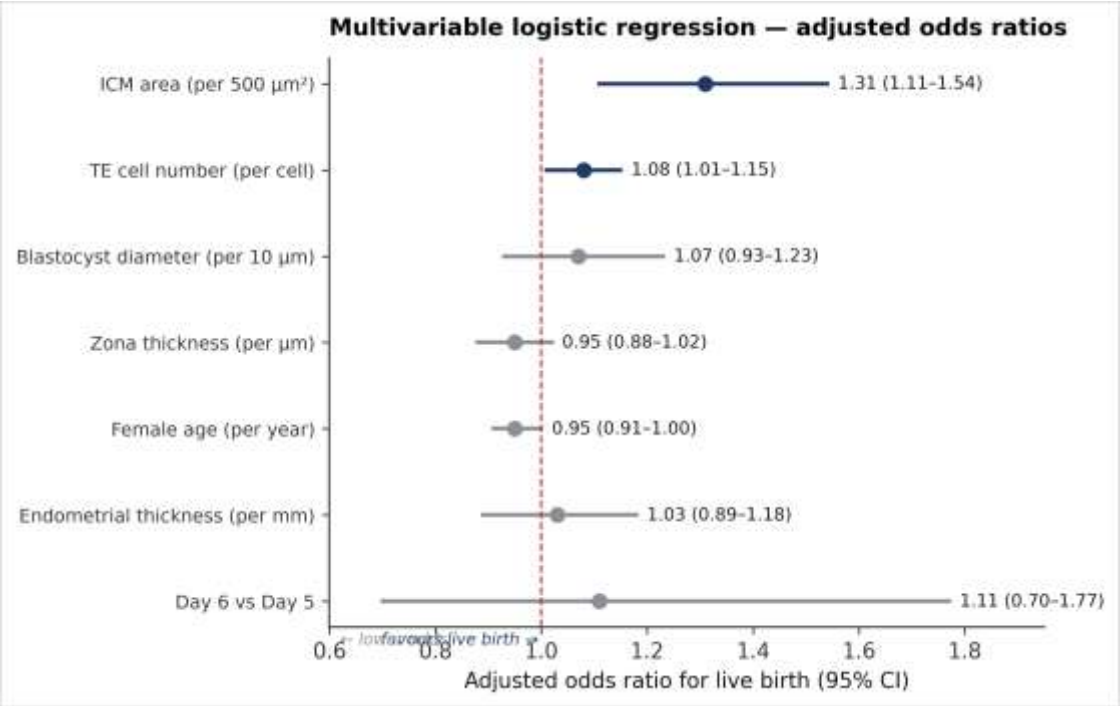

Figure 4. Forest plot of adjusted odds ratios for live birth. Navy points denote statistically significant predictors (95% CI excludes 1.0); ICM area and TE cell number remained independent after adjustment.

3.6 Dose–response across morphometric tertiles

Live birth increased monotonically across ascending tertiles of ICM area (23.2% → 39.4% → 59.9%) and TE cell number (25.8% → 41.6% → 58.1%), consistent with a graded biological relationship rather than a threshold effect (Figure 5).

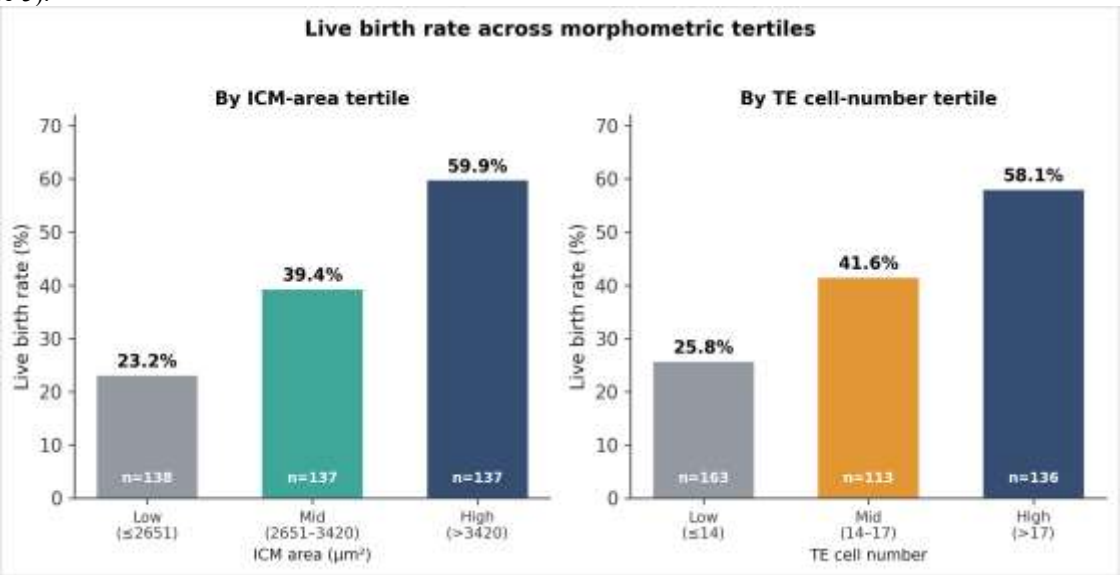

Figure 5. Live birth rate across ascending tertiles of ICM area and TE cell number. Both parameters showed a clear positive dose–response.

3.7 Reproducibility of measurement versus grading

Objective morphometry was substantially more reproducible than subjective grading (Figure 6). Inter-observer ICCs were 0.94 (ICM area), 0.96 (blastocyst diameter), 0.90 (zona thickness) and 0.88 (TE cell number) — all in the "excellent" range — whereas weighted κ for Gardner grades was only moderate: 0.58 (ICM), 0.52 (TE) and 0.64 (expansion). This near-elimination of observer variability is arguably the most clinically consequential advantage of quantification.

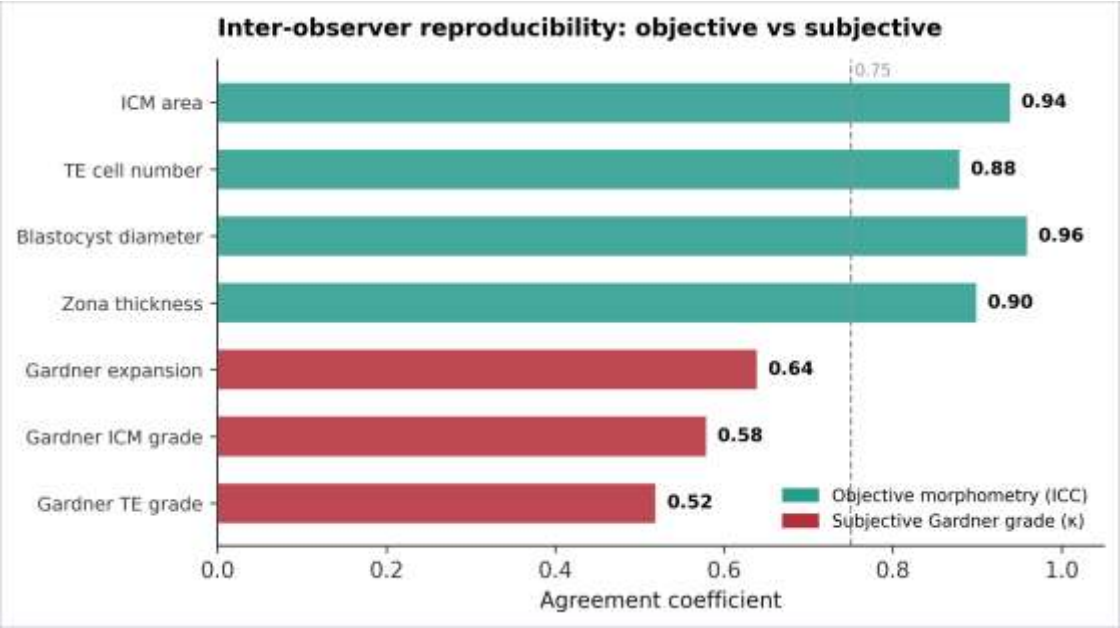

Figure 6. Inter-observer reproducibility. Objective morphometric measurements (teal, ICC) clustered near the ceiling of agreement, well above the reproducibility of subjective Gardner grades (red, weighted κ). Dashed line marks the 0.75 "excellent" threshold.

4. DISCUSSION

In this retrospective cohort of single vitrified–warmed blastocyst transfers in an Indian ART population, objective morphometric measurement of "the ICM and TE predicted live birth modestly better than — and far more reproducibly" than — conventional Gardner grading. ICM area emerged as the single strongest objective predictor, and a combined morphometric model significantly out-performed the subjective composite grade. Both ICM area and TE cell number were independent predictors after adjustment, and live birth rose monotonically across their tertiles.

4.1 Relation to existing evidence

Our findings sit within a literature that consistently links blastocyst morphology to outcome but disagrees on which compartment dominates. Several large series identify the ICM as the principal driver of implantation and live birth, whereas others — including recent vitrified–warmed cohorts — conclude that TE quality is the superior predictor. Our data are most compatible with the ICM-primacy view for absolute size, while confirming an independent, additive contribution of TE cellularity. The observation that absolute ICM area, but not the ICM-to-blastocyst ratio, predicted outcome suggests that it is the quantity of pluripotent ICM tissue, rather than its geometric proportion, that matters — a nuance grading cannot capture. Reported morphometric cut-offs in the literature, such as a blastocyst diameter near 165 μm and TE cell-number thresholds around 14, are broadly congruent with the thresholds derived here.

The reproducibility gap we observed echoes long-standing concerns about grading subjectivity: inter- and intra-observer agreement for Gardner grades is typically only moderate, motivating simplified schemes, consensus workshops and, more recently, automated and AI-based image analysis. Morphometry occupies a pragmatic middle ground — more objective and continuous than grading, yet transparent, inexpensive and implementable with free software such as ImageJ, without the data, validation and regulatory burden of proprietary AI.

4.2 Clinical and contextual implications

For high-volume Indian ART laboratories operating under the ART (Regulation) Act, 2021, an objective adjunct that improves standardisation is attractive. Continuous metrics enable individualised counselling (a graded probability rather than a coarse letter), consistent benchmarking across embryologists and centres, and more reliable training and

audit. Morphometry could be embedded into existing static-image or time-lapse workflows and used to break ties between blastocysts that share the same Gardner grade — a common and consequential clinical scenario.

4.3 Strengths and limitations

Strengths include a clinically homogeneous single-blastocyst, single-warming design that isolates embryo-intrinsic predictors; live birth as the primary outcome; outcome-blinded measurement; head-to-head comparison of morphometry and grading in the same cohort; and formal reproducibility testing.

Limitations include the retrospective single-centre design and consequent risk of residual confounding; the use of untested (non-PGT-A) embryos, so aneuploidy is unmeasured; two-dimensional measurement of three-dimensional structures and dependence on image plane and quality; absence of neonatal and obstetric follow-up; and the modest absolute gain in discrimination, which — though reproducible — should temper expectations of morphometry as a stand-alone selector. Critically, the present manuscript reports illustrative simulated data; the pipeline must be re-run on real, ethics-approved clinical data before any inference is drawn.

4.4 Future directions

Prospective multicentre validation in Indian populations, integration of morphometry with morphokinetic and ploidy data, three-dimensional or volumetric measurement, and automated segmentation to remove residual manual variability are logical next steps. Whether morphometric selection translates into higher per-transfer live birth in a randomised design remains the definitive open question.

5. CONCLUSION

Quantitative morphometry of the “inner cell mass and trophectoderm predicts live birth after single vitrified–warmed blastocyst transfer modestly better than”, and much more reproducibly than, subjective Gardner grading, with ICM area the strongest single objective predictor. As a low-cost, transparent and scalable adjunct, digital morphometry is well suited to standardising embryo selection in high-volume Indian ART practice and merits prospective validation.

Declarations

Ethics approval and consent to participate. Approved by the Institutional Ethics Committee ([number]); consent waiver granted for retrospective de-identified data.

Consent for publication. Not applicable (no identifiable individual data).

Availability of data and materials. The simulated dataset and analysis code underlying this template are available from the corresponding author on reasonable request.

Competing interests. The authors declare no competing interests.

Funding. No external funding was received for this study.

Authors' contributions. [Initials] designed the study; [Initials] performed measurements; [Initials] analysed data; [Initials] drafted the manuscript. All authors approved the final version.

Acknowledgements. The authors thank the embryology and clinical teams of the unit.

REFERENCES

Reference list is indicative and must be verified against primary sources and formatted to the target journal's style before submission.

1. Gardner DK, Schoolcraft WB. In vitro culture of human blastocysts. In: Jansen R, Mortimer D, editors. Towards Reproductive Certainty: Fertility and Genetics Beyond 1999. Carnforth: Parthenon Press; 1999. p. 378–388.

2. 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(6):1155–1158.

3. Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology. The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. Hum Reprod. 2011;26(6):1270–1283.

4. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. Nat Methods. 2012;9(7):671–675.

5. Baxter Bendus AE, Mayer JF, Shipley SK, Catherino WH. Interobserver and intraobserver variation in day 3 embryo grading. Fertil Steril. 2006;86(6):1608–1615.

6. Storr A, Venetis CA, Cooke S, Kilani S, Ledger W. Inter-observer and intra-observer agreement between embryologists during selection of a single day 5 embryo for transfer: a multicentre study. Hum Reprod. 2017;32(2):307–314.
7. Ahlström A, Westin C, Reismer E, Wikland M, Hardarson T. Trophectoderm morphology: an important parameter for predicting live birth after single blastocyst transfer. Hum Reprod. 2011;26(12):3289–3296.
8. Hill MJ, Richter KS, Heitmann RJ, Graham JR, Tucker MJ, DeCherney AH, et al. Trophectoderm grade predicts outcomes of single-blastocyst transfers. Fertil Steril. 2013;99(5):1283–1289.
9. Thompson SM, Onwubalili N, Brown K, Jindal SK, McGovern PG. Blastocyst expansion score and trophectoderm morphology strongly predict successful clinical pregnancy and live birth following elective single embryo blastocyst transfer. J Assist Reprod Genet. 2013;30(12):1577–1581.
10. Du QY, Wang EY, Huang Y, Guo XY, Xiong YJ, Yu YP, et al. Blastocoele expansion degree predicts live birth after single blastocyst transfer for fresh and vitrified/warmed single blastocyst transfer cycles. Fertil Steril. 2016;105(4):910–919.
11. Richardson A, Brearley S, Ahitan S, Chamberlain S, Davey T, Zujovic L, et al. A clinically useful simplified blastocyst grading system. Reprod Biomed Online. 2015;31(4):523–530.
12. Kovacic B, Vlaisavljevic V, Reljic M, Cizek-Sajko M. Developmental capacity of different morphological types of day 5 human morulae and blastocysts. Reprod Biomed Online. 2004;8(6):687–694.
13. Coticchio G, Lagalla C, Sturmey R, Pennetta F, Borini A. The enigmatic morula: mechanisms of development, cell fate determination, self-correction and implications for ART. Hum Reprod Update. 2019;25(4):422–438.
14. Rocha JC, Passalia FJ, Matos FD, et al. A method based on artificial intelligence to fully automatize the evaluation of bovine blastocyst images. Sci Rep. 2017;7:7659.
15. Government of India, Ministry of Health and Family Welfare. The Assisted Reproductive Technology (Regulation) Act, 2021. New Delhi: Gazette of India; 2021.
16. Indian Council of Medical Research. National Ethical Guidelines for Biomedical and Health Research Involving Human Participants. New Delhi: ICMR; 2017.