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ARTICLE

Decoding embryo development: the effect of clinical variables in morphokinetics and artificial intelligence quality scoring

BIOGRAPHY

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KEY MESSAGE

Time to expanded blastocyst as assessed by artificial intelligence was the most impactful morphokinetic event on an embryo quality score associated with patient factors, pregnancy outcomes and live birth and ploidy status.

ABSTRACT

Research question: Do morphokinetic events (MKS) and patient parameters affect resulting embryo quality scores, and how does this relate to pregnancy outcomes as assessed by a time-lapse incubator using an artificial intelligence (AI) embryologist support tool?

Design: Retrospective study analysing data from 6024 embryos retrieved over 1636 cycles. The dataset comprised 3778 donor oocytes and 2246 autologous oocytes. Additionally, 3309 biopsied embryos were included in the PGT-A analysis. Outcome data were derived from 1355 transferred embryos. All embryos were assessed using a time lapse-based AI system (CHLOE EQ™), which assigns an embryo quality score from 0 to 1 based upon established morphokinetic benchmarks. The AI embryo quality score was assigned on day 5 of embryo development. Analysed patient parameters were patient age, fresh or frozen oocyte status and use of own or donor oocytes. Twenty-three MKS were analysed. The effect of morphokinetics on the incidence of ploidy was also assessed.

Results: Clinical outcomes are affected by embryo quality and MKS as detected by an AI software. Time to expanded blastocyst (tEB) was the morphokinetic parameter with the strongest correlation coefficient with embryo quality score (−0.816). As the patient's age increases by 1 unit, embryo quality score decreases significantly and time to achieve expanded blastocyst increases by 0.47 h. Similar trends were observed with frozen oocytes, which showed a 2.1 h increase in tEB compared with fresh oocytes. Autologous oocytes were associated with a 6.08 h longer tEB compared with donor oocytes. Additionally, euploid embryos reached tEB 4.72 h earlier than aneuploid embryos. For a one unit increase in embryo quality score, the odds of achieving clinical

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KEYWORDS
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pregnancy increased by 21.7% and the odds of achieving ongoing pregnancy or live birth increased by 18.5%. Oocyte sources had an effect on miscarriage rates; the use of frozen oocytes resulted in higher miscarriage rates than observed when fresh oocytes were used.

Conclusions: AI can successfully evaluate embryo quality and can assist embryologists in decision making. Furthermore, this AI model can delineate the effect of various clinical factors on resulting outcomes.

INTRODUCTION

Embryo development and implantation is a complicated but vital process in human reproduction. It is the result of a highly organized process that requires a coordinated level of synchronization between the uterus and blastocyst to be successful (*Cha et al., 2012*; *Kim and Kim, 2017*; *Muter et al., 2023*). With so many variables at play, it is difficult to control an embryo's environment and understand what differentiates success from failure. As a result, the process is not completely well elucidated and poses a challenge for embryologists and researchers when trying to identify embryos with the highest likelihood of achieving ongoing pregnancy.

A key component of successful embryo implantation is good-embryo quality and preimplantation development (*Oron et al., 2014*; *Zhu et al., 2020*; *Muter et al., 2023*). Patient age is a critical factor that can affect embryo quality and development (*Hardarson et al., 2008*; *Oron et al., 2014*). For example, it is well known that the implantation rates, as well as embryo quality for women over 38 years, are significantly lower than the resulting outcomes for patients aged younger than 35 years (*Hardarson et al., 2008*; *Oron et al., 2014*; *Reig et al., 2020*).

Another important variable that influences developmental and implantation rates as well as embryo quality is if the gametes are fresh or frozen. Although clinical pregnancy and live birth rates have not been shown to be affected, *Li et al. (2022)* previously reported that vitrification has been shown to decrease the quality of oocytes compared with fresh oocytes. This topic, however, is controversial, as recent studies have shown no effect of cryopreservation status on resulting outcomes (*Wirleitner et al., 2016*). With the advent and common use of vitrification, the use of frozen–warmed donor oocytes has become common practice. Many studies have demonstrated high levels of efficiency when using frozen donor oocytes (*Yeh et al., 2014*).

Upon fertilization, morphokinetic events (MKS) within the developing embryo can be annotated through time lapse imaging (TLI) to monitor subsequent embryo development. Important MKS include the time at which a pronuclei appears (tPNa), time to syngamy (tPNf), time to cell divisions (t2, t3, t4, t5, t6, t7, t8 and t9+), time to morula (tM), time to start of blastulation (tSB), time of blastulation (tB) and time to expanded blastocyst (tEB). Specific intervals can then be calculated from these MKS, including t3-tPNf (cell cycle 1, CC1), t3-t2 (CC2), t4-t3 (S2), t5-t3 (CC3), t5-t2, t8-t5 (S3), t8-t4, t8-t2, and tEB-tSB. Each event has an optimal time range, and deviations can indicate decreased embryo quality, implantation, ongoing pregnancy rates and ploidy (*Chamayou et al., 2013*; *Conaghan et al., 2013*; *Desai et al., 2014*). These data can be used to predict the live birth likelihood and can also be analysed to understand the quality of an embryo.

Manual assessment of embryo grading specially at the blastocyst stage is subjective (*Fruchter-Goldmeier et al., 2023*). For example, the most commonly use blastocyst grading system was initially described by *Gardner et al. (2000)*. This grading system, however, does not take into account embryonic developmental history, rate of expansion and other important characteristics of the blastocyst itself. Therefore, the application of new technologies, such as artificial intelligence (AI), has the potential to reduce the biasness of manual embryo grading (*Salih et al., 2023*).

Our previous research has demonstrated the predictive and analytical capabilities of an AI-based embryo assessment tool, for predicting the likelihood of a successful implantation and live birth retrospectively (*Lucio et al., 2022*). These models are particularly useful in assisting embryologists by automatically annotating the key MKS. Therefore, the aim of the present study was to determine if MKS and patient variables affect or influence the resulting embryo quality score and how this relates to pregnancy outcomes as assessed by a TLI-based AI embryologist support tool.

MATERIALS AND METHODS

Patients

A retrospective study was conducted analysing data from 6024 embryos retrieved over 1636 cycles between 2019 and 2023 across five clinics within a European IVF network. Only embryos derived from intracytoplasmic sperm injection with complete morphokinetic data were included and used for statistical analysis. Embryos that arrested before tEB were excluded from the study. The dataset was composed of 3778 donor oocytes (2960 fresh plus 818 frozen) and 2246 autologous oocytes (1976 fresh plus 270 frozen). Additionally, 3309 biopsied embryos (2947 euploid plus 362 aneuploid; mosaics were omitted) were included in the preimplantation genetic testing for aneuploidy (PGT-A) analysis. The observed high euploid rate was partially due to the predominant use of donor oocytes in addition to the database being composed of only blastocyst with complete MKS annotations and age data. For statistical purposes, the cryopreservation section encompassed donor and autologous oocytes. Similarly, the analysis of oocyte donor status included fresh and frozen oocytes. For all statistical analysis involving patient age, a continuous age variable was used. For tables and figures, however, patients were stratified into age groups with corresponding sample sizes following Society for Assisted Reproductive Technology classification: <35 years (n = 1120); 35–37 years (n = 108); 38–40 years (n = 95); 41–42 (n = 14); and over 42 years (n = 9). In all cases, oocyte age was taken for patient age. Pregnancy outcome data were derived from 1355 embryos transferred between 2021 and 2023.

In the present study, the mean age of the frozen egg donors was 25.8 years (18–33), and the mean age of the fresh donors was 24.6 years (18–33) The age range was identical for fresh and frozen egg donors. The mean age of autologous patients using fresh oocytes was 36.7 years (22–47), whereas the mean age for those using frozen oocytes was 37 years (26–44).

Study variables

Patient parameters analysed in this study included patient age for autologous oocytes and donor age for donor oocytes, the fresh or frozen status of oocytes and the use of autologous or donor oocytes. Twenty-three AI annotated MKS were analysed, comprising 14 absolute MKS (tPNa, tPNf, t2, t3, t4, t5, t6, t7, t8, t9, tM, tSB, tB and tEB) and nine MKS intervals (CC1, CC2, S2, S3, t5-t2, t8-t2, t8-t4 and tEB-tSB). These MKS were used to track embryo development from the appearance of pronuclei to the blastulation stage. The effect of MKS on the incidence of ploidy was assessed. Additionally, the influence of these MKS on clinical pregnancy rates and ongoing and live birth rates was evaluated. Clinical pregnancy is defined as an embryo with a detectable heartbeat, typically observed by ultrasound at about 5 to 6 weeks of gestation. Ongoing pregnancy is defined as a pregnancy beyond 20 weeks of gestation. Live birth and ongoing pregnancy were analysed as a single joint variable for statistical significance.

Artificial intelligence model

An AI decision support tool (CHLOE EQ^{TM}) that evaluates early embryo development through acquired embryo time-lapse videos was used. The model incorporates a deep learning-based application that uses convolutional neural networks to automatically analyse time-lapse videos of developing embryos, retrieved from a TLI. This AI model provides embryologists with automated data-driven evaluations, including automatic annotations and predictive algorithms, to assist them in making informed decisions on the use of embryos in treatment. In the present study, the focus is on the AI capability of assessing MKS. Other variables, however, have been taken into consideration by AI, such as the generation of a single embryo quality score above and beyond morphokinetics. This embryo quality score is numerically described on a scale from 0 to 1. The corresponding embryo quality score increases as embryo quality score increases. Further details on how the embryo quality score was calculated are described in detail by *Erlich et al. (2022)*.

CHLOE embryo quality training was previously carried out using over 50,634 manually tagged embryo images from over 455 TLI videos and validated using a test set of 9511 images from 90 TLI videos. The results were assessed for detection of true positives and false positives pixel-by-pixel accuracy as related to the embryo's image. Further details on how this model was developed and trained are explained in detail by *Erlich et al., (2022)*. Data were provided by a variety of international clinics. These data represent a diverse representation of embryo culture conditions and follicular stimulation protocols, which should reduce the noise of the dataset. In the present study, none of the clinics that provided data were used for training the CHLOE embryo quality AI model.

Statistical analysis

Several dependent variables were analysed in the present study. These include MKS (tEB), embryo quality score, clinical pregnancy, ongoing pregnancy and live birth, and miscarriage (loss of clinical pregnancy between 8 and 12 weeks). For pregnancy analysis, only single embryo transfers were included. Appropriate statistical tests were selected for each dependent variable according to their statistical properties. In the case of regression analysis, patient and donor age was accounted for as potential confounding variables. For statistical analysis, continuous age was used. For illustrative purposes, age was split into the Society for Assisted Reproductive Technology age groups as previously described for tables and figures.

Multivariate linear regression was used to measure effects on tEB after verifying that the statistical assumptions required for this method were not violated. Homoscedasticity was confirmed by examining the residuals and normality of the data was confirmed by a quantile–quantile plot. With pregnancy and pregenetic testing outcomes as the dependent variables, multivariate logistic regression was used. The other dependent variables required the use of non-parametric analysis methods of Spearman's Rank Correlation and Mann–Whitney U test where appropriate. Because of the prevalence of multicollinearity between MKS, they were analysed individually rather than co-variables in a regression model. This ensures that the interaction between the MKS is not biasing the results. Correlation of all MKS with embryo quality score was measured using Spearman's rank correlation. One MKS, tEB, was selected as a representative of the effect of all MKS on embryo quality score, as it was the MKS with the strongest correlation. All statistical analysis was conducted in R Studio (Posit, Boston, MA, USA) using R version 4.3.1. (*R Core Team 2024*)

Internal review board statement

The Internal Review Board (IRB) of Instituto Bernabeu certified that the present project met the protocol's necessary suitability requirements in relation to the study's objectives (approval date 3 October 2023). The investigator's qualifications and available resources were deemed appropriate for the study, and that the procedures for obtaining informed consent were adequate. Instituto Bernabeu's IRB confirmed that the handling of study information complied with current legislation on data protection and confidentiality, as outlined in Organic Law 15/1999 on Data Protection.

RESULTS

All MKS, including intervals, were found to be significantly correlated to embryo quality score as determined by Spearman's Rank Correlation (all $P < 0.001$) (FIGURE 1A and FIGURE 1B). Because of the prevalence of multicollinearity in MKS, they were analysed individually. When analysing the effects of MKS in this study, the event with the most correlation to embryo quality score was tEB (ρ (7267) = −0.816, $P < 0.001$) (FIGURE 1A). In a similar manner, the interval between tEB and tSB was the most representative interval morphokinetic on embryo quality score (ρ (7267) = −0.624). Ultimately, tEB was selected to represent the effect of all the MKS on corresponding embryo quality scores. This suggests that as tEB increases, the embryo quality score tends to decrease.

The above results indicate an effect of MKS on the resulting embryo equality score. Therefore, additional analysis was conducted to assess the correlation between the patient factors on these MKS and the resulting embryo quality score. Effects of patient factors (patient age, oocyte fresh or frozen status and oocyte own or donor status) on resulting embryo quality scores were assessed.

Results from Spearman's rank correlation indicate a weak negative correlation between patient age and embryo quality score (ρ (7221) = −0.233, $P < 0.001$), as patient age increased, the embryo quality score tended to decrease. For example, based on continuous age analyses, the mean embryo quality score for a 28-year-

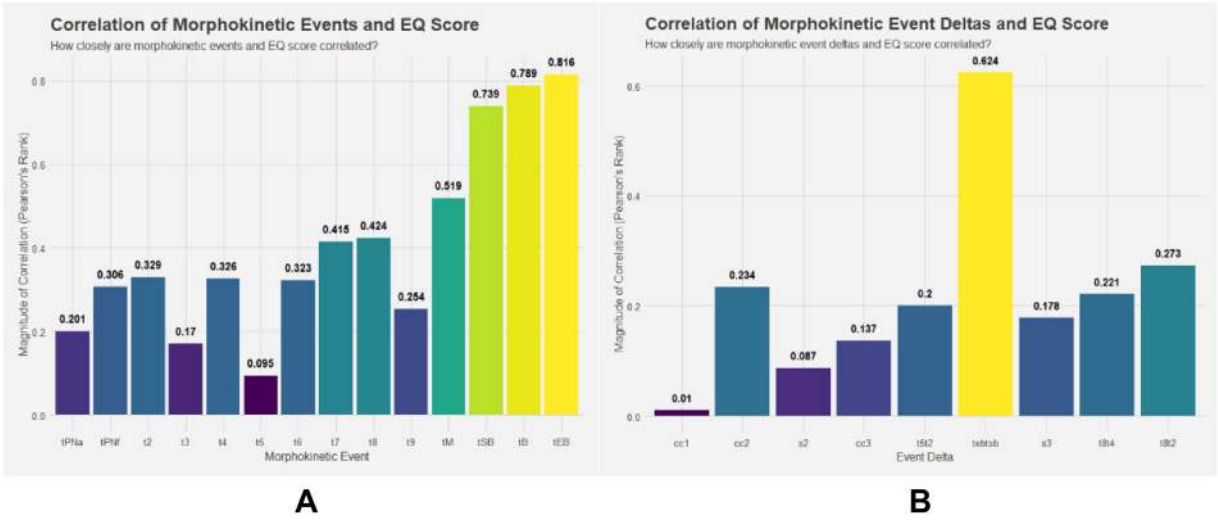

FIGURE 1 Magnitude of Spearman's rank correlation of (A) absolute values and (B) interval values on morphokinetic events and embryo quality score. Embryo quality score determined by CHLOE EQ^{TM}; (n = 6024 embryos). All correlations are negative, and tested $P < 0.001$. CC1, Cell cycle 1=t3-tPNf; CC2, Cell cycle 2=t3-t2; CC3, Cell Cycle 3=t5-t3; S2, synchrony 2= t4-t3; tB, time of blastulation; tEB, time to expanded blastulation; tM, time to morula; tPNa, time at which a pronuclei appears; tPNF, time to syngamy; tSB, time to start of blastulation; t2–t9, time to cell division.

old patient is 0.916, whereas the mean embryo quality score for a 37-year-old patient is 0.789. The simple linear regression measuring the effect of patient age on tEB was statistically significant ($P < 0.001$) as well. For each unit increase in patient age, there was a mean increase in tEB of 0.47 h (0.44–0.51). For example, the mean tEB for 28-year-old patients is 109.7 h, whereas the mean tEB for 37-year-old patients is 117.5 h. Results showing the effect of age on embryo quality score and tEB are presented in FIGURE 2A and FIGURE 2B

Data analysed via Mann–Whitney U test suggested that fresh (autologous and donor) oocytes had significantly greater embryo quality scores than frozen oocytes ($z = -4.21$, $P < 0.001$). Blastocysts resulting from fresh oocytes had a mean embryo quality score of 0.757, whereas the mean embryo quality score of blastocysts resulting from the use of frozen oocytes was 0.714. Multivariate linear regression analysis also found the oocyte's cryopreservation status was significantly correlated to tEB, with frozen oocytes representing an average increase of 2.10 h (1.41–2.79) compared with fresh oocytes ($P < 0.001$). The mean tEB of blastocysts resulting from fresh oocytes was 115.6 and 117.7 for frozen oocytes.

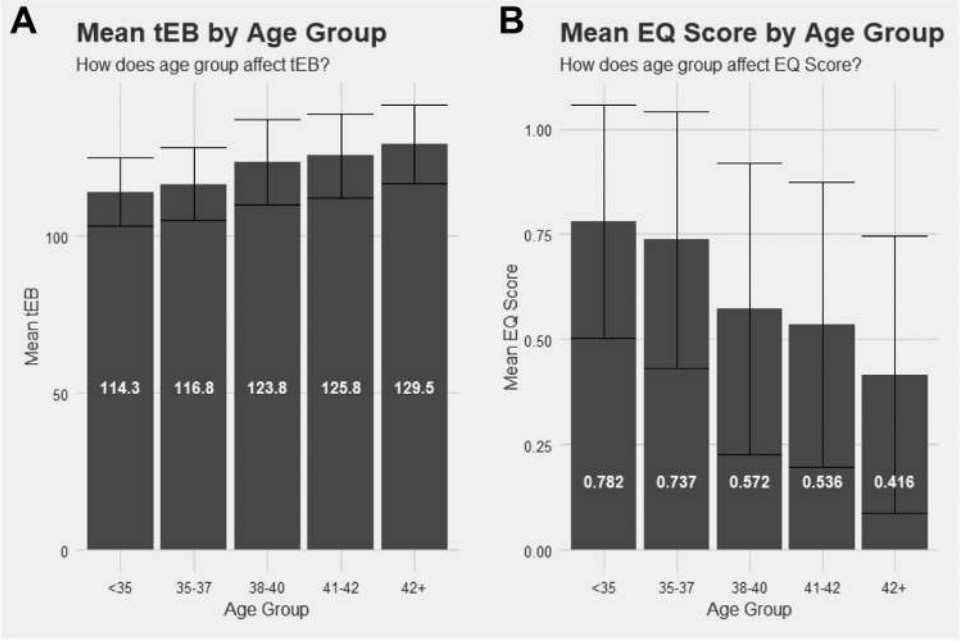

FIGURE 2 Changes in mean (A) time to expanded blastulation (tEB) and mean (B) embryo quality score as age increases. Depicted via linear regression with confidence intervals; (n = 6024 embryos). All tested $P < 0.001$. EQ score, embryo quality score.

Analysis with Mann–Whitney U test also indicated that donor oocytes (fresh and frozen) had significantly greater embryo quality scores than autologous oocytes, ($z = -16.24$, $P < 0.001$) Specifically, blastocysts originating from own oocytes presented a mean embryo quality score of 0.671 and donor oocytes of 0.799. According to multivariate linear regression, autologous oocytes were also significantly associated with an average increase in tEB of 6.08 h (5.55, 6.62) compared with the use of donor oocytes ($P < 0.001$). The mean tEB for use of autologous oocytes was 119.7 h compared with 113.6 h for donor oocytes. Results for these variables are presented in TABLE 1.

Looking at a subset of transferred embryos ($n = 1355$), the effect of patient factors and the correlation with tEB and embryo quality score on clinical pregnancy outcomes was determined. The multivariate logistic regression model of embryo quality score on the likelihood of achieving a clinical pregnancy was statistically significant ($P < 0.001$). For a one unit increase in embryo quality score, the odds of achieving clinical pregnancy increased by 21.7% (15.3–29%). For example, an embryo with an embryo quality score of 0.6 has a predicted probability of achieving clinical pregnancy of 36%, whereas an embryo with an embryo quality score of 0.9 has a predicted probability of 50% (FIGURE 4A). A similar result was found when evaluating ongoing pregnancy and live birth. For a one unit increase in embryo quality score, the odds of achieving ongoing pregnancy and or live birth increased by 18.5% (11.9–26%) ($P < 0.001$). In a similar manner, an embryo with an embryo quality score of 0.6 has a predicted probability of achieving live birth of 35%, whereas an embryo with a score of 0.9 has a predicted probability of 39% (FIGURE 4B). Upon further analysis, no cor relation was found between tEB and clinical pregnancies ($P = 0.371$) or live birth ($P = 0.478$) (TABLE 1).

TABLE 1 RESULTS

Morphokinetic events and embryo quality score			
Event	Rank correlation		*P*-value
tEB	−0.816		
tEB-tSB	−0.624		<0.001[a]
Mean (±SD) tEB and embryo quality score for age group			
Age group, years	Mean tEB (HPI)	Mean embryo quality score	
<35	114.25 (±8.2)	0.782 (±0.19)	Both <0.001[b]
35–37	116.76 (±9.5)	0.737 (±0.23)	
38–40	123.76 (±0.3)	0.572 (±0.28)	
41–42	125.77 (±10.0)	0.536 (±0.29)	
>42	129.50 (±10.9)	0.416 (±0.34)	
Mean (±SD) tEB and embryo quality score for female factor			
Oocyte source	Mean tEB (HPI)	Mean embryo quality score	
Fresh	115.6 (±9.2)	0.757 (±0.22)	Both <0.001[c]
Frozen	117.7 (±9.4)	0.714 (±0.25)	
Autologous	119.7 (±10.2)	0.671 (±0.26)	Both <0.001[c]
Donor	113.6 (±8.2)	0.799 (±0.19)	
Mean (±SD) tEB and embryo quality score for ploidy status			
Ploidy status	Mean tEB (HPI)	Mean embryo quality score	
Euploid	116.23 (±9.6)	0.723 (±0.23)	
Aneuploid	121.24 (±8.7)	0.667 (±0.23)	Both ≤0.001[c]
Mean (±SD) tEB and embryo quality score for clinical pregnancy			
Clinical	Mean tEB (HPI)	Mean embryo quality score	
Yes	107.42 (±5.1)	0.964 (±0.07)	
No	107.79 (±5.2)	0.951 (±0.06)	Embryo quality score <0.001[d]
Mean (±SD) tEB and embryo quality score for ongoing and live birth			
Ongoing and live birth	Mean tEB (HPI)	Mean embryo quality score	
Yes	107.42 (±5.3)	0.964 (±0.07)	
No	107.72 (±5.1)	0.953 (±0.06)	Embryo quality score <0.001[d]

Embryo quality score calculated by CHOLE EQ™.

[a] Spearman's rank correlation.

[b] Linear regression for tEB and Spearman's rank correlation for embryo quality score. Continuous age used for statistical analysis; age groups presented here for illustrative purposes. Both embryo quality score and tEB are significant at $P < 0.001$.

[c] Linear regression for tEB and Mann–Whitney U test for embryo quality score. Both embryo quality score and tEB are significant at $P \leq 0.001$.

[d] Logistic regression. Embryo quality score $P < 0.001$, tEB: clinical pregnancy $P = 0.371$; ongoing and live birth $P = 0.478$.

HPI, hours post insemination; tEB, time to expanded blastocyst; tSB, time to start blastulation.

The effect of age on pregnancy outcome and live birth was also assessed. The multivariate logistic regression model assessing the effect of patient age on the likelihood of achieving a clinical pregnancy was statistically significant ($P < 0.001$) (FIGURE 3). For a one unit increase in age, the odds of achieving clinical pregnancy decrease by 3% (1–5%). For example, a patient aged 25 years has a predicted probability of achieving clinical pregnancy of 52%, whereas a patient aged 40 years has a predicted probability of 40%. A similar result was found when evaluating live birth. For a one unit increase in age, the odds of achieving live birth decreases by 1% (1–2%) ($P = 0.002$). Specifically, a patient aged 25 years has a predicted probability of achieving live birth of 37%, whereas a patient aged 40 years has a probability of 32%. Ages are stratified into groups in FIGURE 3 as defined by SART; clinical pregnancy rate is presented in FIGURE 3A, live birth and ongoing pregnancy rates in FIGURE 3B and mean embryo quality score in FIGURE 3C. The average embryo quality score tended to decrease in all age groups as age increased, except for the group aged 41–42 ($P < 0.001$). This is most likely due to the small sample size in this age group ($n = 14$; 15 single-embryo transfers only).

The effects of oocyte status (fresh versus frozen) on the likelihood of miscarriage

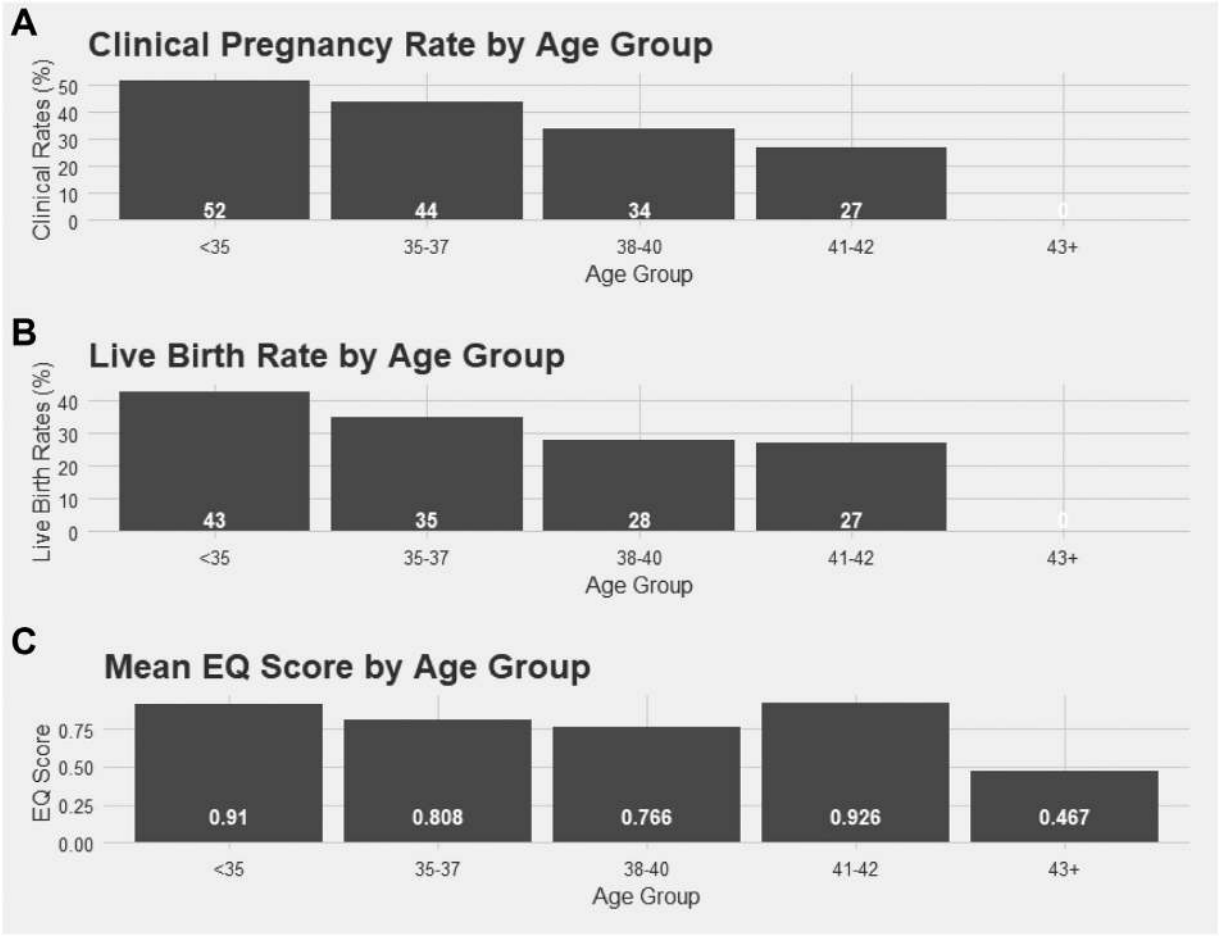

FIGURE 3 (A) Clinical pregnancy; (B) live birth rates and (C) mean embryo quality (EQ) score for transferred embryos by age group. Pregnancy rates presented as a percentage of total number of transferred embryos per group; ($n = 1355$ transferred embryos). Continuous age use for analysis: (A, B) logistic regression; (C) linear regression. All tested $P \leq 0.002$.

were also assessed. The multivariate logistic regression model was statistically significant ($P < 0.001$); compared with fresh oocytes, the odds of frozen oocytes resulting in a miscarriage increased by 24.1% (6.58–44.10%). The predicted probability of miscarriage after the use of frozen oocytes was 20%; in contrast, after the use of fresh oocytes, the predictive probability of miscarriage was 16%. The investigators, however, found no statistical differences when comparing embryo quality scores between patients that resulted in miscarriages compared with patients in the ongoing pregnancy group ($P = 0.751$).

To address the issue of miscarriage, a further analysis of 3309 embryos that underwent PGT-A was conducted. Euploid embryos were found to have significantly greater embryo quality scores than aneuploid embryos ($z = -4.76$, $P < 0.001$). The mean embryo quality score for euploid embryos was 0.723 and 0.667 for aneuploid embryos. Compared with aneuploid blastocysts with multivariate logistic regression, euploid embryos saw a decrease in tEB of 4.72 h (3.43–6.01) ($P < 0.001$), with the mean tEB for aneuploid blastocysts was 121.2 h in contrast to 116.2 h for euploid blastocysts (TABLE 1).

DISCUSSION

Morphokinetic events provide a detailed description of embryo development, resulting embryo quality and outcomes. Artificial intelligence models trained on embryo development using MKS data can be used to assess the quality of an embryo and predict the likelihood of live birth. This has been demonstrated in our previous research (*Lucio et al., 2022*). These MKS are interrelated, and the development of a competent blastocyst depends on their organized occurrence. Artificial intelligence models can automatically annotate these events during an embryo's development using TLI. Deviations from optimal MKS timings and intervals can result in lower embryo quality, decreased ploidy, implantation and pregnancy rates (*Chamayou et al., 2013*; *Desai et al., 2014*; *Karavani et al., 2020*). As a result, specific MKS and associated intervals are predictive of embryo quality, ploidy, implantation and pregnancy rates.

The use of an AI platform to assess embryo quality has the advantage of removing or reducing the inherent subjectivity noted in manual embryo grading (*Fruchter-Goldmeier et al., 2023*). It is a common fact that manual embryo grading is greatly affected by the experience and existing view of the blastocyst in question (*Richardson et al., 2015*; *Kromp et al., 2023*).

Recent research has found how patient factors can affect embryo quality, such as male and female gamete origin, and how

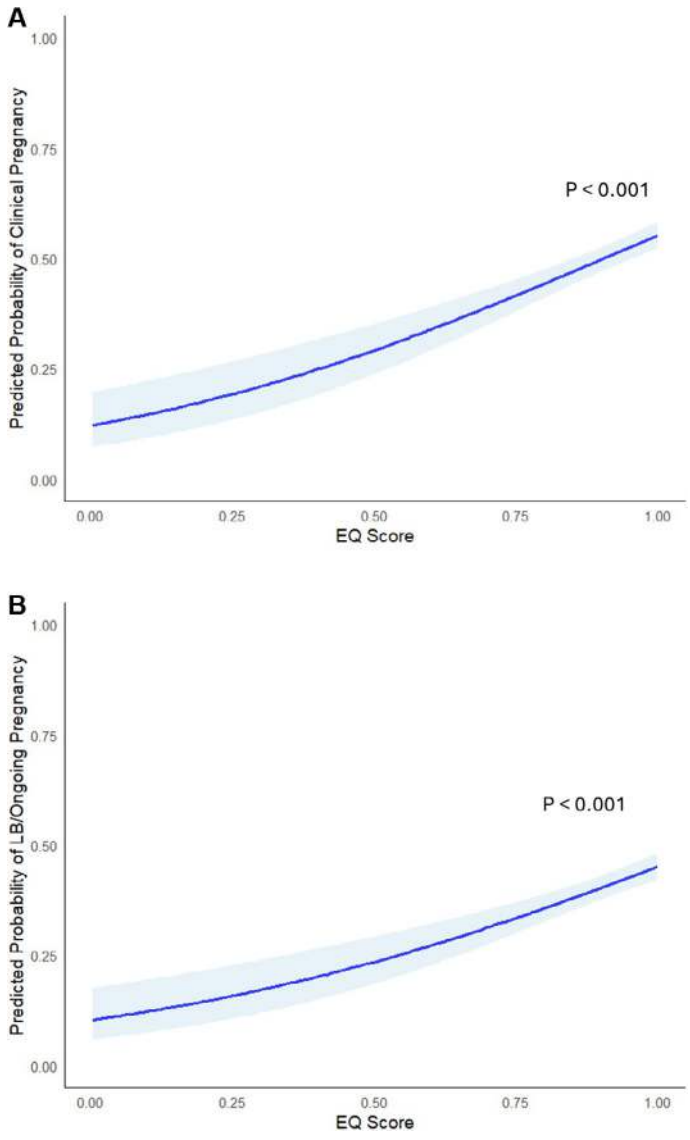

FIGURE 4 Predicted probabilities of (A) clinical and (B) live birth and ongoing pregnancy by embryo quality (EQ) score as obtained via logistic regression. Presented as a probability curve with confidence intervals; (n = 1355 transferred embryos). Both $P < 0.001$.

they correlate with certain MKS and resulting intervals (*Karavani et al., 2020*; *Karagianni et al., 2024*). Results from *Karagianni et al. (2024)* indicated that vitrified oocytes had slightly lower clinical outcomes compared with fresh oocytes (*Karagianni et al., 2024*). Findings from this study indicate that specific MKS and intervals could have high predictive value for embryo quality and likelihood of live birth.

Results from the present study demonstrate that all MKS had a significant effect on embryo quality scores (FIGURE 1). Notably, tEB was found to be the most impactful event on embryo quality score. The longer it took for an embryo to complete blastulation, the lower the resulting embryo quality score. Other investigators have also identified that events related to the time to morula and blastulation were crucial in embryo development and outcomes (*Desai et al., 2014*; *Harada et al., 2019*). *Desai et al. (2014)* observed that morphokinetics associated with morulation, start of blastulation, blastocyst formation and the time to expanded blastocyst are also key events linked with normal embryo development. These investigators also reported that embryos with slower morphokinetic development also had limited developmental potential (*Desai et al., 2014*). The function of Na/K pumps in the trophectoderm is vital for successful expansion of the blastocoele cavity (*Watson, 1992*; *Houghton et al., 2003*). These results demonstrate the importance of cohesive blastocoele formation. Previous studies have described that MKS indicate embryo quality, deviations from optimal MKS ranges significantly reduce embryo quality and the likelihood of live birth (*Chamayou et al., 2013*; *Conaghan et al., 2013*; *Desai et al., 2014*). Furthermore, it has been demonstrated that embryos that take longer to reach later MKS stages, such as t8 or blastulation, have lower embryo quality (*Karavani et al., 2020*). In the present study, results indicate that later MKS events such as tEB are critical events for the development of competent blastocysts. Results from the present study demonstrate that the AI-based embryo quality score is a summation of all MKS and, therefore, the encompassing embryo quality score is more objective at grading the embryo as a whole than using individual MKS events. This AI score represents the overall embryo development.

The effect of certain patient factors, such as age, oocyte status (fresh versus frozen) and origin of oocytes (autologous versus donor) on embryo development were analysed. The comparison of these variables was found to affect the resulting embryo quality score.

Results indicate that, as patient age increased, corresponding embryo quality score decreased. Other investigators have described that the implantation rates as well as resulting embryo quality for women over the age of 38 years was significantly lower than the resulting outcomes for patients under the age of 35 years (*Hardarson et al., 2008*; *Oron et al., 2014*; *Reig et al., 2020*). In the present study, results also indicated a negative correlation between patient age and resulting embryo quality score. These results demonstrate a clear correlation between patient age and embryo quality score.

The effect of patient age was found to be a significant variable on tEB. Other investigators have demonstrated that age affects events related to the surrounding blastocoele development after compaction (*Ezoe et al., 2023*). Results of the present study indicate that patient age affects the time for an embryo to complete the expansion process (tEB), thereby subsequently influencing the resulting embryo quality score.

The effect of oocyte cryopreservation was a significant variable on subsequent embryo quality score, regardless of oocyte origin. In the present study, it was found that fresh oocytes had significantly higher embryo quality scores than frozen oocytes. Other investigators have also demonstrated that cryopreservation may affect subsequent embryo development (*Li et al., 2022*; *Montgomery et al., 2023*). Recently, *Montgomery et al. (2023)* also reported a significant delay in early cleavage between the two- and eight-cell stage as well as time to compaction. These investigators also reported a significantly shorter compaction phase in vitrified oocytes compared with fresh controls.

A limitation of the present study is that, for statistical purposes, both donor and autologous oocytes that were cryopreserved were combined. Therefore, the effect of oocyte sources within the cryopreserved gamete pool were not separately analysed. Similarly, the analysis of donor status included fresh and frozen oocytes. The influence of factors such as age and infertility on the subsequent development rate of frozen autologous oocytes may have been affected.

Results in the present study also indicate that the use of donor oocytes resulted in higher embryo quality scores. Previous studies have also observed that embryos derived from donor oocytes were of higher quality (*Yeh et al., 2014*). The results of the present study suggest that the use of donor oocytes eliminates the effect of age and underlying causes of infertility on subsequent embryo development. Moreover, before selection, oocyte donors undergo a thorough screening process to ensure the selection of optimal oocytes, thereby minimizing patient variables that could affect subsequent development. Results from this study confirm that the use of donor oocytes is a significant variable on embryo quality. The MKS were also affected by origin of oocytes. Autologous oocytes were significantly associated with an average increase in tEB compared with the use of donor oocytes. Data presented in this study demonstrate that the origin of oocytes affects MKS that occurred after embryonic activation and are associated specifically with time to completion of blastocoele formation (tEB).

In addition to determining the effects of patient variables on resulting embryo quality scores and MKS, investigators have also evaluated the effect of fresh and frozen oocyte status on the likelihood of miscarriage rates. When compared with fresh oocytes, the odds of frozen oocytes resulting in a miscarriage increased. Furthermore, a meta-analysis from 2006 found that the use of frozen oocytes was associated with higher miscarriage rates (*Oktay et al., 2006*). *Crawford et al. (2017)*, however, reported no decrease in ongoing pregnancy when using donor frozen oocytes. In the present study, miscarriage rates may be attributable to the act of cryopreservation as previously noted by other researchers (*Crawford et al., 2017*). Additionally, the influence of the cause of patient infertility may become more apparent after cryopreservation compared with the use of screened donor oocytes.

As previously determined, the MKS that most correlated with embryo quality score was tEB. Therefore, it was important to determine the effect of patient factors and the correlation with tEB and embryo quality score on clinical pregnancy outcomes. Results indicate that the embryo quality score is predictive of clinical pregnancy. Other investigators have also previously described that embryo quality scores as derived by AI are predictive of clinical pregnancy rates (*Barnes et al., 2023*; *Weng et al., 2023*). In the present study, however, no correlation was found between tEB and clinical pregnancy. The embryo quality score is a value generated through AI, and is, therefore, a comprehensive score that encompasses all aspects of embryo development, including all MKS; the embryo quality score was correlated with clinical pregnancy.

A similar result was also found when evaluating ongoing pregnancy and live birth. Results indicate that embryo quality score can successfully be used to predict ongoing pregnancy and live birth. Furthermore, as the patient's age increases, the odds of achieving live birth decrease. Data suggest that the likelihood of live birth increases and miscarriages decreases as a corresponding embryo quality score increases. These data align with previously described AI models, indicating that resulting embryo quality scores generated through AI are predictive of live birth and miscarriage (*Amitai et al., 2022*; *Chavez-Badiola et al., 2024*). As previously described for ongoing pregnancy and live birth, tEB was also found to not be a significant MKS leading to live birth. This is not surprising as the AI core includes much more than only the time to expanded blastocyst as mentioned above.

In recent years, PGT-A has become common practice throughout current IVF clinics. Therefore, the application of AI to further select euploid embryos for transfer has become increasingly important. *Miller et al. (2024)* (in press), have demonstrated that subtle morphokinetic differences exist between euploid embryos that ultimately resulted in a live birth compared with those resulting in miscarriage. Therefore, analysis was conducted to determine if embryo quality score was predictive of ploidy status. Results demonstrated that normal embryos had significantly higher embryo quality score than abnormal embryos. Previous researchers have also indicated that embryo quality scores are indicative of ploidy status (*Gómez et al., 2022*; *Barnes et al., 2023*). Aneuploid embryos had a significant increase in tEB. It has also been described that an AI model trained on MKS from TLI data can be used to predict embryo ploidy (*Huang et al., 2021*). This suggests that some MKS are affected by ploidy status and, therefore, may have higher predictive value.

The application of AI in assisted reproduction, specifically in embryo selection, should be considered as a tool to assist the embryologist to select the best embryo for biopsy, cryopreservation and replacement. This AI tool is designed to support embryologists in clinical decision-making within the context of IVF clinics. It is intended as an aid, not a replacement, and should not be used as the sole basis for clinical decisions at this stage of its development.

The present study also underscores the importance of the application of AI in assisted reproduction. A limitation in this study, however, is that only embryos with full MKS annotations were included in this analysis. The exclusion of poor developing embryos from the cohort should be taken into consideration for the prediction of clinical outcomes. An additional limitation in the present study is the fact that this was a retrospective study that could create information or data availability biasness.

Results demonstrate that MKS alone do not predict outcomes without additional parameters and variables at play in the background analysis by AI, as demonstrated by the lack of statistical correlation between tEB and clinical pregnancy as well as with ongoing

pregnancy and live birth. The time-lapse based AI model used in this study evaluates early embryo development through acquired embryo time-lapse videos. This is a deep learning-based application that uses convolutional neural networks to automatically analyse time-lapse videos of developing embryos. In addition, this AI technology considers more than just MKS for developing the resulting embryo quality score. The resulting embryo quality score is a summation of all the morphokinetic and morphological events along with their weighted values in conjunction with specific measurements of many cytoplasmic attributes. At present, ongoing research is being conducted to further elucidate how all these MKS and developmental parameters interact with each other and can be further studied independently.

In conclusion, embryo selection based on AI has many potential applications in the IVF laboratory today. For example, the use of this technology can be used to further standardize embryo grading. The use of MKS can also be used as an additional selection criterion for the transfer of fresh embryos. Furthermore, this MKS can be used to select the best euploid embryo for transfer based on developmental history. This AI tool has the potential to revolutionize fertility treatment by enhancing the effectiveness of selection of the most viable embryos for implantation and live birth.

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AUTHORS' ROLES

KW, PP and AZ: conceptualization, methodology, project administration, writing the original draft and editing; KK: data curation, methodology, formal analysis, software and writing the original draft; TL: data curation, software and formal analysis; JT and MCT: conceptualization, investigation, writing the original draft and reviewing; AM: investigation and writing the original draft; JG, AR-A, ND, MH, AB and RB: investigation, resources, software and reviewing; AB and CH: conceptualization, project administration, and reviewing.

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