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The Importance of Time of ICSI (T0) In Time-Lapse Embryo Imaging

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Abstract

When a time-lapse system is installed in an IVF lab, embryologists must decide the time of starting a time-lapse project (t0) as there are no recommendations about t0 and the implications of setting t0 are still unknown. The aim of our study was to analyse the impact of using different settings for the time of ICSI (t0) on the value of the biomarker as well as on Machine Learning algorithms' performance. Four morphokinetic biomarkers were analysed on 220 embryos included in the study: 117 Live-Birth embryos and 103 aneuploid embryos. Different t0 configurations were compared in this analysis: t0 relativized for each oocyte; mid-time ICSI t0; t0 relativized on extrusion of the second Polar Body; and t0 relativized on the Pronuclear Fading. The analysis consisted of a calculus of the mean, standard deviation and the min-max range for each biomarker, outcome group and t0 configuration. Furthermore, a Machine Learning algorithm (XGB) was performed for each t0 configuration through V-Fold Cross-Validation and the Confusion Matrix's results were obtained. Biomarkers' mean values were very similar between t0 relativized for all oocytes and mid-time ICSI. Machine Learning XGB accuracy was 5.5% lower for mid-time ICSI compared with relativized t0 (66.5% vs 72.0%, respectively). Nonetheless, after removing long-time ICSI procedures, mid-time ICSI model accuracy improved by 2.4% while the models based on the other t0 configurations obtained decreased accuracies. Relativizing t0 for each oocyte could lead to better time-lapse predictive models' performance especially when the ICSI procedure lasted more than 45 minutes.

Keywords

Artificial Intelligence, time-lapse, Machine Learning, morphokinetics, time of ICSI.

Graphical abstract

Time of ICSI (t0) and its implication on Machine Learning morphokinetic algorithm' accuracy

BACKGROUND

When a time-lapse system is installed in an IVF lab, embryologists must decide the time of starting a time-lapse project (t0) as there are no recommendations about t0 and the implications of setting t0 are still unknown.

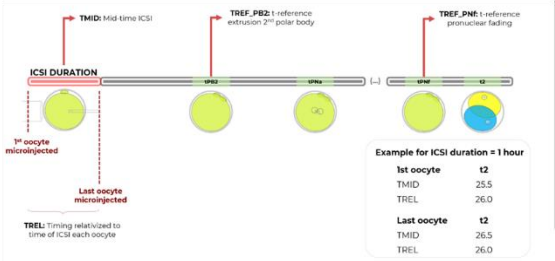
AIM OF THE STUDY

Analyse the impact of using different settings for the time of ICSI (t0) on the value of the biomarker as well as on Machine Learning (ML) algorithms' performance.

DESIGN

Four morphokinetic biomarkers (t3, t5 and their respective nuclear fading prior to cleavage) were analysed on 220 embryos.

Different t0 configurations were compared in this analysis: Mean comparison, min-max range and ML: eXtreme Gradient Boosting (XGB)



RESULTS

Classical statistics did not show differences neither when working with mean values nor when evaluating min-max values, with the exception of the minimum value for LB embryos, where the difference of that value was ~0.5h between TREL and TMID.

The Machine Learning XGB accuracy was 5.5% lower for mid-time ICSI compared with relativized t0 (66.5% vs 72.0%, respectively). After removing long-time ICSI procedures, mid-time ICSI model accuracy improved by 2.4%

CONCLUSION: Relativizing t0 for each oocyte could lead to better time-lapse predictive models' performance especially when the ICSI procedure lasted more than 45 minutes.

Introduction

In the last decade, time-lapse imaging in Embryology has been widely widespread in in vitro fertilization (IVF) laboratories [1, 2, 3], leading to numerous studies about morphokinetic [4, 5, 6] and morphodynamic biomarkers [7, 8, 9] and their association with the genetic status [10, 11, 12], blastocyst development [13, 14, 15] and implantation potential [16, 17, 18, 19, 20]. The Live-Birth potential has also been studied [21, 22, 23, 24, 25] although the selection of non-implanted embryos as the contrary reference to Live-Birth embryos (LB) could negatively impact the algorithms' performance due to misclassification [26]. A highly variable agreement was found when performing different algorithms [27] and it has not been possible to establish any generalized predictive algorithm yet [28]. For this reason, the construction of each centre's own algorithms has been recommended [29, 30, 31, 32, 33, 34].

When a time-lapse system (TLS) is introduced and installed in the IVF lab, the provider company usually gives the technical information in order to make it work properly, such as equipment maintenance, gas supply requirements or the software associated with the TLS. However, no information is given about the time of starting a time-lapse project (t0) and embryologists are responsible for deciding when to set t0. This point is used as a start time for the morphokinetic biomarkers (t-times) measured in hours post insemination/injection [35]. In the proposed guidelines for time-lapse monitoring [35], t0 was defined as the time at which insemination occurs in conventional IVF, and as the time of sperm injection per oocyte (TREL). In case of impossibility to record t0 to each oocyte, t0 was proposed as the mid-time point from when injection began and ended for the

patient's cohort of oocytes (TMID). In some clinics, t0 is recorded as the time of the first oocyte injected and in other clinics t0 is recorded as the time of the last oocyte injected [36]. Previous studies comparing t0 for conventional IVF and ICSI showed a lag in conventional IVF biomarkers as the sperm could need a certain time to reach to the oolemma and trigger fertilization, while in ICSI the sperm was already laid into the cytoplasm. These differences can be avoided by standardizing on a specific t-time (t-reference, TREF) such as pronuclear fading [37], although the timing between t0 and t-reference time would be wasted. The multiple possibilities for t0 are schematized in Figure 1. Nevertheless, the implications of the different options for t0 on the biomarkers and on the predictive models based on biomarkers are still unknown.

For this reason, the aim of our study was to analyse the impact of using different settings for the time of ICSI (t0) on the value of morphokinetic biomarkers as well as on the Machine Learning (ML) algorithm's performance.

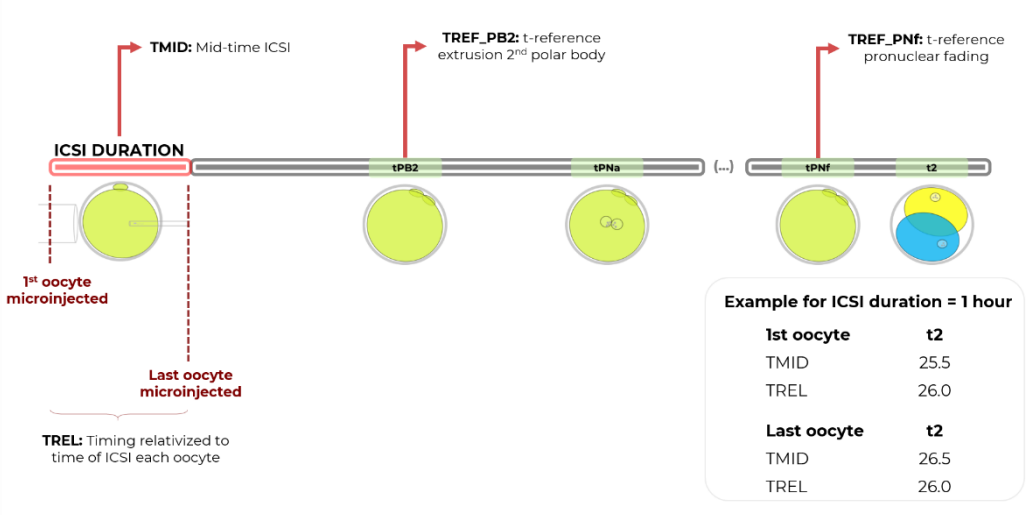


Figure 1. Scheme of the different options for t0 and an example of the values obtained for the morphokinetic biomarker "t2" for two kinetically identical oocytes but with different time of injection in a 1-hour ICSI procedure.

Materials and Methods

Study Population

This project was a retrospective observational study comprising 220 embryos from 117 IVF cycles, carried out by the ART Consultancy CONSULTFIV from Valls (Tarragona, Spain) with data collected from embryos cultured at Institut Conceptum IVF lab in Reus (Tarragona, Spain). Baseline characteristics are shown in Supplementary Table 1. This study and its protocol were approved by the Ethics Committee of Hospital Clinic de Barcelona (reference HCB/2021/1006).

Morphokinetic analysis

Manually annotation of morphokinetic biomarkers was performed on Primo Vision Analyzer software. "Start time" of ICSI was registered at the time of injection of the first oocyte and the time of injection of each oocyte (t0-ICSI) was registered in the excel spreadsheet exported from the software. "End time" of ICSI was also registered after the injection of the last oocyte. The unit of morphokinetic parameters (MKP) was hours post-insemination (hpi) and the unit of morphokinetic intervals was hours (h). With this data, two datasets were parallelly created depending on the chosen t0: t0 Mid-time ICSI (TMID) was obtained by subtracting the half of "End time" value to each biomarker value, while t0 relative per oocyte (TREL) was estimated by subtracting the value of t0-ICSI to each t-time value. In this project, a total amount of 6 biomarkers were essential to be registered. Time of extrusion of second polar body (tPB2) and time of pronuclear fading (tPNf) were used for obtaining two standardised datasets by subtracting the value of the reference timing to each morphokinetic value (TREF_tPB2 and TREF_tPNf, respectively) as displayed in Figure 1. Four more biomarkers were also registered per embryo: t3, t5, the time of fading of the nucleus of the cell cleaving to t3 (tNf Cc2a) **[**38**]** and the time of fading of the nucleus of the cell cleaving to t5 (tNf Cc3), as depicted in Figure 2.

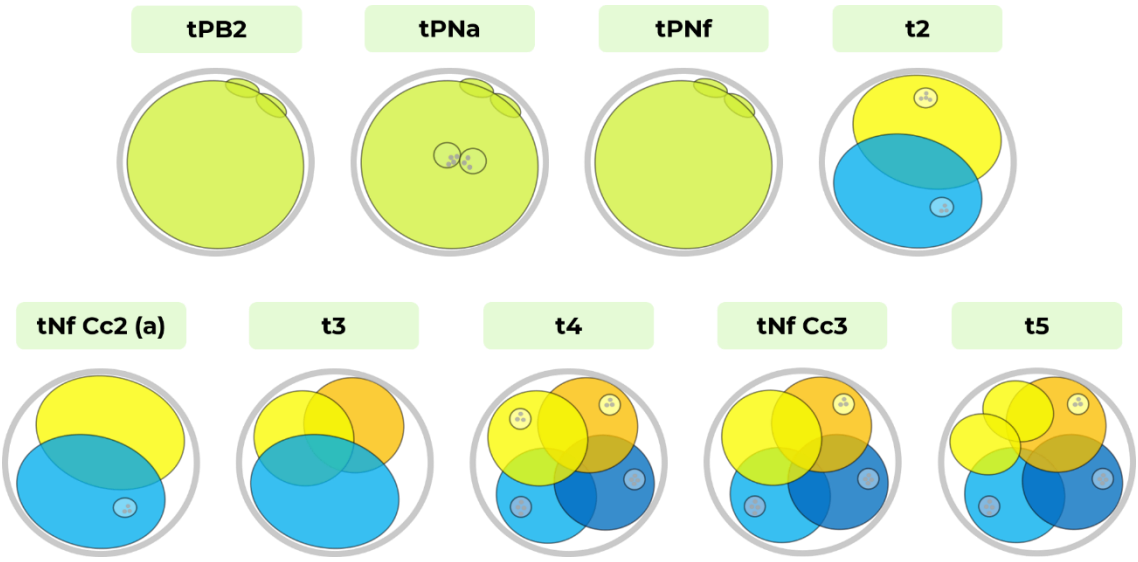


Figure 2. Diagram of morphokinetic biomarkers annotated in this project. tPNa, t2 and t4 were not needed to be registered but are included in this diagram for facilitating its comprehension.

Experimental Design

The study consisted on comparing the effect of different t0 configurations (TREL, TMID, TREF_tPB2, TREF_tPNF, as shown in Figure 1) on the values of morphokinetic parameters (tNf Cc2a, t3, tNf Cc3, t5) depending on the known outcome of the embryo (Live-Birth or Aneuploid) and also on the performance of a Machine Learning algorithm using morphokinetic parameters for predicting the known outcome of the embryo (Live-Birth or Aneuploid) **[**26**]**. There were included a total of 220 analysed embryos, 117 were Live-Birth embryos and 103 were aneuploid embryos after PGT-A of a single blastomere at Day 3. Embryos without a known outcome result (Live-Birth or aneuploid) or with unreported morphokinetic parameters were excluded from the project. The analysis was compounded by 3 sections:

(i) The mean and standard deviation (SD) was computed for each studied MKP, outcome of the embryo and t0 configuration. T-student test or Mann-Whitney U-test was performed when suitable for the outcome variable comparison (LB vs aneuploid) for each biomarker and t0 setting. Two-sided P-values of 0.05 or less were considered to indicate statistical significance.

(ii) The minimum and maximum value was listed for each MKP, outcome of the embryo and t0 configuration. Furthermore, differential for minimum and maximum values for each studied MKP and outcome of the embryo was computed as TREL-TMID.

(iii) A Machine Learning algorithm **[**39**]** corresponding to eXtreme Gradient Boosting (XGB) was trained and v-fold cross-validated by 80% train set and 20% validation set **[**40**]**, with the same 4 MKP as input variables and outcome of the embryo as output variable. The algorithm was repeated 10 times for each t0 configuration and all confusion matrix's metrics were recorded, focusing on the accuracy (%).

The third section was expanded by repeating all the ML performance with an additional condition: embryos belonging to ICSI procedures lasting more than 45 minutes were considered as "Long-time ICSI" and were excluded from the datasets before running the ML algorithm.

Results

Aneuploid embryos registered higher lagging in all morphokinetic parameters for each t0 setting ($p<0.05$) as presented in Table 1. Despite there were no statistical tests applied between t0 configurations, values for each MKP for the same embryo outcome between TREL and TMID were very similar, registering +0.1h in TREL with respect to TMID.

	TREL	TREL	TMID	TMID	TREF_tPB2	TREF_tPB2	TREF_tPNf	TREF_tPNf
Biomarkers hpi	Aneuploid	LB	Aneuploid	LB	Aneuploid	LB	Aneuploid	LB
tNf Cc2 mean (SD)	36.09 (4.39)*	33.31 (3.46)*	35.99 (4.39)*	33.22 (3.44)*	32.01 (4.09)*	30.62 (3.34)*	13.19 (3.07)*	12.06 (1.32)*
t3 mean (SD)	37.98 (4.50)*	35.24 (3.48)*	37.89 (4.49)*	35.14 (3.47)*	34.84 (4.16)*	32.54 (3.37)	15.09 (3.17)*	13.99 (1.43)*
tNf Cc3 mean (SD)	50.75 (6.50)*	47.08 (5.01)*	50.65 (6.51)*	46.99 (5.00)*	47.71 (6.21)*	44.41 (4.92)	27.85 (5.01)*	25.83 (3.10)*
t5 mean (SD)	52.55 (6.61)*	48.79 (5.10)*	52.46 (6.62)*	48.69 (5.09)*	49.51 (6.30)*	46.12 (5.01)*	29.65 (5.17)*	27.54 (3.22)*
hpi = hours post-insemination; SD = Standard Deviation, LB = Live-Birth * All pairwise comparison between Aneuploid and LB for each biomarker and t0 configuration was statistically significant $p<0.05$								

Table 1. Mean and SD results for each t0 setting

The minimum and maximum values analysis (Table 2) obtained concordant results with the mean comparison, finding less than 0.2h of difference between the minimum and maximum values for each MKP in the comparison of TREL versus TMID. However, the differential for the minimum value for each MKP belonging to LB category was near 0.5h between TREL and TMID (Table 3).

	TREL	TREL	TMID	TMID	TREF_tPB2	TREF_tPB2	TREF_tPNf	TREF_tPNf
	Aneuploid	LB	Aneuploid	LB	Aneuploid	LB	Aneuploid	LB
tNf Cc2	29.47 - 62.82	26.47 - 45.48	29.55 - 62.69	25.93 - 45.62	24.98 - 53.05	23.72 - 43.48	10.42 - 38.63	8.77 - 18.33
t3	31.80 - 65.63	28.15 - 47.07	31.88 - 65.51	27.68 - 47.20	26.90 - 55.87	25.47 - 45.07	11.90 - 41.45	10.53 - 19.92
tNf Cc3	40.38 - 82.12	36.22 - 68.72	40.45 - 82.33	35.68 - 68.85	36.16 - 77.70	33.47 - 66.72	21.47 - 56.37	19.95 - 41.57
t5	42.43 - 83.95	37.55 - 69.87	42.36 - 84.17	37.02 - 70.00	38.16 - 79.53	34.80 - 67.87	23.13 - 59.03	21.03 - 42.72

Table 2. Min-Max values for each analysed t0 setting and biomarker (hpi min - hpi max)

	Min value Differential	Max value Differential	Min value Differential	Max value Differential
	Aneuploid	Aneuploid	LB	LB
tNf Cc2	-0,08	0,13	0,47	-0,14
t3	-0,08	0,12	0,47	-0,13
tNf Cc3	-0,07	-0,21	0,54	-0,13
t5	0,07	-0,22	0,53	-0,13

Table 3. Differential for minimum and maximum values between TREL and TMID.

The Machine Learning XGB accuracy was 5.5% lower for mid-time ICSI compared with relativized t0 (66.5% vs 72.0%, respectively). After removing long-time ICSI procedures, mid-time ICSI model accuracy improved by 2.4% while the models based on the other t0 configurations obtained decreased accuracies (Figure 3).

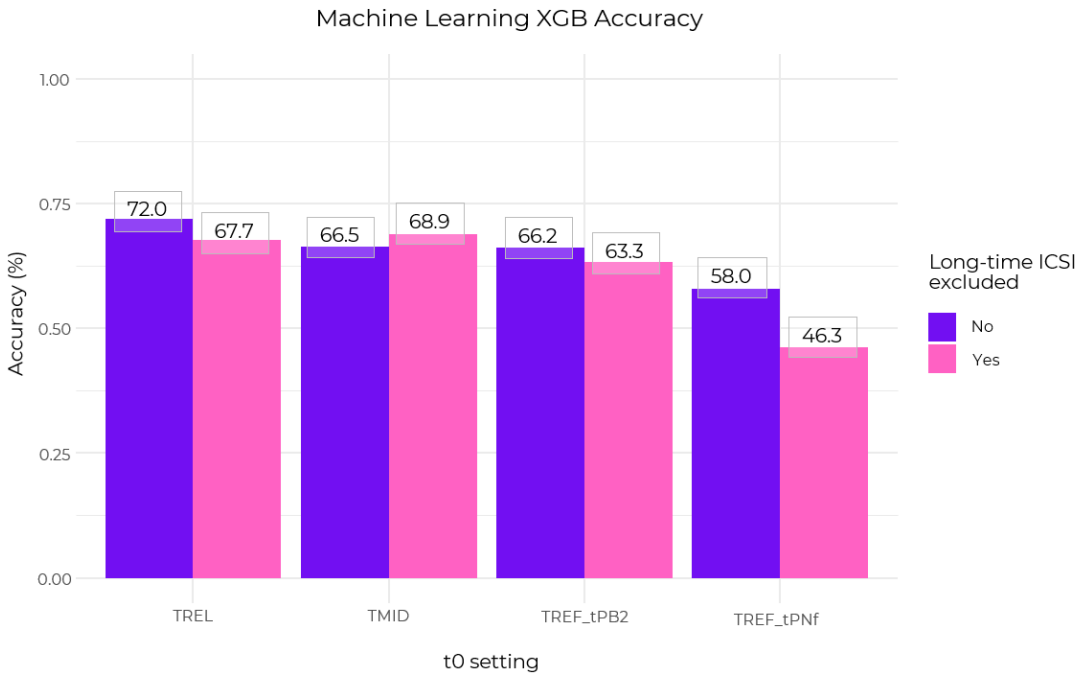


Figure 3. Machine Learning XGB accuracy for the prediction of Live-Birth versus aneuploid for each t0 setting. Violet bars belong to full datasets while pink bars belong to datasets excluding long-time ICSI procedures (>45 minutes)

XGB: eXtreme Gradient Boosting

Discussion

Despite the use of time-lapse imaging on embryos has been routinized during the last decade, the manual annotation has been suspicious of human bias, mainly for those events more linked to subjectivity such as pronuclear appearance or features after 8-cell stage **[**41**]**. According to this, automation of annotation has been proposed to overcome this issue **[**42**]**. Nevertheless, the time of ICSI could be affecting both manual and automated annotation as the magnitude for the value of an event is, by definition, hours post insemination when it is not relativized for another specific t-time.

When analysing morphokinetics, we split the embryos into LB and aneuploid categories with the purpose of avoiding the risk of masking detectable differences when analysed together. Moreover, comparing LB and aneuploid could involve lower Label Noise than other comparisons **[**26**]**. Our results confirmed the developmental delay of aneuploid embryos **[**43**]**, which could be detected in all t0 configurations.

Classical statistics did not show differences either when working with mean values nor when evaluating min-max values, with the exception of the minimum value for LB embryos, where the difference of that value was ~0.5h between TREL and TMID. This could be explained as these timings belonged to a fast cleaving embryo which was microinjected during the first rounds of a long-time ICSI procedure. Consequently, MKP values in TMID showed that a LB embryo could reach t5 in 37.0h while, in fact, it reached t5 in 37.5h in TREL. Our results suggested that TREL could be the t0 configuration with the most reduced dispersion between the annotated time and the real time, while TMID could involve a certain number of embryos unproperly parameterized.

The Machine Learning performance comparison exposed that the patterns used for embryo classification by the eXtreme Gradient Boosting were sensitive to the time of ICSI. According to this, relativizing each MKP to the time of ICSI of each oocyte could lead to better ML accuracy as the value of each MKP could be closer to the real value rather than in other t0 configurations. Nevertheless, the duration of the ICSI procedure could also play a role in the t0 impact. When long-time ICSI procedures (>45 minutes) were excluded from the analysis, TMID's accuracy was improved suggesting that the patterns observed by Machine Learning algorithms could be sensitive to those decimal points that were inaccurately given/withdrawn to the embryo when using mid-time t0. Embryos microinjected in the middle of the ICSI procedure would not have any t0 impact in TMID as TREL and TMID would converge in this scenario. Conversely, the removal of long-time ICSI procedures had a negative effect on the algorithm's performance, probably due to the decreased sample size (-39 LB, -34 aneuploid).

ICSI duration could depend on the IVF lab protocols, embryologist skills or semen sample characteristics. Recording each t0 by round of ICSI could be more accurate than using TMID and despite it could be seen as a tedious practice, once routinized it became easy if the start time of the project is the start time of the ICSI procedure and a t0 can be recorded for each oocyte in the TL platform or in the exported spreadsheet.

Thus, if we desired to get the best results when building predictive models using morphokinetics or even to export and extrapolate morphokinetic biomarkers to other IVF labs, t0 should be considered for the aforementioned implications. The alternative to TMID and TREL could be using other events for relativizing MKP. In this study, we tried relativization with time of 2nd polar body and time of pronuclear fading, despite we obtained poorer results, mainly when t-times were relativized with pronuclear fading. In our case, this could suggest that the timing of the first cell cycle **[**44**]** could play a role in detecting patterns associated with aneuploidy or viability that could be wasted when the MKP was relativized several hours later.

In conclusion, relativizing t0 for each oocyte could lead to better time-lapse predictive models' performance especially when the ICSI procedure lasted more than 45 minutes.

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