Use of time-lapse technology on fertilization verification, embryo evaluation, and utilization: A national survey in Japan

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BACKGROUND: Time-lapse technology (TLT) has emerged as a significant advancement in the field of assisted reproductive technology (ART), providing continuous observation of embryos. However, limited information exists on the adoption of TLT across ART facilities and the clinical implications of its application in embryo evaluation and fertilization verification. The existing literature has not yet comprehensively examined how TLT data are utilized to optimize ART outcomes, particularly in Japan, where ART practices are highly prevalent.

OBJECTIVES: This study aimed to investigate the adoption rate of TLT and its clinical effects on fertilization verification, embryo evaluation, and utilization of ART in Japan.

STUDY DESIGN: An online survey was conducted from December 23, 2022, to January 16, 2023, in 616 ART facilities *with both email and mailed notices*. The survey investigated the utilization of TLT in each facility's evaluation of oocyte morphology, fertilization, embryo culture, and morphology.

RESULTS: Overall, 345 responses were analyzed. Of these, only 42.6% confirmed fertilization at 16 to 18 hours after insemination. Most facilities defined normally fertilized eggs as 2 pronuclei (2PN; 53.3%) or a combination of a second polar body extrusion and 2PN (44.9%). Overall, 54.6% of the facilities had adopted TLT, and 76.9% to 96.9% of these facilities used TLT images for fertilization verification. At these centers, the use of 0PN embryos decreased, whereas the use of 2.1PN embryos increased. The rates of culture medium supplemented with antioxidants and hyaluronan were significantly higher in facilities with TLT than in those without TLT. TLT images were used for embryo evaluation in 94.3% of the facilities, while 31.0% used a combination of TLT images and artificial intelligence-based scoring systems.

CONCLUSIONS: While TLT use is widespread in Japan, its application in evaluating fertilization and embryo development stages varies across facilities. Reaching a consensus on the optimal use of the TLT system will enhance the effectiveness, safety, and efficiency of ARTs.

Key words: add-on, assisted reproductive technology, time-lapse technology, fertilization verification, embryo selection, embryo transfer

Introduction

Fertilized eggs are sensitive to culture environment, with factors such as pH,[1,2] temperature,[3] oxygen concentration,[4] and oxidative stress[5] implicated in embryonic development. In conventional in vitro fertilization (cIVF), variability in practices regarding the timing and frequency of observations, ranging from day 2 to day 7 after insemination, is expected. Frequent observation of embryos for morphological evaluation outside the incubator negatively affects embryonic development. A time-lapse technology (TLT)-equipped incubator, which has an in-built microscope and camera, is expected to enable embryo culturing under stable and uninterrupted conditions.[6] Furthermore, consecutive observation using TLT facilitates the accurate assessment of the development speed and duration of the cell cycle in embryos. TLT reveals novel events of unknown biological significance that were not observed in previous fixed-point observations, such as two evenly sized pronuclei (PN) plus one smaller PN (microPN),[7] direct cleavage,[8] perivitelline threads,[9] fertilization cones, and cytoplasmic waves.[10] These embryonic developmental behaviors, known as morphokinetics, are reportedly useful for embryo selection in single-embryo transfer.[11] In addition, TLT images have been recently used for artificial intelligence education and training in embryo assessment, and some studies have reported the efficacy of artificial intelligence for embryo selection.[12,13]

TLT has also benefited from routine work. It allows clinicians to perform retrospective embryo observations, especially regarding the timing of fertilization checks, which is a critical step in IVF treatment. Although the Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of

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The authors report no conflict of interest.

This research did not receive any specific grants from funding agencies in the public, commercial, or not-for-profit sectors.

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2666-5778/$36.00

http://dx.doi.org/10.1016/j.xagr.2024.100397

AJOG Global Reports at a Glance

Why was the study conducted?

To assess the adoption rate of time-lapse technology (TLT) and its clinical effects on fertilization verification, embryo evaluation, and utilization of assisted reproductive technology in Japan.

Key findings

TLT was adopted in 54.6% of facilities in Japan.
At the facilities using time-lapse images for fertilization verification, the use of 0 pronuclei (0PN) embryos decreased, whereas the use of 2.1PN embryos increased.

What does this add to what is known?

Establishing a consensus regarding how and when to evaluate fertilization after insemination using a TLT will contribute to a better understanding of the regulatory mechanisms of embryogenesis, as well as to the effectiveness, safety, and efficiency of assisted reproductive technologies.

Embryology recommend assessing normal fertilization at 16 to 18 hours postinsemination,[14] a recent retrospective, multicenter analysis showed that the optimal time to assess fertilization for oocytes cultured in standard incubation is 16.5±0.5 h postinsemination. This suggests that the current consensus requires modification to minimize the chances of missing fertilization.[15] Therefore, determining how and when to evaluate fertilization after insemination is vital. Although the ESHRE guidelines state that only 2PN embryos should be used for embryo transfer,[16] the handling of these embryos has not been clearly defined. To ensure the effectiveness and safety of ART, it is necessary to clarify the scientific significance of these events and the current practices related to embryo utilization. Accordingly, the Japanese Society for Reproductive Medicine's Academic Committee on Oocyte and Embryo Development and the Embryology Special Interest Group conducted a fact-finding survey on the actual adoption rate of TLT and the utilization of recorded images, with the aim of establishing a consensus regarding oocyte, fertilization, and embryo development for ART.

Materials and Methods

Ethical approval

The ethical exemption was granted by the Institutional Review Board of Fukushima Medical University in accordance with the national legislation under the "Ethical Guidelines for Life Science and Medical Research." This reassures that there was ethical oversight and that the conclusion on the "waiver" was not self-judged, even if the reasons given are correct. The respondents provided their consent to publish the results of the fact-finding survey by answering these questions. All procedures were performed according to relevant guidelines and regulations.

Methods and timelines for conducting the survey

All ART facilities registered with the Japan Society of Obstetrics and Gynecology (n=616) were recruited for this survey, and a Google questionnaire was used. On December 22, 2022, a notice regarding the fact-finding survey was sent via regular mail and email. The survey started on December 23, 2022, with a response deadline of January 16, 2023. Corrections were made for facility closing/consolidation (5 facilities), cessation of IVF treatment (three facilities), and additions/new openings (three facilities). Ultimately, 616 facilities were included.

Questionnaire content

The questionnaire comprised the following sections: oocyte morphology evaluation, fertilization evaluation, embryo culture evaluation, embryo morphology evaluation, and other evaluations.

Statistical analyses

Descriptive statistical analysis was performed for representative indicators from the information collected for the entire surveyed facility. Open-ended responses were provided if the facility selected "other" in response to the multiple-choice questions. Wherever possible, these responses were recoded to fit one of the original response alternatives, and additional categories were created for group responses. The response percentages for each question were calculated by excluding missing data. Data analysis was performed using JMP software (SAS, Cary, NC).

Results

Gathering information

Duplicate responses (4 facilities) were removed from the 353 responses obtained, resulting in 345 valid responses. Of the 271 facilities that did not consent to this survey, the final response rate was 56.0% (345/616). One respondent per facility was included. Among the respondents, 87.0% (300/345) were embryologists, 11.3% (39/345) were physicians, and 1.7% (6/345) were other clinicians (Figure 1, A).

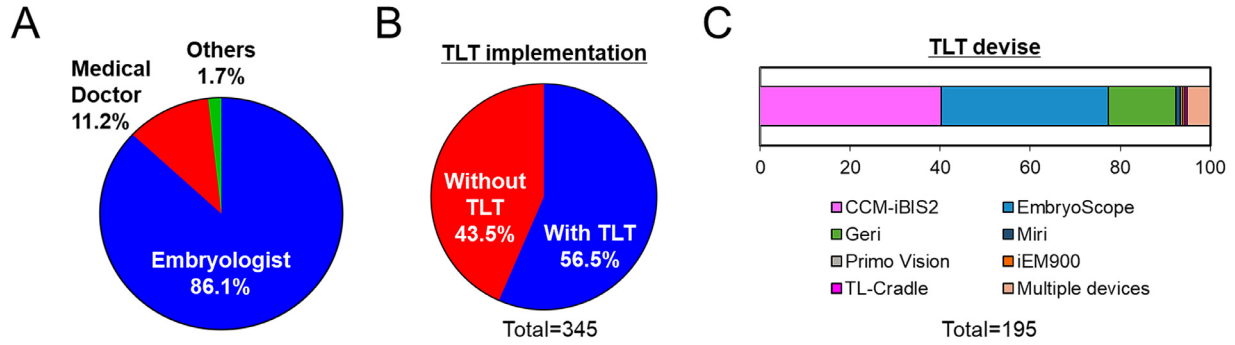
TLT adoption

Next, we investigated the status of TLT adoption and utilization. The adoption rate of TLT per facility was 56.5% (195/345; Figure 1, B). The majority of those with only one type of system had either the CCM-iBIS (Astec, 40.2%), EmbryoScope (Vitrolife, 37.1%), or Geri (Merk, 14.9%) system, with the remainder having at least one Miri TL, Primo Vision, iEM900, or TL-Cradle. Approximately 5.2% of the facilities had adopted two types of TLT devices (Figure 1, C).

Utilization of TLT images in fertilization check

Although some facilities had adopted TLT, 23.1% (45/195) of them did not use TLT images for evaluating insemination in cIVF (Figure 2, A). This frequency decreased to 3.1% (6/195) during the intracytoplasmic sperm injection (ICSI) cycles (Figure 2, B). Furthermore, the utilization rate of TLT images for fertilization verification

FIGURE 1
Adaption of time-lapse technologies (TLT)



(A) Percentage of questionnaire respondents. (B) Percentage of facilities that have adopted TLT. (C) Percentage of TLTs used at each facility.

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varied among TLT devices (Supplementary Table 1). Compared with Geri, the utilization rate after ICSI was significantly higher in the facilities where EmbryoScope was introduced.

Effects of introducing TLT in fertilization check

In most facilities that adopted TLT, the fertilization check was retrospectively performed using time-lapse sequences. Therefore, the survey at the time of the fertilization check was conducted only in the facilities without TLT. Among 143 facilities without TLT, most (42.6%; 61/143) checked for successful fertilization at 16 to 18 hours after insemination, and only 39.9% (57/143) checked for fertilization at 18 to 20 hours (Figure 2, C). Overall, 4.8% (7/143) checked before 15 hours and 52.4% (75/143) after 19 hours (Figure 2, C).

Most facilities (98.3%, 339/345) defined 2PN (53.3%, 184/345) or a combination of second-polar body extrusion and 2PN (44.9%, 155/345) as normally fertilized oocytes (Figure 2, D). However, 0PN zygotes were used in more than half of the facilities for cIVF cases (60.0%, 207/345, Figure 2, E) and intracytoplasmic sperm injection (ICSI; 56.2%, 194/345, Figure 2, F) for embryo transfer. 1PN zygotes were used in both cases of cIVF (80.9%, 279/345) and ICSI (74.5%, 257/345) for embryo transfer. 2PN with micro PN zygotes, referred to as 2.1PN, were utilized in approximately 50% of the facilities for cIVF cases (174/345) and in 52% of the facilities for ICSI cases (180/345) for embryo transfer (Figure 2, E and F).

The introduction of TLT did not influence the fertilization criteria; most facilities defined 2PN as normally fertilized oocytes (Figure 2G). However, the utilization of abnormally fertilized oocytes differed significantly between institutes with and without TLT (Figures 2, H and I). The utilization of 0PN zygotes was reduced by the introduction of TLT in both the cIVF and ICSI cycles ($p<.0001$). Conversely, the use of 2.1PN zygotes significantly increased with the introduction of TLT in both the cIVF and ICSI cycles ($p=.0207$ and $p=.0019$, respectively).

Effects of introducing TLT in culture medium

The introduction rates of the antioxidant-supplemented medium, granulocyte-macrophage colony-stimulating factor-supplemented medium, and hyaluronic acid-supplemented medium were 23.4% (81/345), 14.5% (50/345), and 57.1% (197/345), respectively (Table 1). Additionally, 33.6% of facilities did not introduce these types of media.

IVF add-ons, such as TLT, have been incorporated in various ways to improve IVF outcomes, including protection against oxidative stress and supporting implantation. Such efforts are particularly common in private clinics without bed facilities compared with other facilities in Japan, including university hospitals.[17] Therefore, we investigated the actual conditions of the culture media that could be used in conjunction with TLT. The introduction rates of antioxidant-supplemented medium and hyaluronic acid-supplemented medium were significantly higher in institutes with TLT than in those without ($p = 0.0352$ and $p = 0.0194$, respectively; Table 1). The basic medium was used more frequently in institutes without TLT ($p = 0.0039$).

Utilization of TLT images in embryo evaluation

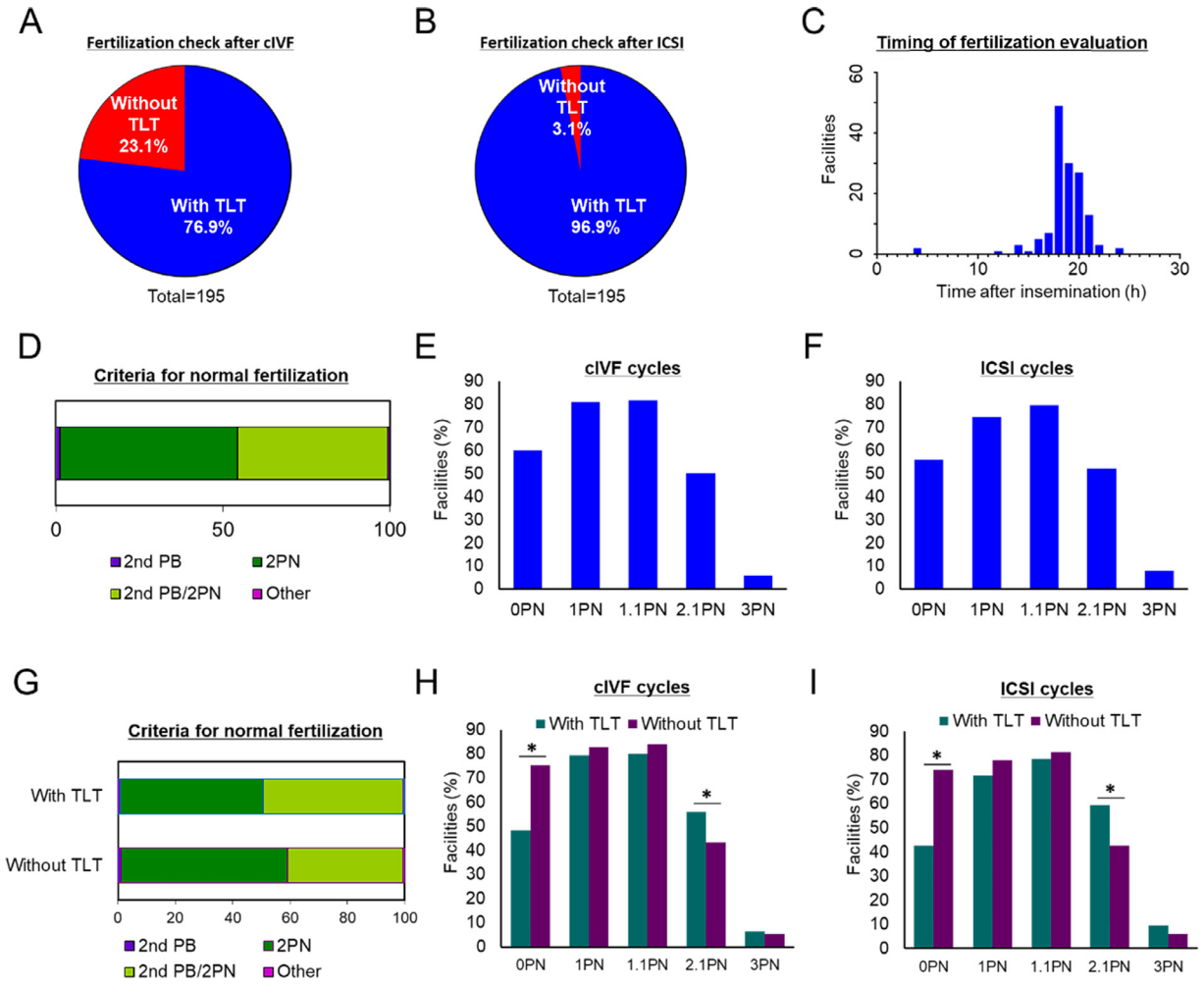
TLT images were used for embryo evaluation in 94.3% (184/195) of the facilities. Embryos were evaluated at 57 facilities (31.0%) using a combination of TLT images and artificial intelligence-based scoring systems.

Criteria for embryo evaluation and cryopreservation at the cleavage stage

Most facilities used Veeck's criteria for embryo evaluation and cryopreservation criteria at the cleavage stage (Table 2). The blastomere number, blastomere uniformity, and degree of fragmentation were frequently observed to assess the embryo quality.

The implementation rate of Veeck's criteria was significantly higher in institutes without TLT ($p=.0375$). In contrast, in institutes with TLT, the proportion of in-house grading systems

FIGURE 2
Introduction of time-lapse technologies (TLT) alters utilization of abnormally fertilized eggs



Percentage of facilities utilizing TLT to confirm fertilization for (A) conventional in vitro fertilization (cIVF) and (B) intracytoplasmic sperm injection (ICSI). (C) Number of facilities stratified by timing of fertilization evaluation. (D) Criteria for normal fertilization. Percentage of facilities transferring embryos developed from anomalous fertilizations in (E) cIVF and (F) ICSI. (G) Criteria for fertilization with or without TLT. Percentage per facility using abnormally fertilized eggs for (H) cIVF and (I) ICSI with or without TLT.

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TABLE 1
Type of embryo culture medium used in institutes with or without time-lapse technology

Supplements	Total (n=345)	With time-lapse technology (n=195)	Without time-lapse technology (n=150)	*p* value[a]
Antioxidants, n (%)	81 (23.4)	54 (27.7)	27 (18.0)	.0352
Granulocyte macrophage colony-stimulating factor, n (%)	50 (14.5)	31 (15.9)	19 (12.7)	.3981
Hyaluronic acid, n (%)	197 (57.1)	122 (62.6)	75 (50.0)	.0194
None of these, n (%)	116 (33.6)	53 (27.2)	63 (42.0)	.0039

[a] Comparison between facilities with time-lapse technology and those without time-lapse technology.

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TABLE 2
Criteria for embryo evaluation and cryopreservation at the cleavage stage

	Total (n=345)	With time-lapse technology (n=195)	Without time-lapse technology (n=150)	*p* value[a]
Embryo evaluation				
Veeck criteria, n (%)	314 (91.0)	172 (88.2)	142 (94.7)	.0375
Istanbul consensus, n (%)	20 (5.8)	14 (7.2)	6 (4.0)	.2103
In-house grading systems, n (%)	67 (19.4)	52 (26.7)	15 (10.0)	.0001
KIDScore, n (%)	2 (0.6)	2 (1.0)	–	
Others, n (%)	2 (0.6)	2 (1.0)	–	
Observation point				
No. of blastomeres, n (%)	319 (92.5)	179 (91.8)	140 (93.3)	.5915
Size of blastomeres, n (%)	139 (40.3)	75 (38.5)	64 (42.7)	.4299
Uniformity of blastomeres, n (%)	298 (86.4)	161 (82.6)	137 (91.3)	.0186
Presence of fragments, n (%)	220 (63.8)	114 (58.5)	106 (70.7)	.0194
Degree of fragments, n (%)	310 (89.9)	171 (87.7)	139 (92.7)	.1293
Morphokinetics, including abnormal cleavage, n (%)	215 (62.3)	161 (82.6)	54 (36.0)	<.0001
Presence of multinucleated blastomeres, n (%)	105 (30.4)	61 (31.3)	44 (29.3)	.6966
Features of cytoplasm, n (%)	1 (0.3)	1 (0.5)	–	
Criteria for cryopreservation				
Not perform cryopreservation at the cleavage stage, n (%)	20 (5.8)	11 (5.6)	9 (6.0)	.8875
Veeck criteria, n (%)	283 (87.1)	154 (83.7)	129 (91.5)	.0379
Istanbul consensus, n (%)	19 (5.8)	13 (7.1)	6 (4.3)	.2846
In-house grading systems, n (%)	75 (23.1)	57 (31.0)	18 (12.8)	.0001
KIDScore, n (%)	2 (0.6)	2 (1.1)	–	
Others, n (%)	2 (0.6)	2 (1.0)	–	
Physician's decision, n (%)	2 (0.6)	1 (0.5)	1 (0.7)	.8498
No criteria, n (%)	6 (1.8)	2 (1.1)	4 (2.8)	.2455

[a] Comparison between facilities with time-lapse technology and those without time-lapse technology.

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was significantly higher than that in institutes without TLT (p=.0001, Table 2). A similar trend was observed for the cryopreservation criteria (Table 2). Regarding the observation points used to determine embryo grade, both types of institutes focused on the number of blastomeres and degree of fragmentation. Furthermore, blastomere uniformity and the presence of fragments were used more frequently in institutes without TLT than in institutes with TLT (p=.0186 and p=.0194, respectively). Morphokinetics was more often used as an evaluation factor in institutes with TLT (p=.0001). The evaluation of multinucleation was comparable between both types of institutes.

Criteria for embryo evaluation and cryopreservation at the blastocyst stage

Most facilities used Gardner's classification for the embryo evaluation and cryopreservation criteria at the blastocyst stage (Table 3). The number of cells in the trophectoderm was frequently observed to evaluate the blastocyst quality.

The embryo evaluation method was comparable across the institutes, and Gardner's classification was used most frequently in institutes regardless of the application of TLT (Table 3). A similar trend was observed for the cryopreservation criteria (Table 3). The observation timepoints were similar among the institutes. However, institutes with TLT focused more on the number of cells in the trophectoderm and the morphokinetics of blastocyst evaluation (p=.0038 and p<.0001, respectively).

Discussion

We conducted a survey of ART facilities in Japan to investigate the adoption and utilization rates of TLT. We found that 54.6% of the facilities had adopted TLT.

TABLE 3
Criteria for embryo evaluation and cryopreservation at the blastocyst stage

	Total (n=345)	With time-lapse technology (n=195)	Without time-lapse technology (n=150)	*p* value[a]
Embryo evaluation				
Gardner's classification, n (%)	334 (96.8)	187 (95.9)	147 (98.0)	.2705
In-house grading systems, n (%)	43 (12.5)	25 (12.8)	18 (12.0)	.8191
iDAScore, n (%)	6 (1.7)	6 (3.1)	–	
KIDScore, n (%)	4 (1.2)	4 (2.1)	–	
Observation point				
Size of blastocysts, n (%)	239 (69.3)	139 (71.3)	100 (66.7)	.3570
Size of blastocoel, n (%)	170 (49.3)	91 (46.7)	79 (52.7)	.2691
Presence of hatching, n (%)	115 (33.3)	58 (29.7)	57 (38.0)	.1068
Size of inner cell mass, n (%)	253 (73.3)	147 (75.4)	106 (70.7)	.3259
No. of cells in inner cell mass, n (%)	252 (73.0)	142 (72.8)	110 (73.3)	.9153
Density of inner cell mass, n (%)	243 (70.4)	139 (71.3)	104 (69.3)	.6942
Uniformity of cells in inner cell mass, n (%)	137 (39.7)	75 (38.5)	62 (41.3)	.5889
No. of cells in trophectoderm, n (%)	316 (91.6)	186 (95.4)	130 (86.7)	.0038
Density of cells in trophectoderm, n (%)	254 (73.6)	147 (75.4)	107 (71.3)	.3973
Uniformity of cells in trophectoderm, n (%)	200 (58.0)	111 (56.9)	89 (59.3)	.6530
Presence of fragments, n (%)	133 (38.6)	68 (34.9)	65 (43.3)	.1094
Degree of fragments, n (%)	128 (37.1)	71 (36.4)	57 (38.0)	.7619
Morphokinetics, n (%)	230 (66.7)	155 (79.5)	75 (50.0)	<.0001
Morphology of the oocyte, n (%)	1 (0.3)	–	1 (0.7)	
Criteria for cryopreservation				
Gardner's classification, n (%)	323 (93.6)	179 (91.8)	144 (96.0)	.1130
In-house grading systems, n (%)	64 (18.6)	42 (21.5)	22 (14.7)	.1036
iDAScore n (%)	3 (0.9)	3 (1.5)	–	
KIDScore, n (%)	2 (0.6)	2 (1.0)	–	
Physician's decision, n (%)	2 (0.6)	1 (0.5)	1 (0.7)	.8520
No criteria, n (%)	4 (1.2)	1 (0.5)	3 (2.0)	.2008

[a] Comparison between facilities with time-lapse technology and those without time-lapse technology.

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However, in the case of cIVF, approximately 23% of the facilities did not use recorded TLT images for the evaluation of fertilization. Furthermore, embryos with abnormal fertilization were often used for embryo transfer, except for 3PN, even in institutions that had adopted TLT. These findings suggest that biologically abnormal phenomena detected using TLT were not sufficiently considered for embryo selection and that consensus on the establishment of evaluation methods from data mining is lacking.

A previous study suggested that >50% of ART facilities do not have a sufficient number of embryologists relative to the number of ART cycles.[18–20] TLT can eliminate time constraints for embryo observations. Therefore, TLTs may have been implemented to address the insufficient number of embryologists and improve workflow in the laboratory.

The shortage of personnel at these centers may be related to insufficient personnel to assess the recorded image data and enable the rescue of ICSI. For cIVF, approximately 23% of the centers that adopted TLT did not use TLT images for the fertilization evaluations. Notably, few facilities did not use TLT images, even for ICSI, where cumulus cells were removed from cumulus-oocyte complexes (COCs) at the time of insemination. Although each facility

might expect a higher fertilization rate if COCs are cultured overnight during cIVF and may not be able to observe the fertilization of oocytes bound to cumulus cells, the possibility that a system cannot be established to assess images should also be considered.

In previous research, the presence of 0PN[21] or microPNs in zygotes[7] following fertilization has been documented, with a significant portion believed to result from unsuccessful fertilization. Static observations have routinely identified 0PN zygotes, even in cases where 2PN confirmation was not established during post-insemination fertilization checks. Notably, 0PN zygotes experiencing PN breakdown before the recommended static observation interval can develop further if cultured. However, the clinical utilization of 0PN zygotes varies among institutes owing to the associated risk of transferring embryos derived from anomalous fertilizations.[22,23] The advent of TLT has revolutionized the clinical landscape, allowing continuous observation of PN appearance and disappearance. In our study, despite the risk of transferring embryos derived from 1PN or 3PN zygotes,[22] approximately 75% of the institutes without TLT still employed 0PN zygotes. Although successful pregnancies and live births have been reported from embryos derived from 0PN zygotes,[21,24–26] a cautious approach recommends preimplantation genetic testing for aneuploidy (PGT-A) for 0PN-derived embryos to mitigate the risk of hydatidiform mole formation.[24,27] The clinical efficacy of PGT-A, although questioned in systematic reviews,[28] aligns with efforts to enhance fertility outcomes, as recognized by the Human Fertilization and Embryology Authority.[29] In Japan, cytogenetic validation using PGT-A is primarily limited to clinical studies. Despite a decrease in the use of 0PN zygotes to 40% to 50% with the introduction of TLT, it is noteworthy that TLT alone does not conclusively confirm the existence of 0PN fertilization.[22,23] Hence, further investigation is warranted to understand cases where 0PN-derived embryos are transferred despite TLT observation.

The implementation of TLT resulted in a notable reduction in the utilization of 0PN zygotes, coupled with a notable increase in the utilization of 2.1PN embryos. Given the dynamic changes in pronuclear dimensions over time, a continuous monitoring approach is important. In laboratory settings without TLT integration, embryos with microPNs are at risk of misclassification as 3PN entities owing to the inherent difficulty of confirming the origin of microPNs. In contrast, the adoption of TLT allows for precise discrimination of the presence of microPNs within an embryo, thereby warranting consideration as a viable candidate for subsequent embryo transfer procedures.

The type of embryo culture medium varied between institutes regardless of TLT adoption. Institutes adopting TLT preferred to use media supplemented with antioxidants or hyaluronic acid, while the number of laboratories where the simplified medium was used was higher in institutes that did not employ TLT. This could be because of the differences in laboratory budgets or varying levels of attention to embryo culture practices among the institutes. Further research is required to determine the selection method for the embryo culture medium and its association with the introduction of TLT.

Since the publication of Veeck's criteria and Gardner's classification, these methods have been used for embryo evaluation at the cleavage and blastocyst stages in Japan. In institutes without TLT, static observation is performed to evaluate embryo quality according to Veeck's criteria or Gardner's classification. Therefore, researchers have focused on the factors associated with each evaluation criterion during embryo evaluation. Conversely, institutes with TLT have emphasized morphokinetics, including the appropriate time intervals for cell division, blastulation, and expansion, as well as abnormal biological events. Furthermore, the number of institutes that have introduced embryo evaluation systems using TLT and artificial intelligence has recently increased (31.0%); thus, novel criteria for embryo evaluation should be developed. Among artificial intelligence tools, the intelligence data analysis score (63.2%) had the highest utilization rate (Supplementary Table 1). Future research should regularly survey embryo evaluation methods.

Study limitations

This study had some limitations. First, because the survey was open-ended, it may have included respondents who were not TLT professionals. Additionally, facilities with existing TLT tend to increase the number of TLTs, making it challenging to determine the full impact across the entire facility. However, the response rate may have limited the impact of this bias on the analysis. Second, it is difficult to infer why some respondents did not reply to postal reminders in addition to emails. Since the postal address is publicly available, we believe that communication-related bias was limited. Third, in Japan, participation in advanced studies is required for performing PGT-A, and embryo evaluation alone may not be sufficient due to strict regulations. Finally, since the survey items did not include questions about the type of culture media (sequential or single-step media), the type of culture media may be a confounding factor in this analysis.

Conclusions

More than half of the ART facilities in Japan have introduced TLT; however, some of these facilities did not use the recorded TLT images to evaluate either fertilization or the embryos. Our study shows that even the facilities with TLT often transfer embryos developed from anomalous fertilizations, except those with multipronuclear oocytes. The technology enabling continuous observation of embryos has enabled the effective utilization of fertilized eggs that would otherwise have been discarded. Achieving a consensus on the optimal use of the TLT system and the management of abnormal embryos will contribute to a better understanding of the regulatory mechanisms of embryogenesis, as well as to the effectiveness, safety, and efficiency of ARTs. ■

CRediT authorship contribution statement

Mitsutoshi Yamada: Writing – original draft, Validation, Supervision, Project administration, Investigation, Formal analysis, Data curation, Conceptualization. **Kenji Ezoe:** Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Satoshi Ueno:** Writing – original draft, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Osamu Yoshino:** Supervision. **Toshifumi Takahashi:** Supervision.

Acknowledgments

We thank all registered facilities for their cooperation in responding to the survey. We would also like to express our sincere gratitude to the Japanese Society of Reproductive Medicine (JSRM) for their generous support in conducting the survey. We would like to thank Editage (www.editage.jp) for English language editing.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.xagr.2024.100397.

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