# Appendix: KIDScore™ D3

Appendix: KIDScore D3, version 2.0

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# 1 Introduction

This appendix provides information on the KIDScore D3 model. The appendix should be used in conjunction with the user manual for the KIDScore decision support tool.

KIDScore D3 is defined by Vitrolife based on the knowledge and experience extracted from our available KID data (see the user manual for the EmbryoViewer software for a definition of KID data).

The model focuses on which embryos to avoid rather than which embryos to select. It is thus a model that is based on avoidance criteria rather than selection criteria. The model will apply the avoidance criteria to the embryos and assign a low score to the embryos with the statistically lowest probability of implanting and a higher score to the embryos with a statistically higher probability of implanting.

## 1.1 Important restrictions and warnings

All users of the tool must agree to read and understand this user manual, observe the restrictions on use and read the following warnings.

Users should contact Vitrolife immediately to report any incident and/or injury to a patient, operator or maintenance employee that occurred as a direct or indirect result of operating the tool and associated hardware. Any serious incident that has occurred in relation to the tool should be reported to the competent authority of the Member State in which the user is established.

| **RESTRICTIONS ON USE** |
| --- |
| • All rights in the KIDScore models belong to Vitrolife. Your installation and use of a model are subject to the terms of the end-user license agreement.<br>• You may not copy, modify, decompile, reverse engineer, disassemble or convert a KIDScore model or assign, transfer, sell, rent or lease a model to any third party. |

| **WARNING** |
| --- |
| • KIDScore models may only be used by clinic personnel who have been properly trained in their function and applicability by Vitrolife. Users must be qualified to operate the tool and qualified to perform procedures associated with tool use in accordance with local qualification standards. |

| **WARNING** |
| --- |
| • The KIDScore model assigns a score to each embryo. The embryos with the lowest scores have the statistically poorest chance of implanting, and the embryos with the highest scores have the statistically best chance of implanting. However, this does not necessarily imply that the embryo with the highest score is the one most suitable for transfer. The decision about which embryo(s) to transfer must always be made by the user after an assessment of the quality of all relevant embryos. |

| **WARNING** |
| --- |
| • KIDScore models may not be used for any other purpose than intended and specified by Vitrolife as such usage may result in incorrect decisions being made by the embryologist. |

## 1.2 General cybersecurity recommendations

Users are advised and expected to take the following measures to reduce cybersecurity risk in order to ensure that the device will work as designed in the intended user environment:

- Ensure that personnel are properly trained in cybersecurity awareness
- Prevent physical access to the equipment by unauthorized users
- Use strong passwords (at least eight characters including both uppercase and lowercase letters, numbers and at least one special character).

Users must inform Vitrolife A/S without any undue delay upon becoming aware of a cybersecurity vulnerability incident or any suspected security events.

For details about how to reduce cybersecurity risk, please refer to the separate guide on this subject provided by Vitrolife.

## 1.3 Intended use

KIDScore D3 is a decision support tool intended to be used together with a time-lapse incubator as part of fertility treatment.

## 1.4 Indications for use

The KIDScore D3 tool provides decision support for prediction of the likelihood of embryos developing to the blastocyst stage by scoring them according to their statistical viability.

Adjunctive information provided by KIDScore D3 aids in the selection of embryo(s) for either transfer on day 3, freezing or continued embryo development when, following morphological assessment on day 3, there are multiple embryos deemed suitable for transfer or freezing.

The KIDScore D3 tool is only to be used with the EmbryoScope and EmbryoScope+ time-lapse incubator systems.

## 1.5 Intended users

Embryologists, other laboratory personnel and clinic staff at IVF clinics trained by Vitrolife A/S-certified instructors.

## 1.6 Clinical benefit

As an accessory to a medical device, KIDScore D3 provides the indirect clinical benefit of improving the decision-making process by providing support for selection of embryos incubated in the incubator(s) connected to the system.

# 2 What KIDScore D3 is suitable for

KIDScore D3 is defined for use in standard incubation conditions and is suitable only for:

- Treatments with day 3 transfer
- ICSI and IVF treatments
- Incubation conditions:
    - 4% reduced oxygen – 20% ambient oxygen
    - Temperature level between 36.5°C and 37.5°C (97.7°F to 99.5°F).

## 2.1 What KIDScore D3 is NOT suitable for

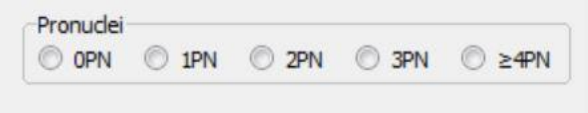
KIDScore D3 is NOT suitable for:

- Treatments where the status of the embryo is evaluated by performing a biopsy or any other disruptive embryo procedure during the culture period.

# 3 Mandatory annotation variables

As a minimum, the following variables that are used by KIDScore D3 must always be annotated when the model is used:

- **Pronuclei** (number of pronuclei):




- **tPNf** (time from insemination until pronuclei have faded)
- **t2** (time from insemination to complete division to two cells)
- **t3** (time from insemination to complete division to three cells)
- **t4** (time from insemination to complete division to four cells)
- **t5** (time from insemination to complete division to five cells)
- **t8** (time from insemination to complete division to eight cells)

  For the model to function as intended, it is important to annotate t8 if the embryo cleaves to 8 cells within 66 hours. If t8 has not been annotated, the model will instead use the number of cells at 66 hours.

If one or more of these annotations are missing for an embryo, the model cannot assign a score to that specific embryo. In such cases, the software will display the score **NA** for the embryo.

# 4 Definition of the possible scores

The model assigns the scores by comparing the embryos to the model criteria one criterion at a time until the process stops, either because the embryo did not pass one of the criteria in the sequence or because the last criterion in the model was reached.

Below is a specification of the scores that KIDScore D3 may assign to the embryos:

| Score | Description |
|---|---|
| 0 | • The embryo is not 2PN. |
| 1 | • The initial development was too fast or the embryo displayed a direct cleavage from one to three cells. |
| 2 | • The embryo was very slow to develop. |
| 3 | • Embryo development was irregular, and the development pace increased from day 2 to day 3. |
| 4 | • Embryo development was irregular, and the development pace slowed down from day 2 to day 3<br>and/or<br>• The number of cells annotated at 66 hours was not as expected. |
| 5 | • The embryo passed all of the avoidance criteria included in the model. |

**Table 1: Scores assigned by the KIDScore D3 model**

| CAUTION |
|---|
| • Embryos that have been assigned the same scores by the model are not necessarily directly comparable. The model applies only morphokinetic criteria to the embryos. Additional criteria such as morphology may also be indicative of embryo quality, which means that two equally scored embryos may still be different in some aspects. |

| NOTE |
| --- |
| • If embryos are incubated for less than 66 hours after fertilization in your clinic, only the embryos for which it is possible to annotate t8 earlier than 66 hours can be assigned the score 5. A shorter incubation time will therefore reduce the number of embryos with the highest score. |

# 5 KIDScore D3 clinical study results

## 5.1 KIDScore D3 validation study overview

The safety and effectiveness of KIDScore D3 were investigated in a prospective study. The study was performed as a single arm, multicenter clinical study conducted at six sites in the United States of America (NCT03740828). This was a non-interventional clinical study in which KIDScore D3 was not used during patient treatment. Briefly, the purpose of the study was to collect data to evaluate the safety and effectiveness of the ability of KIDScore D3 as an adjunct to morphological grading to predict which embryos are most likely to develop to the blastocyst stage. Imaging data were collected from embryos cultured to day 5. Embryologists were masked to imaging data, and evaluation was only based on morphology and KIDScore D3 scores.

This study aims at analyzing the utilization of established morphology methods with adjunct outcome of an algorithm (KIDScore D3) that provides a score (1-5) derived from timings of morphokinetic events.

## 5.2 KIDScore D3 validation study protocol

A double-blinded multicenter study designed to evaluate the odds ratios and other measures for outcomes of methodologies used for embryo assessment: Day 3 morphology alone and day 3 morphology with KIDScore D3 results as adjunct information.

### 5.2.1 Selection of study data

The data included embryos from 81 patients who had undergone blastocyst transfer cycles. The patients underwent (IVF) treatment using their own eggs or donor eggs.

These data are a subset taken from a total collection of 4,152 embryos from 1,338 treatments in which all sibling embryos have been annotated for the morphokinetic events required by KIDScore D3. These data originated from treatments that were carried out in European IVF clinics in the period 2009-2014.

A subset of the above data set was derived by taking only the treatments into consideration in which at least five embryos in total were actively dividing as the embryologist must make a choice of two embryos from an embryo cohort larger than two.

A random subset of 81 treatments that fulfilled the above criteria was selected.

For all embryos, time of blastulation (h) was recorded. If this annotation occurred within 115 hours, the embryo was considered to have formed a blastocyst. Otherwise, it was recorded as not forming a blastocyst.

All of the above-mentioned 1,338 treatments were carried out as day 5 transfers, and none of the data from these treatments were used to develop KIDScore D3. KIDScore D3 was developed solely based on day 3 transfer data.

### 5.2.2 Panelists and study phases

The six panelists in the study were all currently in practice and from a range of geographical areas within the United States of America. Four of the panelists were experienced lab directors while the remaining two were embryologists.

Each panelist reviewed the day 3 morphology data from a cohort of embryos scored by a highly experienced embryologist. Morphology was graded according to Society for Assisted Reproductive Technology (SART) embryo morphology grading reports as described further below. The data included:

- Number of cells
- Fragmentation (0%, < 10%, 10-25%, > 25%)
- Symmetry (perfect, moderately asymmetric, severely asymmetric).

In addition, the age of the patient was provided.

The data were presented to the panelists as full treatments cohorts.

The study consisted of two different parts as described below:

- **Part I**: Each panelist was supplied with the above morphological information and then evaluated:
    - Embryo category (A: Good, B: Fair+, C: Fair-, D: Poor)
    - Blastocyst prediction (blastocyst, arrested)
    - The top 2 embryos from all the treatment's embryos.

The embryologist was blinded to KIDScore outcome.

- **Part II:** Hereafter, an adjunct assessment was performed in which each panelist received the same information as in part I, with the addition of KIDScore score values (1, 2, 3, 4 or 5) and a description of the parameters and the characteristics of the embryos falling into each group. Here, the morphological assessment and the adjacent KIDScore score were used to evaluate:
    - Blastocyst prediction (blastocyst, arrested)
    - The top 2 embryos from all the treatment's embryos.

The embryologist was blinded to part I results by randomly shuffling the patients in the spreadsheets and changing the patient ID. In addition, there was a blinding period of at least two weeks between the two parts.

For both parts I and II, panelists were not provided with any information on morphology beyond the provided day 3 morphology.

The participating embryologists did not collect the morphological data. Hence, this study was not designed to consider variation among embryologists in embryo morphology data collection. They were presented with the morphological data collected beforehand by an experienced clinical embryologist who was blinded to clinic, age and KIDScore outcome. The collection of morphological data was performed based on high-resolution (500 x 500 pixels) Hoffmann contrast images taken at 66 hours in an EmbryoScope with 7 focal planes for each embryo.

## 5.3 KIDScore D3 validation study endpoints

The study has one primary endpoint and three secondary endpoints as described below.

### 5.3.1 Primary endpoint

The primary endpoint of this study was to assess the association between the adjunct prediction of blastocyst outcome and the actual blastocyst outcome. The purpose was to determine if KIDScore D3 is informative for embryos graded as A, B, or C using day 3 morphology category assignment. For those good/fair embryos, the blastocyst odds ratio (OR) for the adjunct prediction is required to be statistically significantly greater than 1. This will demonstrate that adjunctive use of KIDScore D3 leads to embryologist predictions for day 5 blastulation that are informative for outcome (i.e. blastocyst formation: Y/N).

### 5.3.2 Embryo-level diagnostic performance measures (secondary endpoint)

The specificity (proportion of embryos that did not form blastocysts that were predicted to not form blastocysts, TN/N) was calculated for morphology alone prediction and compared to the specificity for adjunct prediction.

Comparable calculations were performed for sensitivity (proportion of embryos forming blastocysts that were predicted to form blastocysts, TP/P), negative predictive value (NPV, proportion of embryos predicted to not form blastocysts that did not form blastocysts, TN/[TN+FN]) and positive predictive value (PPV, proportion of embryos predicted to form blastocysts that did so, TP/[TP+FP]). Furthermore, the negative likelihood ratio (NLR, [1 – sensitivity]/specificity) and the positive likelihood ratio (PLR, sensitivity/[1 – specificity]) were calculated.

The above measures were given both as an overall and for each panelist, assessing the performance measures without and with adjunct prediction.

### 5.3.3 Top 2 embryo analysis (secondary endpoint)

This analysis included the top 2 embryos selected by each panelist based on morphology alone. The assessment of whether or not the OR is significantly greater than 1 was carried out by the use of a generalized linear mixed model (GLMM).

### 5.3.4 Treatment-level analysis (secondary endpoint)

For the performance assessments of treatment-level analyses, the below definitions were used. These definitions correspond to a data assembly and hence differ from the definitions at embryo level.

*True positive (TP)*: 1-2 embryos from the treatment were predicted as “Blastocyst,” and one of these formed a blastocyst, or

> 2 embryos were predicted as “Blastocyst,” and at least one of the top 2 as selected by the panelist formed a blastocyst.

*False negative (FN)*: None of the embryos from the treatment were predicted as “Blastocyst,” but at least one formed a blastocyst, or

1-2 embryos were predicted as “Blastocyst,” but none formed a blastocyst, and at least one other embryo formed a blastocyst, or

> 2 embryos were predicted as “Blastocyst,” but none of the top 2 as selected by the panelist formed a blastocyst, and at least one not in the top 2 formed a blastocyst.

*False positive (FP)*: Some embryos from the treatment were predicted as “Blastocyst,” but none of the embryos from the treatment formed a blastocyst.

*True negative (TN)*: None of the embryos from the treatment were predicted as “Blastocyst,” and none formed a blastocyst.

Sensitivity and specificity derived from these data assembly definitions were calculated for all panelists and for each single panelist. This was done in order to assess the treatment-level performance measures without and with adjunct prediction.

## 5.4 Inclusion and exclusion criteria

The inclusion and exclusion criteria were limited as the study cohort was intended to reflect a typical IVF treatment.

Inclusion criteria: Women undergoing fresh in vitro treatment using own or donor eggs.

Exclusion criteria: Less than five actively dividing embryos.

# 5.5 KIDScore D3 validation study results

## 5.5.1 Primary endpoint results

The primary endpoint for the study was met with statistical significance. The overall OR for adjunct prediction was calculated by the sponsor to be 4.13 and significantly greater than 1 (95% CI: [3.48; 4.90], p < 0.0001). Thus, KIDScore D3 is informative for blastocyst outcome.

| | KIDScore D3 |
|---|---|
| Odds ratio | 4.13 |
| 95% CI | 3.48-4.90 |
| p value | < 0.0001 |

**Table 2: Odds ratios and 95% confidence intervals for KIDScore D3**

## 5.5.2 Secondary endpoint results

### 5.5.2.1 Embryo-level diagnostic performance measures

Table 3 shows the overall results of part I and part II for KIDScore D3 for embryo-level performance.

| | Part I | Part II |
|---|---|---|
| Odds ratio | 3.43 | 4.13 |
| Sensitivity | 77 | 85 |
| Specificity | 51 | 42 |
| Accuracy | 62 | 61 |
| NPV | 73 | 78 |
| PPV | 56 | 54 |
| PLR | 1.56 | 1.46 |
| NLR | 0.46 | 0.35 |

**Table 3: Overall performance of KIDScore D3 in part I and part II**

#### 5.5.2.2 Top 2 embryo analysis

This analysis includes the top 2 embryos selected by each panelist (based on part I). The KIDScore D3 score is informative for the blastocyst prediction on the subset of the top 2 embryos selected by the embryologist. The odds ratio was 16.3 with a confidence interval of 5.83-45.7 and thus significantly higher than 1.

| | Part I | Part II |
|---|---|---|
| Odds ratio | 3.64 | 16.32 |
| 95% CI | 2.03-6.54 | 5.83-45.7 |
| p value | < 0.001 | < 0.001 |

**Table 4: Odds ratios and 95% confidence intervals for the subset of top 2 embryos for KIDScore D3**

#### 5.5.2.3 Treatment-level analysis

For the treatment-level analysis, several of the embryologists had a specificity of 0%. Thus, measures such as PLR, NLR and OR that depend on specificity are limited and not meaningful.

| | Part I | Part II |
|---|---|---|
| Sensitivity | 83% | 82% |
| Sensitivity range | 76%-86% | 80%-84% |

**Table 5: Sensitivity for the treatment-level analysis for KIDScore D3**

On average, KIDScore D3 did not change the sensitivity. For KIDScore D3, the odds ratio was not significant. Thus, the benefit of KIDScore D3 cannot be said to be established on the treatment level compared with traditional morphology.

## 5.6 KIDScore D3 validation study discussion and conclusion

The above findings demonstrate that the adjunct use of KIDScore D3 improves the ability of the embryologist to select embryos for transfer compared with morphology alone. For the primary endpoint, it was shown that the adjunct use of KIDScore D3 was informative for predicting blastocyst outcome.

For the individual embryo use (secondary endpoint), it was shown that the adjunct use of KIDScore D3 compared with pure morphology increased the odds ratio from 3.43 to 4.13. The odds ratio for the adjunct use was significantly higher than 1, and thus the adjunct use was informative for the blastocyst outcome.

If only the top 2 embryos were considered (secondary endpoint), it was also shown that adjunct use of KIDScore D3 was informative for blastocyst prediction on this subset. In addition, the odds

ratio was also higher for the adjunct use of KIDScore D3 vs the use of only morphology (16.3 and 3.6, respectively). For the treatment-level analysis (secondary endpoint), it could not be demonstrated that there was a benefit of adjunct use of KIDScore D3 compared with traditional morphology.

No adverse events were encountered during the study.

# 6 Symbols and labels

| Label | Description | Note |
|---|---|---|
|  | Declaration by the manufacturer that the device meets all of the applicable requirements in the Medical Device Regulation (EU) 2017/745 | - |
|  | Medical device | - |
|  | Unique device identifier | - |
|  | Manufacturer name and address | See section 8. |

# 7 Disposal of waste

In order to minimize the waste of electrical and electronic equipment, waste must be disposed in accordance with the Directive 2012/19/EU on waste electrical and electronic equipment (WEEE) as amended by Directive (EU) 2018/849. This includes: PCBs (lead-free HASL), switches, PC batteries, printed circuit boards and external electrical cables. All components are in accordance with the RoHS 2 Directive 2011/65/EU, which states that new electrical and electronic components do not contain lead, mercury, cadmium, hexavalent chromium, polybrominated biphenyls (PBB) or polybrominated diphenyl ethers.

# 8 Contact information

Urgently need help? Call our service hotline for support:

+45 7023 0500

(available 24 hours a day, 7 days a week)

**E-mail support: support.embryoscope@vitrolife.com**

(response within two working days)

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