Consistent annotation for better evaluation – a guide on definitions for morphokinetics

Annotations constitute the base on which embryo evaluation can be performed using time-lapse monitoring in the IVF clinic.
The embryo developmental events that can be detected with time-lapse technology are immense. Events relevant for annotation ideally reflect embryonic potential in the specific clinical setting. Therefore it is important to define which events are relevant for the evaluation of embryos in your clinical setting.

Annotations should be objective and definitions should be the same across operators in order to perform meaningful evaluations.
This technote describes definitions of variables most commonly used in embryo assessment with time-lapse. These definitions will help you attain consistent annotations and thereby objective evaluations in your clinic and further streamline the understanding of embryo developmental events within the clinic and beyond the clinic.

Evaluation of embryos with KIDScore models require only few annotations, however this technote describes a more extensive selection of variables.

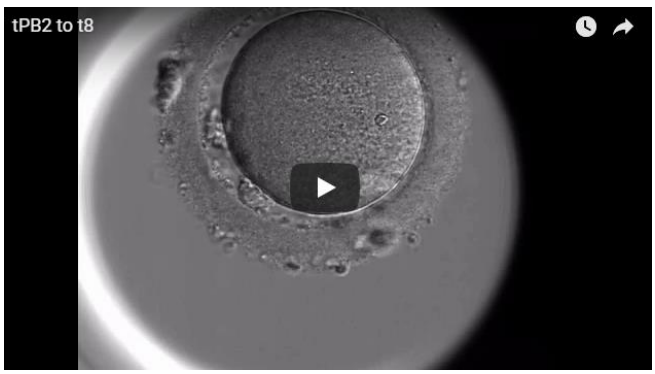
Time-lapse assessment

The first step on the way to reach consistency of annotations within a clinic is to agree on definitions of each annotated variable.
Time-lapse facilitates a more precise and objective method of embryo assessment than with static embryo monitoring. This is due to the continuous monitoring provided by time-lapse technology.

This continuous monitoring allows you to visually detect changes in embryo stages and morphology in a precise manner.

Morphokinetics – assessing embryo stages

With time-lapse the exact time that an embryo transits into a new stage can be determined with precision.
To do this, visual differences from one image to the next should be registered as annotations.
With morphokinetic variables, annotating the first time that an embryo is observed to be in a certain stage ensures a consistent and objective annotation strategy.



Annotation of fertilization events and blastomere cleavages: tB2, tPNa, tPNf, t2, t3, t4, t5, t6, t7 and t8

Variables from tPB2 to t8 represent distinct events that are detectable by differences from one image to the next.
To annotate those, the first image for which the stage is observed is annotated.
tPB2; time of extrusion of 2nd Polar Body: annotate at the first image in which the 2nd polar body is observed.
tPNa; time of ProNuclei appearance: annotate at the first image in which all pronuclei can be observed.
tPNf; time of ProNucei fading: annotate at the first image in which all pronuclei have faded.
t2-t8; time of cleavage to 2 etc cells: annotate at the first image in which a distinct separation of cell membranes can be observed, i.e. mark the exact time that the embryo progresses into another developmental stage.

The video to the left illustrates the definitions of morphokinetic variables from tPB2 to t8. View the full video at https://info.vitrolife.com/consistent-annotation-for-better-evaluation-a-guide-on-definitions-for-morphokinetics

TechNOTE

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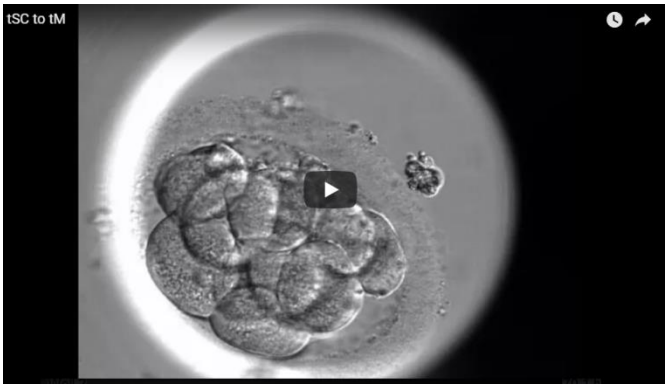
Annotation of morula and blastocyst formation

Morula and blastocyst formation are both processes that are not observed as instantaneous occurrences but rather observed as reached through gradual, subtle changes.

In order to reach consistency when annotating developmental steps during morulation and blastulation, definitions are based on distinctive features during the gradual processes.

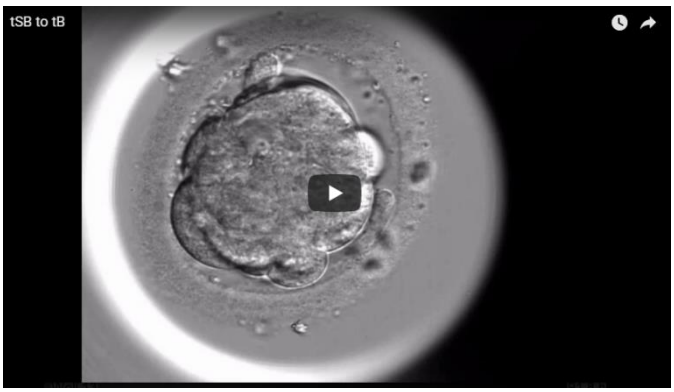
time of Starting Compaction (tSC): the first time that membranes between some of the blastomeres of the future morula are no longer distinct.

time of Morula (tM): the first image in which a compacted morula includes all the blastomeres that will later take part in the formation of the blastocyst. This solves the question of how to handle partial compactions as excluded cells can be accounted for



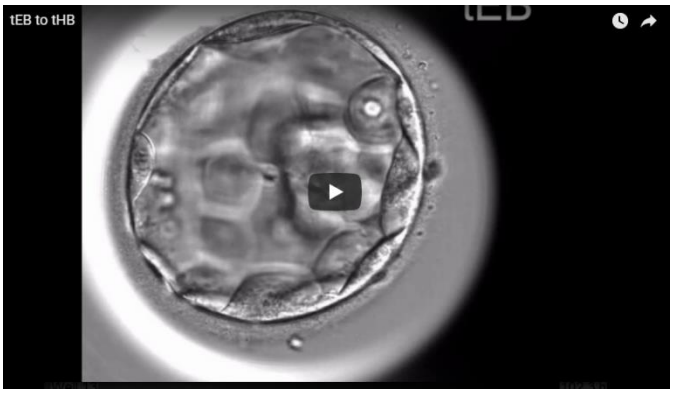
time of Starting Blastulation (tSB): the first time that a sign of cavity formation is observed. As the blastocoel cavity grows during blastulation, going back in the image sequence from a definite blastocyst stage can be helpful to attain this annotation.

time of Full Blastocyst (tB): the last image before expansion starts. This is recognised as the last image before the zona pellucida is pushed by the growing blastocyst. This is a very distinct hallmark during blastocyst development and therefore easy to annotate precisely and consistently.



time of Expanding Blastocyst (tEB): blastocyst expansion can go on for several hours and therefore a defined characteristic during this process is necessary to obtain accuracy during embryo analysis. Importantly, this should be informative on another level than previous parameters as otherwise annotation would be dispensable. Therefore, we characterize tEB as the time at which the blastocyst has expanded so much that the zona pellucida has reached half of its original thickness, which can be measured and thus represents a truly objective assessment.

time of Hatching Blastocyst (tHB): the first image at which a sign of hatching is observed.



View the full videos at https://info.vitrolife.com/consistent-annotation-for-better-evaluation-a-guide-on-definitions-for-morphokinetics

Note that for some variables precise and consistent annotation is easier if the video sequence is followed backwards in time, i.e. from a time of definite observation to the excact time of first observation.
This is especially helpful for variables which occur gradually and hence do not evoke extensive changes between consecutive images such as e.g. time of ProNuclear appearance (tPNa) and time of Starting Blastulation (tSB).
The above definitions reflect time-lapse annotations as recommended by Vitrolife and to some extend based on the definitions of Ciray et al., 2014: Hum Reprod 29(12): 2650-2660