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Which morphological scoring system is relevant in human embryo development?

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ABSTRACT

Objectives: In the past several scoring systems were proposed for early human development aiming to assist in the identification of the best embryos. Scoring criteria are usually assessed at static developmental time points by microscopy. For almost every scoring system controversial results on its benefit can be found in the literature. With the introduction of time-lapse imaging static assessment of developmental parameters needs to be revised. The objective of this study was to critical review the strategy of static assessment by using an embryo monitoring system to study time-dependent variations of scoring criteria.
Study design: Human oocytes were subjected to intracytoplasmic sperm injection and subsequently incubated in an embryo monitoring device. Images from individual oocytes were captured at given time intervals allowing a time-lapse analysis of early embryo development.
Main outcome measures: Scoring of pronuclear morphology, early cleavage and embryo morphology up to day 3 of development was performed at standard time points and compared to the morphological fate present in time intervals prior and after standard assessment.
Results: Pronuclear morphology showed a high variability within very short time intervals. First cleavage can be observed at very early time points questioning the criterion “early cleavage”. Embryo morphology can change within short time intervals and thus may be misleading if assessment is done at a static time point.
Conclusions: Scoring of early embryo development has limitations if based on static observation only. Time-lapse imaging will lead to revised scoring systems emphasizing the need for a new look on embryological parameters.

1. Introduction

Scoring of gametes and embryos has been used since the beginning of assisted reproduction, initially to characterize embryo development and later to assist in selecting embryos with a high implantation potential [1]. Obviously an embryo has to reach a certain cell number at a given day [2] and can be further characterized by its morphological appearance based on the equal or unequal size of blastomeres and the degree of fragmentation [3]. This type of scoring has been extended to all cleavage stages from the 2-cell embryo up to expanded blastocysts and is in principle still in use today. In view of the increasing demand to move towards single embryo transfer, further scoring criteria were defined and applied in numerous studies, like pronuclear morphology [4], early cleavage [5], multinucleation [6] and others.

Scoring has to be regarded as an intervention to embryo culture as the dish holding the embryos must be removed from the incubator for microscopy. This may potentially compromise embryo development due to a possible atmospheric change in temperature and pH as well as due to changes in O_2 and CO_2 tension. Therefore the time needed for any scoring event has to be restricted and usually most embryologists consider a single observation at every day of embryo culture to be acceptable for embryo development and in order to enable scoring of zygotes or embryos, although this approach is a static event.

Most studies presenting positive outcome with new scoring criteria do stimulate others to look into the nature of this type of scoring and very often the parameters applied in follow-up studies are slightly modified in order to further improve the outcome. However, for nearly every scoring criterion initially beneficial reports are followed by others which do not see an improvement.

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One example is pronuclear scoring, where numerous publications based on different approaches of weighting individual pronuclear markers clearly are in favor of this technique [4,7–14]. On the contrary, the results of other publications culminate in the conclusion that it is not justified to spend additional time for scoring zygotes if the outcome is questionable and of no benefit at all [15–19].

However, for pronuclear scoring the possible influence of the method used for fertilization as well as the time on the changing appearance of the underlying cellular markers has been shown [10,20]. This leads directly to the drawback of most scoring algorithms which are in place today, namely that scoring is done at a single time point. Early studies on kinetics in embryo development have shown, that from the beginning embryonic growth is a progressive and dynamic process [21] and that e.g. fragments may appear and disappear and thus even bias morphological classifications [22]. However, kinetic studies were not widely applied as setting up time-lapse recording without interfering with embryo development was not as easy. Recently several devices were presented which allowed for continuous monitoring of early embryo development by automated time-lapse imaging [23–27]. The availability of this technique prompted us to look into possible variations of some of the most relevant scoring criteria used in human embryology, namely pronuclear scoring, early cleavage and embryo morphology.

2. Material and methods

2.1. Oocyte retrieval, embryo culture and time-lapse imaging

Stimulation, oocyte retrieval, denudation and ICSI were performed according to standard protocols. For all steps related to embryo culture a sequential culture medium was used (gamete medium/fertilization medium/cleavage medium/blastocyst medium, Cook, Ireland). Immediately after ICSI injected oocytes were placed in individual wells within a special culture slide (EmbryoSlide, Unisense Fertilitech, Aarhus, Denmark) which was placed inside an embryo incubation and monitoring system (EmbryoScope, Unisense Fertilitech). The safety and reliability of the incubation system has been proven in a recent publication [25]. Incubation was performed in culture medium (cleavage medium, Cook) under oil at 6% CO_2 in a reduced oxygen atmosphere (5% O_2) at 37 °C and without humidification. The embryo monitoring system was programmed to sample in intervals of 20 min from every position in the slide an image stack consisting of individual images taken from seven focal planes. Following each round of image acquisition by the EmbryoScope all images and related data were transferred to a viewing station (EmbryoViewer, Unisense Fertilitech) and stored in a database. The database allowed for adding further patient related information, for viewing time-lapse images from individual growing embryos and for annotations of individual images.

2.2. Annotation of time-lapse sequences

For the annotation and analysis we used the tools implemented in the time-lapse software which allow to mark a certain event within the time-lapse sequence and to use this mark for comparison. For this study we marked and evaluated the fate of the pronuclei and the initiation of cleavage events (1 to 2 cell, 2 to 3 cell and so on). All annotations were done by one investigator and cross-checked (MM) by the other investigators (JL, MK). In case of divergent annotations these were discussed, however, there was no pronounced inter- or intra-observer variability.

2.3. Evaluation of scoring criteria

Based on time-lapse imaging the distribution of the nucleolar precursor bodies (NPB) was evaluated at 14–15, 16–18 and 19–20 h after ICSI and it was noted if it changed or not. As the study was conducted within a routine clinical program some 2PN stage oocytes were cryopreserved prior to 19–20 h and could be not evaluated at these time points. The distribution of NPB in both pronuclei was classified as asymmetric, symmetric or perfectly aligned (corresponding to patterns 1,2,4,5/OA,3 and OB presented by [10]). The time point of the first cleavage was determined and compared to the standard value defined for assessing early cleavage by a single observation at 25–26 h after ICSI [5,28]. Changes in embryo morphology and blastomere number on day 2 were assessed for a time period of 38–42 h after ICSI and changes were noted.

3. Results

3.1. Pronuclear scoring

When we analyzed pronuclear morphology based on the distribution of the nucleolar precursor bodies at 14–15, 16–18 and 19–20 h after ICSI we found that from 14–15 h to 16–18 h a similar pronuclear score was present in 75% (159/212) pronuclear stage oocytes, whereas 25% (53/212) showed a change in the pattern which resulted in another pronuclear score (Fig. 1). Due to cryopreservation of supernumerary 2PN oocytes at 16–18 h, investigating a possible change in pronuclear score from 16–18 h to 19–21 h was only possible for 65 pronuclear stage oocytes. From these, 66.2% showed at both time points a similar pronuclear score whereas 33.8% showed a change in the pronuclear score. The documented changes were mostly from an asymmetric distribution of NPB towards a symmetric or perfectly aligned distribution. The change from a symmetric to an asymmetric pattern was observed only occasionally.

3.2. Early cleavage

Precise analysis of the exact timing of the first cleavage in 59 embryos which were cultured beyond the 2PN stage revealed a range from 22 to 36 h for this event, the mean being 26.7 ± 3.2 h. According to the definition of early cleavage 57.6% (34/59) of the embryos had cleaved by 25–26 h and 42.3% (25/59) cleaved later. Regarding the optimal cleavage time of 25–28 h as proposed from time-lapse imaging [29], 27.1% (16/59) of the embryos did cleave before 25 h: 42.3% (25/59) between 25 and 28 h and 30.5% (18/59) after 28 h.

3.3. Embryo morphology on day 2/day 3

When we looked at embryo morphology and blastomere number at 38 h, 40 h and 42 h we found that within the overall time span of 4 h 49.1% (28/57) of embryos changed either morphology or blastomere number thus leading to a different score. When the same evaluation was done for the time points at 40 h and 42 h. 32.6% of the embryos would have been scored differently. The analysis revealed that the likelihood for embryo morphology changes resulting in a better or worse score from one time point to another was the same (Fig. 2).

4. Discussion

Scoring of human embryo development is mostly done at one given time point and is then considered as a representative score for that oocyte or embryo. This has been reviewed in a recent consensus paper on embryo assessment by ALPHA and ESHRE [30]. The limitations of this approach have been already discussed 16 years back [31]. Already then it was proposed to use time-lapse cinematography for a more reliable classification. Probably due to the increased use of extended culture to the blastocyst stage scoring at earlier stages of embryo development - whether static or by time-lapse - was not given a high priority. However, the discussion about multiple pregnancies and the implementation of single embryo transfer has stimulated the use of sequential scoring strategies [32–34] as a possibility to sub-select the one and single embryo which will implant at the highest rate while avoiding or reducing the risk of multiple gestation.

The availability of time-lapse monitoring of embryo development allows for analyzing scoring strategies and their potential value. Hence we took this approach to look into some prominent scoring strategies. When we analyzed changes for the different

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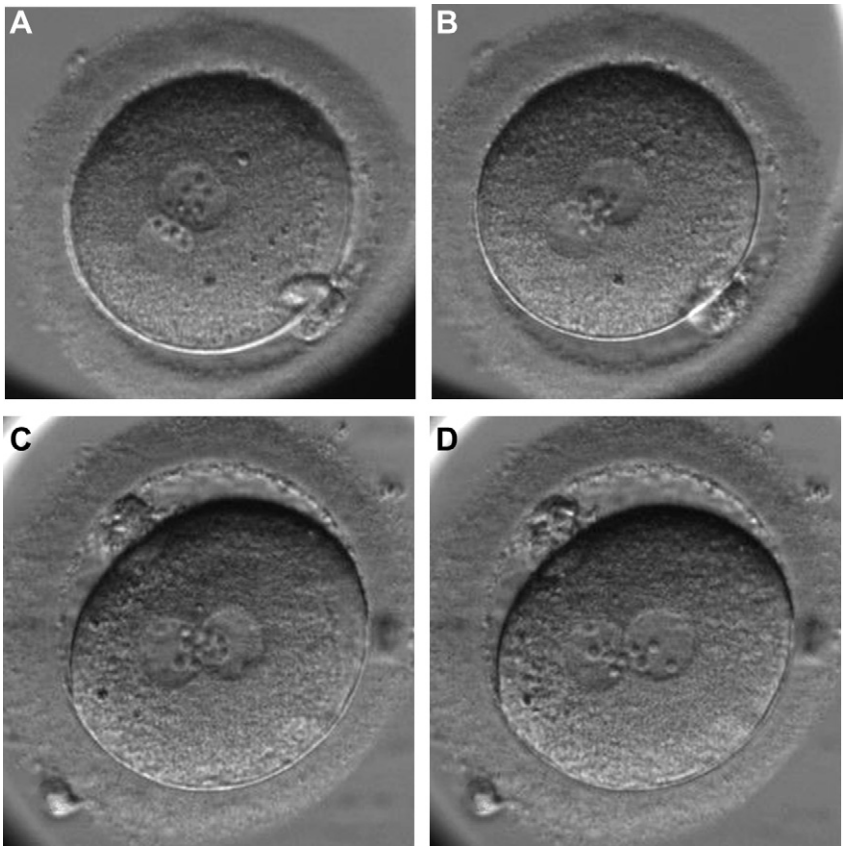

Fig. 1. Dynamics of pronuclear morphology. Two examples of a change in pronuclear morphology are shown. In the first the distribution of the NPB changed from an asymmetric pattern at 17 h (A) to a symmetric pattern at19 h after ICSI (B). The second shows the change from a symmetric pattern at 18 h (C) to an asymmetric pattern at 21 h (D).

scoring markers by time-lapse imaging we intentionally chose those time points for a sub-evaluation which are used in the routine laboratory for static observations (Table 1). Usually pronuclear morphology is assessed 16–20 h [35], early cleavage at 25–26 h [5,28] and day 2 embryo morphology around 40–42 h after ICSI [31]. Our evaluation clearly shows that pronuclear morphology at 16–20 h after ICSI is prone to undergo changes within a short time period. Therefore an annotation done at 16 h may be no longer valid 1 h later and may even change again at a later time point. Such changes may easily result in different scores of pronuclear pattern, as the majority of the proposed pronuclear scoring systems do rely on pronuclear morphology. It may also explain in part the contradictory situation in the literature, where based on static observation, some authors reported no benefit when pronuclear scoring was applied [15–19].

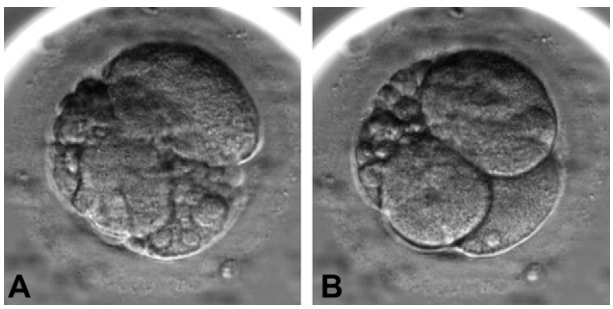

Fig. 2. Changes of embryo morphology after cleavage. An example of an embryo is shown with a highly irregular morphology at 37.1 h after ICSI (A) and regular blastomeres and less fragments at 41.7 h after ICSI (B).

Our results on the first cleavage show that this event may occur as early as 22 h and as late as 36 h post ICSI in our cohort of embryos. A recent study by time-lapse imaging revealed that embryos with too early cleavage fail to implant [29] and that the optimal time window for the first cleavage which is associated with high implantation is 25–28 h. In our evaluation 43% of the embryos were within this category. However, by using the common definition for assessing early cleavage at 25–26 h by a single observation [5,28], 57% of embryos would have been chosen as best although half of them had already divided earlier at 20–24 h after ICSI. Embryos with "too early cleavage" will most likely form embryos with a too fast development and for these it is well known that they do have a lower chance to implant [2] or are prone to aneuploidy [36]. The common selection strategy would overlook these as well

Table 1
Scoring markers assessed at different time points.

Pronuclear morphology	Same pattern		Different pattern
16–18 h: 212 2PN oocytes compared to 14–15 h:	75% (159/212)		25% (53/212)
19–20 h: 65 2PN oocytes[a] compared to 16–18 h:	66.2% (43/65)		33.8% (22/65)
Time of first cleavage	<25 h	25–26 h	>26 h
$n = 59$	27.1% (16/59)	30.5% (18/59)	42.4% (25/59)
"Early" cleavage	57.6% (34/59)		
Embryo morphology	Same score		Different score
40 h versus 38 h	50.8% (29/57)		49.1% (28/57)
42 h versus 40 h[b]	67.3% (33/49)		32.6% (16/49)

[a] Numbers for 2PN oocytes are lower at 19–20 h as some have been frozen at the 2PN stage after the second assessment.
[b] Numbers of embryos analyzed are lower at 42 h versus 40 h as some were removed for transfer in-between 40 h and 42 h.

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as those which will divide shortly after 26 h and according to recent data these would still have a high chance for a viable pregnancy.

Scoring embryos on day 2 for morphology and number of blastomeres revealed that for half of these embryos the initial classification and score changed within the time range which is used in most laboratories for embryo annotation. As morphological scoring was found to change in both directions, half of the embryos which scored low at 38–40 h looked top 2–4 h later. This phenomenon has been described earlier [31] and was attributed to the fact that embryos which are in the process of cleavage at the time point of observation do look sometimes very bad and hence will be given a lower score. In this study we did not proceed culturing to the blastocyst stage. Therefore one may argue that extended culture to the blastocysts stage would overcome the limitations which were described here. However, if the ultimate aim is to transfer only one embryo, multiple scoring strategies are highly relevant. In a single static observation strategy an embryo which scored top may ultimately be not the best one as the kinetics and morphological appearance in-between the observational interventions may reveal peculiar cleavage characteristics, phases of fast development followed by slower development and/or periods of multinucleation.

5. Conclusions

With the introduction of time-lapse imaging systems which enable continuous embryo monitoring the relevance of certain scoring criteria like pronuclear morphology and embryo morphology must be critically revised. Single observations at a static time point may be misleading as time-lapse imaging reveals dynamic changes of cell-cycle related markers like the position of nucleolar precursor bodies in pronuclei or the fate of embryo morphology shortly after cleavage. Furthermore, early cleavage must be re-defined as time-lapse imaging allows for sub-categorizing this event into very early, medium and very late cleavage and each of these categories has a different prognostic value. Consequently it may be time to define new scoring criteria and new selection strategies based on the knowledge which we gain with time-lapse imaging. The results presented here are preliminary and a prospective randomized trial is needed to prove the benefit of the concept and to evaluate the value of combined markers for embryo selection based on time-lapse imaging.

Conflict of interest statement

All the authors declare that they have no conflict of interest.

Contribution of authors

MM designed the study and wrote the first draft; JL and MK were involved in data acquisition; JL evaluated the raw results; all authors wrote and approved the final manuscript.

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