ESHRE 2021 Virtual (26 June-1 July 2021)

Questions for the speakers

Session 04: Morphological evaluation for euploidy detection

Computer vision can distinguish between euploid and aneuploid embryos. A novel artificial intelligence (AI) approach to measure cell division activity associated with chromosomal status. - Lorena Bori (Spain)

Q: How did to account for excluded blastomeres?

A: We did not consider excluded blastomeres. The whole thing is just an approximation, which is good enough as a rough proxy for cell activity but we shouldn't take it too literally as representing the exact measurements

Q: Can the system differentiate large fragments from cells?

A: Not really, not at this time. Our early results showed that euploid and aneuploid embryos are visually distinct, significantly enough to merit further computer vision investigation. According to our theory, the assumption was that many aneuploid embryos would "have more cell activity" than most euploid embryos. If true, this would manifest itself in a higher rate of false mitosis, in more cell movement before/after every mitosis event, and in more pseudo-mitosis events that create fragments rather than cells. These events are observable but very difficult to detect and quantify using automated techniques. So, we hypothesized that we could use an easier measure as a proxy for it. Actually, the total length of the cell edges is clearly a proxy for the number of cells and their sizes (gives us an idea of cell cycles). And it is relatively easy to find these edges, with few errors, using computer vision. Now, our next step is to implement a more robust and more detailed AI function to measure the cell activity by using not only cell edge lengths but their movements inside the embryo, classifying cells and fragments separately, and explicitly identifying mitosis events.

Q: How about the fragments? How are they counted?

A: The computer vision code was capable of distinguish small fragments, as they had few pixels, but it was not able to distinguish large fragments at the moment. Our early results showed that euploid and aneuploid embryos are visually distinct, significantly enough to merit further computer vision investigation. According to our theory, the assumption was that many aneuploid embryos would "have more cell activity" than most euploid embryos. If true, this would manifest itself in a higher rate of false mitosis, in more cell movement before/after every mitosis event, and in more pseudo-mitosis events that create fragments rather than cells. These events are observable but very difficult to detect and quantify using automated techniques. So, we hypothesized that we could use an easier measure as a proxy for it. Actually, the total length of the cell edges is clearly a proxy for the number of cells and their sizes (gives us an idea of cell cycles). And it is relatively easy to find these edges, with few errors, using computer vision. Now, our next step is to implement a more robust and more detailed AI function to measure the cell activity by using not only cell edge lengths but their movements inside the embryo, classifying cells and fragments separately, and explicitly identifying mitosis events.

Q: Did you analyze mosaic embryos apart from euploid/aneuploid?

A: In this first approach, mosaic embryos were included in the group of aneuploid embryos.

Q: We know that a small percentage of D7 blastocyst embryos are shown to be euploid by PGTA, how does this model cope with these slower developing euploids?

A: This model would detect that an embryo is aneuploid at the cell stage, between t2 and t8. It does not imply the blastocyst stage, regardless of blastocyst formation at day 5, 6 or 7.

Q: Could you further explain the rationale linking cell edges and aneuploidy?

A: We found that aneuploid embryos reached the blastocyst stage later than euploid embryos (on average). We thought that this might be due to the fact that many aneuploid embryos would "have more cell activity" than most euploid embryos. If true, this would manifest itself in a higher rate of false mitosis, in more cell movement before/after every mitosis event, and in more pseudo-mitosis events that create fragments rather than cells. These events are observable but very difficult to detect and quantify using automated techniques. So, we hypothesized that we could use an easier measure as a proxy for it. Actually, the total length of the cell edges is clearly a proxy for the number of cells and their sizes (gives us an idea of cell cycles). And it is relatively easy to find these edges, with few errors, using computer vision

Q: Did there a difference between day 5 and day 6 blastocysts?

A: In this study we treated all embryos equally, since cell border counting was done at cell stage.

Retrospectively, however, we observed that the rate of aneuploidy was higher in embryos reaching blastocyst later.

In-depth analysis of embryo development: Differences among monosomic, trisomic and chromosomally chaotic embryos compared to euploid embryos. - Fernando Meseguer Estornell (Spain)

Q: Does culture media affect the pace of development or do you need to calibrate for each media?

A: Morphokinetics depends on many factors and the culture medium is one of them. Therefore, it must be calibrated for each medium. In addition, it has been observed that culture conditions such as humidity also affect morphokinetics, due to changes in osmolarity.

Q: How would you use this information in real-life setting? i.e. is it to decide if you should biopsy?

A: This study shows the relative risks that an embryo has one type of aneuploidy or another. Therefore, it is not able to predict the type of aneuploidy. The next step would be to use the relative risks obtained in this study to create a predictive model. According to the predictive capacity of the hypothetical

model, we could decide not to biopsy those embryos that the model considered aneuploid, thus reducing the costs for patients.

Q: Do you plan to create your own AI program or do you plan to use comercial AI program for predicting embryo constitution?

A: We do not use any artificial intelligence program to only predict embryo chromosomal content. Currently, there is no program capable of replacing PGT. Creating our own program based on artificial intelligence is a long and arduous but at the same time very interesting process.

Q: How about the maternal age? is it affected? Did you analyze also mosaic embryos?

A: It is a preliminary study in which we have not seen subpopulations. Although it has been observed that maternal age affects morphokinetic parameters, the older the maternal age the development slows down. In our study we discarded mosaic embryos.

Q: Do you think that your study results might alter for IVF and ICSI derived embryos? culture conditions, embryologists performing the tasks.

A: All the embryos in our study are from ICSI cycles. In the in vitro fertilization laboratory, there is a high standardization of the protocols and the embryologists are highly qualified personnel with many years of experience. In terms of culture conditions, calibration and routine quality controls are carried out so that variations are minimized to the maximum. Furthermore, the sample size is high enough so that if any result is not correct, it cannot affect the results.

Q: Do your study find any difference in age groups with your data?

A: It is a preliminary study in which we have not seen subpopulations.

Q: When looking at delays in development, if you are delayed in one timing doesn't it affect the other timings? Are each timing independent?

A: Multiple scenarios can occur; there may be an independent delay at some point in development, there may be two independent delays, or if we intend to look at morphokinetic parameters very close in time, a delay in one parameter may affect the other.

End-to-end deep learning for recognition of ploidy status using time-lapse videos - Mark Liu (Taiwan R.O.C.)

Q: What is the output? is it probability of aneuploidy and what would be the cutoff to decide to biopsy or not?

A: The output of the model was the confidence score of aneuploidy (Group 1) from 0 to 1. The confidence level greater than 0.5 will be classified as positive (Group 1), and others will be negative

(Group 2). Because of the processing of different ploidy results from PGT-A, our model only provided the confidence scores of aneuploidy, which provide embryologists some references to make the final decision.

Q: Any clinical data between PGT_A and deep learning analysis?

A: In this study, clinical data was not input into the model except videos and PGT-A outcome of embryos. The additional clinical data will be explored in the future.

Q: Can you confirm the % what the number of false negatives "presumed aneuploid but actually would be euploid by PGT-A"?

A: The number of false negatives was 8 out of 111. The false positive rate was 7.2%.

Q: What is the minimum number of videos that are required for deep learning?

A: In theory, the more data collected, the more robust the model performs, so there is no minimum number for deep learning. However, in reality, obtaining sufficient data could be a great challenge, we expect a wider sample size in our future with approximately 1000 or more.

Q: Any data for faster developmental embryos?

A: In this study, we only focused on raw timelapse videos, which do not involve handcrafted annotation of the embryo stage, so the speed of embryo development was not to be discussed.

Embryos with higher mitochondrial DNA ratios show better clinical outcomes in single euploid embryo transfer - Duke Chen (Taiwan R.O.C.)

Q: Have you ever compare the MT ratio by embryo morphological grade?

A: Yes, we compare the Mt ratio with embryo morphological grade, but only compare the TE morphology of embryo. In our experimental result indicated that the average Mt ratio of the TE grade A is 1.04. Grade B is 1.05 and Grade C is 1.01. There is no statistical difference between the Mt ratio and TE morphology.

Q: Did you find differences in general implantation rate D5 versus D6 transfers?

A: Yes. The implantation rate of D5 transfer was higher than D6 transfer (59.5% vs. 44.6%). Besides, the result demonstrates that D5 Biopsied embryos contained a significantly higher Mt ratio than D6 biopsied embryos.

Q: Which could be the biological meaning of your results, different from the classical "less is better"?

A: Although our results were different from the classical “less is better”. But actually, people hold different opinions about this inference. However, the mechanism of correlation between MtDNA

content and implantation results is still unknown. In our opinion, we propose another hypothesis. The previous studies investigate the mitochondria copy number and replication in the oocyte and embryo development. In the oocyte maturation stage, the mitochondria replication was used to supply the energy required for two meiotic divisions. However, the mitochondria no longer replicate at the stage of embryonic development. Therefore, back to our conclusions, we thought that the energy consumed during the embryo development stage comes from the accumulated mitochondria by the mature oocyte. If the blastocysts with a lower Mt ratio and poor implantation results represent that the poor quality of embryos requires more energy consumption to maintain the embryonic development.

Q: As there are differing results when you compare D5 and D6 MT ratio as well as the implantation rate. What could be the best decision in which day to do biopsy?

A: D5 biopsied embryos had a significantly higher mitochondrial DNA ratio than D6 biopsied embryos. The embryos with a higher mitochondrial DNA ratio increase pregnancy rate and implantation rate in single euploid embryo transfer. In our opinion, D5 biopsy is a better biopsy time than D6 biopsy. But it also depend on the embryo have enough TE cell to biopsy on D5, which means that the embryonic development is more robust than that of D6.

Q: Did you examine an association between Mt DNA ratio and the live birth rate?

A: No. But in the future, we will investigate the relationship between Mt DNA ratio and the live birth rate.

Q: Did you see any relationship between the mtDNA ratio and the hatching status?

A: We didn't compare the mtDNA ratio with different hatching status. Because we think the TE morphology may have a better chance to influence the mtDNA ratio. But this is a good suggestion, we will investigate the relationship between the mtDNA ratio and the hatching status in the future.

Q: Although aneuploidy had a higher Mt ratio than euploidy had, a higher Mt ratio correlated with increased pregnancy rate in clinical data. How do you explain it?

A: These contradictory results, the explanation we can think of is the chromosomal abnormalities mainly come from the errors with meiosis during oocyte maturation. The aneuploid embryos need more mitochondria to provide energy and maintain oocysts development. However, the mitochondria no longer replicate at the stage of embryonic development. Therefore, we thought that the energy consumed during the embryo development stage comes from the accumulated mitochondria by the mature oocyte. If the blastocysts with a lower Mt ratio and poor implantation results represent that the poor quality of embryos requires more energy consumption to maintain the embryonic development.

Performance of a commercial artificial intelligence software for embryo selection (Embryoscope/KIDScore™) on predicting biopsied and non-biopsied blastocyst clinical pregnancy according to score subgroups. - Renata Erberelli (Brazil)

Q: Clinical Pregnancy is one outcome - have you looked at miscarriage rate and the impact that may have on final Live Birth Rate - the ultimate aim of IVF

A: In this study, the data of positive cases consider clinical pregnancy that includes positive heartbeat and yolk sac, so we don't consider chemical miscarriage, but we can still have a 5% loss for live births if we consider our clinical abortion rate.

Q: Can you please share any preliminary results regarding ongoing pregnancy rate or live birth rate between the two groups (in contrast to clinical pregnancy rate)

A: For future direction we could compare live birth rates with the subgroup of score, but in our experience, in overall viewer we have a 5% loss when comparing clinical pregnancy and birth rates.

Q: Could you understand which embryos are euploid by morphokinetic analyse?

A: Many studies involving this subject have already been carried out, and no specific parameters were found that could replace the PGS. The most recent studies already involve artificial intelligence assisting this prediction and perhaps the best way to deal with the choice of better embryos is an in-house validation according to the experience of each clinic.

Q: Nothing has been said about other parameters like miscarriage rates or LB rates. Could you tell us your opinion?

A: It is the same answer as the first two questions

Q: Any difference between fresh and frozen embryo, and difference between delyed and faster embryos?

A: Most of our transfers are from frozen embryos, even for cycles without PGT-A. Considering the number of cases, it would not be possible to proceed with this analysis. We do not use the separate morphokinetic parameters, just the embryo viewer score, but faster developing embryos tend to have a better score, but this should be studied carefully.

Q: Is there some correlation between KIDScore and Gardner grading?

A: Initially, we looked the morphological quality of the embryo as a parameter and it seems to us that it does not affect it, but there is low data collected, especially when we separated by embryo morphology, we have seven different group just for quality. But in an initial view biopsy should only be indicated for subgroup 1 (lowest) for embryos with a minimum morphological parameter (3BB or more) and perhaps for patients with a history of previous implantation failure this may apply to low morphology embryos. For future direction we could relate morphological assessment.

Q: Is it the age of the patient (recipient) or the age of the oocyte donor that was used for patient allocations?

A: We had homogeneous groups in relation to age with and without PGT-A. If we separate the group of donations, we would have mostly of the cases performing non-PGT-A. In our center there are no

differences in clinical pregnancy rates in young patients and egg receptors (mostly from egg freezing), so the invasive biopsy technique could be harmful for this age group. However, some couples remain with the performance of the PGT- A for already experienced treatment failures or repeated miscarriages. But this could be a new line of study separating the ages and correlating the score subgroups