ESHRE 2020 Virtual (5-8 July 2020)

Questions for the speakers

Session 28: Revisiting early embryo development

Cell cycle in the preimplantation embryo: mechanisms, errors, consequences and cell fate - Joris Vermeesch (Belgium)

Q: Have you seen different level of mosaicism in blastocysts from lite stimulated, moderate stimulated or "natural" (very low stimulation level) cycle?

A: Many studies suggest that ovarian stimulation does not impact embryo aneuploidy rate. However, more single cell research should be done on blastocyst stage to determine the true level of mosaicism at that stage.

Q: Do we have info on further destiny of blastomeres that result from fusion of two cells at early developmental stages?

A: To my knowledge, there is no follow up data available on such fusions.

Q: Is it possible to use comprehensive PGT at the blastocyst stage and can it detect mosaicism?

A: It is possible to use comprehensive PGT at the blastocyst stage. It can detect mosaicism, but the sensitivity to detect different degrees of mosaicism is under investigation.

Q: Would you like to tell something more about agilent one PGT solution

A: The Agilent OnePGT solution is a sequencing based SNP typing approach and is based on haplarithmisis for the data analysis. It allows comprehensive PGT (combined PGT-M and PGT-A). Briefly: Single or few cell DNA is amplified, the amplified DNA is cut by a restriction digestion reaction and the restricted fragments are PCR amplified and sequenced. This results in a reduced representation library which allows cost-effective sequencing based SNP typing. The SNPs are analyzed as described for haplarithmisis and the copy number profiles are calculated based on sequencing depth. For more information look at Masset et al., Multi-centre evaluation of comprehensive preimplantation genetic test through haplotyping-by-sequencing, Human Reproduction 2019;34(8):1608-1619.

Q: Have you any insight into whether aneuploid (trisomic) cells might ever 'correct' by selectively losing the extra chromosome.

A: Yes, we do know that aneuploidy trisomic cells can undergo a mitotic error leading to a disomic cell. However, direct evidence in preimplantation embryos that this is happening is, to my knowledge, still lacking.

Q: Does the presence of balanced translocation in parent increase the risk of mosaic segmental or complete aneuploidy in blastocysts?

A: The presence of a balanced translocation in the parent leads to a high frequency of embryos with the unbalanced translocation. There is no evidence that the balanced translocation would lead to increased mosaic segmental or increased incidence of complete aneuploidy of other chromosomes. Unfortunately the number of PGT-SR cases in the context of a translocation analysed via haplarithmisis is still very restricted to drive any strong conclusions.

Q: Why during evolution of the human species there was no selection against aneuploidy and mosaicism?

A: My favorite hypothesis is that the increased (segmental) aneuploidy rate during cleavage stage increases the overall (chromosomal) mutation rate. This in turn could for the species would provide a broader scala of variation that can rapidly evolve. For the species this could be of evolutionary advantage.

Q: Based on all these data do you believe that one should go ahead and offer TE-PGTA

A: If you have the choice, trophectoderm PGT-A is more accurate than cleavage stage PGT-A, although the biological discrepancies due to the nature of the sample that is analyzed remain (TE vs ICM).

Now, advanced maternal age seems to be the only indication, where PGT-A has some evidence of beneficence. Currently, the biggest challenge is what to do with mosaic embryos, which are still often discarded. Yet, instead of discarding embryos, one could rank them or give priority for transfer, which can potentially reduce time to pregnancy. However, the benefit of PGT-A in itself is still being debated.

Q: With respect to chromosomal abnormalities in in vivo human embryos. What is your opinion of the data presented by Munne and colleagues

A: I do know the data and we have analyzed the DNA ourselves. So the incidence of aneuploidy reported in the human in vivo embryos, in this one small dataset is correct. However, the dataset was small and it will be essential to expand the embryos analyzed to draw more solid conclusions.

Q: Could you differentiate between aneuploidy levels of ICM and TE? Is TE or ICM more prone to abnormalities?

A: There is evidence that TE is more prone to abnormalities compared with ICM. This distinction between ICM and TE can, however, not be determined during a regular PGT-A cycle. Studies analyzing specifically the TE or ICM do suggest a stronger selection pressure for normal cells in the ICM. That would mean that when performing TE-PGT-A, embryos with abnormal TE and potentially normal ICM are being discarded.

Q: Do you culture your bovine embryos at 20% O2?

A: No, bovine embryos are cultured at 5% 02. We did a comparison between 5% and 20% culture, and 20% 02 reduces embryo quality and blastocyst formation rate in bovine.

Q: Do you think current PGT-A technic is a good way to select embryo?

A: From a technical point of view, currently used NGS-based PGT-A is a rather reliable technique for embryo comprehensive chromosome screening, despite its restrictions. However, international guidelines on embryo transfer policies and position on mosaicism are lacking. Currently, mosaic embryos are still being discarded, although they can still lead to a successful pregnancy albeit at a smaller rate. Importantly, a lot of PGT-A studies have been performed in good prognosis patients, hence a consensus report from the American Society of Reproductive Medicine states that "At present, however, there is insufficient evidence to recommend the routine use of blastocyst biopsy with aneuploidy testing in all infertile patients".

Q: There are different levels of chromosomal alterations in different developmental stages. Do you think the embryo has the capability to correct itself?

A: Rather than speaking about self-correction, I prefer to think there is 'Darwinian' selection at the cellular level, especially after embryo activates its genome on day-3. This can also potentially explain the difference in aneuploidy rate between cleavage embryos and blastocysts. Cells that have lost or gained certain chromosomes, may not grow, not grow as fast as better fit cells, etc… In the trophectoderm this chromosome set could be different as compared with the embryo proper and even within the embryo, this can be variable amongst tissues.

Q: Is there any study been done about THE differences of occurrence of cancer later in Life between PEOPLE originating from IVF and people from natural origin

A: Recently, it was demonstrated that ART-conceived children might be at higher risk of developing childhood cancer (Hargreave, M. et al, 2019, JAMA), but data on long-term health are still too sparse to draw solid conclusion.

Q: If there are all these mosaic embryos and the abnormalities that seem to reduce as the embryo - fetes grows. Is there any value of PGT-A in clinical practice?

A: PGT-A of cleavage stage cells is of no value, since the cellullar variation in the embryo is so high that a single cell does not reflect the constitution of the whole embryo. The aneuploidy incidence in trophectoderm biopsies is much lower and here, multiple cells are characterized. This could be more predictive of the real situation, but never conclusive. As mentioned above, the clinical validity of PGT-A is still being questioned. However, some methods allow the identification of meiotic aneuploidies/trisomies. When observing a meiotic trisomy, it can be expected the trisomy is present in the large majority of cells and hence, the chance for a normal embryo is low.

Q: Do you think shorter culture period in vitro would be better?

A: Based on overall implantation ,pregnancy and live birth rate, there is no difference between day-3 or day-5 embryo transfer. Hence, there seems to be no strong evidence to support that shorter culture is better. What might play are in vitro conditions, in which the embryos are growing, including used culture medium.

Q: In terms of epigenetic risk: Conventional IVF insemination and cleavage stage embryo transfer is preferable to ICSI + blastocyst stage embryo transfer?

A: There are studies suggesting that mode of fertilization (namely, ICSI) and culture medium (rather than embryo culture timing) can induce epigenetic changes. However, it remains unclear whether the epigenetic changes observed are the effect of infertility itself. Additional data is necessary to evaluate all the factors involved in ART, including male/female infertility, stimulation protocols, mode of fertilization and other IVF lab variables.

Q: In view of the extensive abnormalities in embryos, what is the role of PGS- do we discard embryos which may become normal later

A: The critics of PGT-A argue exactly that we may discard embryos that could develop properly. This in turn could reduce the overall success rate of an IVF cycle. There are conflicting studies in literature.