ESHRE 2021 Virtual (26 June – 1 July 2021)

Questions for the speakers

PCC13: Novel technologies in human reproduction

Bioinformatics to determine gene variants implicated in disorders of sexual development - Daniel Rodriguez Gutiérrez (Switzerland)

Q: Did you get the cells for each fetal cell population by pooling the SC data from first and second trimester from the Li-dataset?

A: Yes, we pooled Li-dataset SC data from each cell population into single clusters. Our analysis is more focused on the differences between the cell populations of the testis and less on the evolution of certain population in time. Although a timeline analysis of every gonadal cell population would be of great interest, this is a proof of concept study and the inclusion of so many clusters would generate extremely complex networks to analyze. Nevertheless, additional detailed analysis of the developmental evolution of each gonadal cell population separately would be an excellent complement to the analysis and should be considered in the future

Q: Regarding all DSD, did you study KS (47, XXY) and variants (48, XXXY etc..)? If yes, did you find any candidate genes in somatic cells?

A: Unfortunately, not. The current study was focused exclusively on 46, XY DSD patients. Of course, it would be very interesting and we have plans to expand the analysis to additional DSD patients in the future.

Q: Could you please explain again how you relate the DSD genes with the genes you got from the fetal SC-data?

A: A sex development-related gene list obtained from literature was used to highlight genes in the SC-data generated networks that are known to be related with gonadal development. Based on that, we can identify which genes with variants in our patient's cohort are actually expressed in the different networks and if they directly interact with sex development-related genes, prioritizing the in-depth analysis of those variants.

Organs-On-Chip models of the female reproductive system - Virginia Pensabene (United Kingdom)

Q: Does the phase of the menstrual cycle matter to establish the endometrium OoC?

A: In our model we aim to control precisely the hormonal stimulation, so that we can use the cell response to evidence similarities to the natural endometrial function.

Q: Can you see changes in phases in the endometrium OoC if you change the hormones in the culture medium?

A: yes, we can for example observe different morphological characteristics in endothelial cells and increased production of prolactin for decidualized stroma cells in response to E2 and MPA.

Q: What can the pregnancy model be used to and what is the read-out?

A: it can be sued for toxicology study, for example, and the read out can be either images (with selective staining of different cell types for example in response to specific stimuli) or analysis of the media by ELISA or MASS spectrometry to evidence changes in the media composition.

Q: Your microfluids tech can be used to study implantation model in patients with recurrent abortions and the role of uNK cells?

A: It could, having available patient derived cells.

Q: For how many days you can in vitro culture the embryos in your system?

A: we usually culture them from 1 cell to blastocysts, over 4 days max with murine embryos, but we are developing different protocols for the bovine embryos.

Q: In your embryo-model: will you attempt to culture the embryos further until Day14? erhaps in a more permissive niche to study implantation?

A: We have not looked at prolonged culture, but this is definitely feasible and a good idea.

Q: ¨What is the bottle neck to develop Organ on chip models: chip design or culture media?

A: I would say media, together with cell sources, since microfabrication technologies are quite flexible at the moment.

Q: I was wondering if it is possible to use new drugs or new cell therapies to treat and study endometriosis by using endometrium-on-chip?

A: One of the goal is to optimize the endometrium on chip for testing new drugs or new therapies, so definitely yes, it is a goal of this research.

Q: Would you consider building a system similar to EVATAR to study the embryo development?

A: not at the moment.

Q: What is the most advanced Organ-on-chip model regarding female reproductive organs? What about ovary organ on chip?

A: Thre are many examples and great groups working on different organs, with slightly different approach. There is not a best one at the moment or a commercially available as far as I know.

Q: Do you think there are more challenges using human embryos compared to mouse embryos?

A: absolutely, based on the fact that the availability and possibility to use human embryos are (appropriately) limited. We are starting to explore different genetically altered mice to see if the system we developed supports growth of more specific, less robust murine embryos.

Q: Do you plan to study the male reproductive organs/tissues with your systems?

A: not at the moment.

Q: How would you build a system to study effects of chemicals without background contamination from components of the chip system itself?

A: Yes, that is a goal.

Q: You mentioned you are also working with oocytes; are they from mouse or human?

A: mouse.

Current status of transplantable artificial ovaries - Christiani Amorim (Belgium)

Q: The malignant cells were transplanted to mice, I assume? Were those animals immunocompromised? How long did you wait to see the results?

A: *Yes, they were transplanted to SCID mice for 20 weeks (Soares et al., J Assist Reprod Genet, 2014), similarly to what we did with ovarian tissue from leukemia patients in our precedent study (Dolmans et al., Blood, 2010).*

Q: Did you transplant the malignant cells to different places in the animals?

A: *No, we only transplanted in a peritoneal pocket we created in the internal side of the mouse peritoneum (Soares et al., J Assist Reprod Genet, 2014).*

Q: What are the markers for theca cells that you are using?

A: *All markers in the theca cells involved in the synthesis of steroid hormones (LHR, StAR, CYP11A1, CYP17A, HSB3B1, and HSB3B2). Additionally, CD13 and NOTCH1 (Asiabi et al., Hum Reprod, 2020).*

Q: Do you think that ovarian cells are really necessary for the artificial ovary?

A: *I think they are because some of them will be recruited to differentiate into theca cells.*

Q: How heterogenous is your population of ovarian cells and does it include OSE (ovarian surface ectoderm)?

A: *We have basically ovarian stromal endothelial cells and a small proportion of epithelial cells.*

Q: Does it matter the phase of the cycle (lutein or follicular phase) that you isolate the ovarian cells from human?

A: *Since such information is not recorded in the patient files, we do not really know.*

Q: What would be the best source of somatic cells for the ovarian construct? If patient samples, is there a risk of malignant cells being present in the cell prep?

A: *In theory, we could collect ovarian cells after cancer remission. In this way, we would avoid the risk of accidently having malignant cells.*

Q: Why do you think the follicles do not develop further in the mice-host? what is the next step for improving oocyte maturation in the artificial ovary?

A: *They can develop (Dolmans et al., Reproduction 2008).*

Q: What about decellularized ovarian tissue? Do you think it's a good alternative to graft the isolated follicles?

A: *The problem with decell ovaries is the follicle seeding. Follicle diameter varies in each stage and how could we fit the isolated follicles in the right pores? Some studies have been injecting follicles in the decell tissue, but since the human preantral follicles need a right degree of stiffness to survive, I wonder if this is the best strategy. We have been developing hydrogels from decell ovaries in order to take advantage of the ECM biochemical cues, but our results are still too preliminary.*

Q: You mentioned that recovery rate of follicles grafted in fibrin is 35%. This doesn't seem much; how can this be improved?

A: *While I agree that it does not appear to be a lot, it is important to bear in mind that 1-week xenografting of human ovarian tissue showed a recovery rate of around 27% (Nisolle et al., 2001). So, we are not far from the findings observed with ovarian tissue transplantation. On the other hand, we are trying to improve this rate by tailoring our matrix having in mind our recent knowledge about human ovarian ECM (Ouni et al., Mol Cell Proteomics, 2018; Ouni et al., Hum Reprod, 2020; Ouni et al., Nat Commun, in press).*

Q: There are reports that MSC can improve vascularization/function of ovarian tissue grafts. Could this also play a role in the artificial ovary strategy?

A: *Yes, definitely.*

Single cell technologies: mapping the cell types in human ovaries - Pauliina Damdimopoulou (Sweden)

Q: In the DDX4 positive sequencing exp, what do you think is recognized by the DDX4 antibody. DDX4 or another protein?

A: I believe that the antibody binds to some other protein on perivascular cells, not DDX4. Only the recommended DDX4 antibody (Rabbit anti-DDX4 antibody, Abcam, cat. no. ab13840; Woods & Tilly 2013 Nat Protocols) can isolate perivascular cells, not other DDX4 antibodies we have tested. In addition, only the recommended antibody stains perivascular cells in ovarian tissue sections. Importantly, the perivascular cells do not express DDX4 RNA as shown by the absence of transcript in the scRNA-seq experiments, and the absence of labeling in the RNAscope experiments. In summary, the antibody unspecifically binds something else than DDX4 on perivascular cells.

Q: What is your opinion why the oogonia stem cells have escaped meiosis? What if they will never enter meiosis? What is their fertility potential?

A: I am not convinced that there are oogonial stem cells in human ovaries, so I cannot answer this question.

Q: What is the role of the DDX4-perivascular cells?

A: I do not believe that perivascular cells express DDX4. We do not see any DDX4 RNA in the perivascular cells in our experiments (scRNA-seq, RNAscope). We are able to isolate perivascular cells using the DDX4 antibody recommended for isolation of oogonial stem cells (Rabbit anti-DDX4 antibody, Abcam, cat. no. ab13840; Woods & Tilly 2013 Nat Protocols). Only this rabbit polyclonal antibody works, other antibodies we have tested do not. The collective evidence from our experiments leads us to believe that the antibody simply binds to some other epitope on the perivascular cell, not DDX4.

Q: Do you think that based on the gene expression profile you will able to identify the oocytes that will develop to competent oocytes that will eventually ovulate?

A: That would be amazing! I am very much interested in heterogeneity among cortical follicle populations. Are they all the same? Do they all have the inherent capacity to grow and mature? I don't have any answers but would love to find out!

Q: Do you think populations (clusters) are missing from the cortex?

A: Yes, I know that our data does not contain epithelial cells, which theoretically should be there. We used cryopreserved-thawed tissue samples in our experiments. The handling of the tissue for freezing likely damages the fragile epithelial cell layer. It comes off by gently brushing the ovary, so twisting and turning the sample in a petri dish while trimming for cryopreservation will likely do so too. Further, the six main cell types we present (oocytes, granulosa, immune, perivascular, endothelial, and stromal) have subtypes. For example, the perivascular cluster contains both pericytes and smooth muscle cells, and the immune cell cluster T-cells and monocytes. I think this is a central question: how is a cell type defined, where should the boundaries be drawn.