

## ESHRE 2021 Virtual (26 June – 1 July 2021)

### Questions for the speakers

#### **PCC01: Are we ready for the future of male fertility creation & preservation**

##### **Cryopreservation of low numbers of sperm - Nicolás Garrido Puchalt (Spain)**

**Q: With the dry ice method, how do you sterilise the surface between different samples?**

A: there is no need to sterilize the dry ice surface, since it is designed for a single use.

**Q: In your opinion what's the indication in routine clinical for sperm vitrification?**

A: In my opinion, the cost/benefit ratio indicates that although it is probably improving the recovery rates of motile sperm, compared with traditional methods, the practical benefits are very small, while the workload remains greater. Then, I do not see on which cases vitrification can replace classic sperm freezing providing practical benefits

**Q: How does sperm freezing on dry ice compare to vitrification and slow freezing in terms of 1) survival, total motile count; 2) application?**

A: to my knowledge, there are no studies comparing these, although it seems vitrification provides a small improvement on these parameters. That said, and as I answered above: I do not see on which cases vitrification can replace classic sperm freezing providing practical benefits

**Q: I was wondering if there is an improvement in the embryo usage rate (%Blastocyst/2PN) after ICSI with vitrified vs regular freezing protocols**

A: Good point, but unfortunately, not sure if there is literature out there providing this info.

**Q: Are there freezing protocols that are effective in preserving sperm in testis tissue fragments without need for prior mincing or extraction?**

A: Well, it depends on what we understand as "effective". If you mean with "effective" = some cells that can be utilized survive, then yes. But as far as I know, by mincing one improves cryoprotectant diffusion and accessibility within the cells, providing way better results

**Q: One thing I have not seen in the literature is gradual dilution to remove cryoprotectant after thawing. Have you looked at that?**

A: This is a good observation and point. So far, I am not aware of any paper showing how different cryoprotectant dilution protocols after thawing may affect survival.

This could be a complex project, in need of several samples, due to the great variability on cryosurvival.

We always need to keep in mind what we need these cells for: if ICSI is planned, then small variations on cryosurvival could not be so relevant.

### **Spermatogonial stem cells: Preserving male reproductive potential - Ans van Pelt (The Netherlands)**

**Q: Do spermatogonial subtypes (e.g., Adark and Apale) form one homogenous SSC population in vitro, or do they keep their subgroup-specific profile?**

A: Although we have no clear scientific evidence for this because they are transcriptionally hardly distinguishable from each other, I also indeed think they will all become Apale spermatogonia and some will finally differentiate spontaneously in vitro.

**Q: Great overview! Is there a way to overcome incomplete synapsis during in-vitro spermatogenesis?**

A: The reason why synapsis is incomplete is still not known. More research is required to figure this out. We never should bypass this point and we need to make sure that the checkpoint becomes active to eliminate non-synapsed spermatocytes to avoid aneuploidy.

**Q: Should we strive to incorporate single cell genetic analysis to inform the optimisation of these approaches?**

A: This is a very good idea and will be helpful in optimizing the development of these approaches. However, once approaches are optimized and implemented for clinical purposes, single cell sequencing would be too time consuming and makes the application unnecessary expensive.

**Q: In mouse 2D culture; pachytene checkpoint + SAC are not stringent enough for meiotic arrest. Is meiosis compromised in numbers reaching spermiogenesis?**

A: All in vitro spermatogenesis approaches so far reported are inefficient. So the answer is that your suggestion could very well be the case, but might not be the only reason.

**Q: Would you speculate on comparison between histological spermatogonial sub-types (Adark, Apale) and single cell sequencing 'spermatogonial' states'**

A: Although Adark are proliferative inactive and Apale proliferative active, we have shown earlier by laser dissection microscopy followed by deep RNA seq that there is no or hardly any transcriptomic difference between histologically Adark and Apale spermatogonia (Jan et al., 2017, doi: [10.1242/dev.152413](https://doi.org/10.1242/dev.152413)).

**Q: What will be the key check points to ensure patient's testis is ready to receive the SSC or tissue transplant?**

A: the patient should be infertile with no spermatogenesis present and SSC niches empty to receive SSCs. The testis should have a homogenous appearance by sonography and a clearly visible rete testis.

**Germline gene editing to treat male infertility - Kyle Orwig (U.S.A.)**

**Q: Would you perform gene editing in SSC at the beginning of the propagation culture or during the last step before transplantation?**

A: I would do it as early as possible but a few passages will be necessary to establish robust cultures, do the edit, propagate the properly edited clones and screen for off target events. I would limit the time in culture to the minimum that is absolutely necessary to achieve the above to reduce the possibility of culture artifacts.

**Q: How efficient is CRISPR/Cas9 for modifying germ cells, compared with its efficiency in modifying somatic cells.**

A: I think the efficiency of CRISPR/Cas9 gene editing is probably similar. However, the efficiency of introducing gene editing reagents into germline stem cells is much lower than many somatic cell lines.

**Q: Would you recommend SSC transplantation with gene editing for e.g. sickle cell disease if prepubertal testicular tissue was banked?**

A: I think this is an excellent application of gene editing in patients who have already preserved their prepubertal testicular tissue prior to BMT. Some of those patients are actually already on sickle cell gene therapy trials and may be inclined to use the same therapy to reduce the chance of passing the sickle train to their offspring. Germline gene editing that results in the production of a genetically modified hu an embryo is currently illegal in the United States but would be permissible in some parts of the world.

**Q: Did you ever try to transplant Tex1 corrected cells into BOTH testes? If so...could you reach a situation where the recipient males could breed without ICSI?**

A: We have done that in mice but not specifically in the Tex11 experiment. Restoring natural fertility after SSC transplantation in adult mice is possible but difficult to accomplish. Transplanting into mouse pups is much more efficient and would be my preferred approach if the objective is to restore fertility.