ESHRE 2021 Virtual (26 June-1 July 2021)

Questions for the speakers

Session 05: Genetic Analyses in Andrology

A Genome Wide Association Study in men with unexplained infertility identifies nine SNPs at the FSHB locus to be associated with Follicle Stimulating Hormone level - Maria Schubert (Germany)

Q: Are there other SNPs in the region? Would you be able to find an association / or not with those?

A: For genome-wide-significance level we find the 9 presented SNPs, for (the lower) suggestive-significance level, we find another 64 SNPs that are associated with FSH serum level.

Q: So the mutation lowers FSH level? the function of FSH remains normal

A: The SNP is a variant, not a mutation; and in case of the unfavorable Genotype in the SNP, indeed the FSH level is lower. However, still within the reference range.

Q: Why would you only genotype one of the polymorphisms, and not both?

A: Since all identified SNPs are in very high Linkeage disequilibrium, there is no need to genotype all or two SNPs, one is, in our opinion, sufficient.

Q: Can we treat those patient with exogenous FSH?

A: That is our long-term-goal to treat those men with an unfavorable Genotype in the SNP with FSH.

Correcting a PLCζ mutation in the human germ line to overcome hereditary infertility - Bieke Bekaert (Belgium)

Q: Why did you use AOA if you have attempted to correct PLCz activation deficiency? Do you think this may have resulted in higher embryo abnormalities?

A: Mutations in *PLCZ1* can result in an oocyte activation deficiency (OAD) which results in failed fertilization after ICSI. To overcome these mutations AOA can be used. The translation of *PLCZ1* takes place during the spermiogenesis therefore the correction of *PLCZ1* during ICSI will still result in aberrant/absent *PLCZ1* and therefore failed fertilization. The born baby with the corrected *PLCZ1* will not need AOA to achieve his own offspring.

Assisted oocyte activation (AOA) is already applied in many centers. Different research groups examined children born after AOA and they did not see any impact on the children. The use of AOA could possibly induce embryo abnormality after CRISPR/Cas9 editing. Due to the need of AOA in our project, the information about the possibility to achieve corrected embryos without abnormalities after the combined application of AOA and CRISPR/Cas9 editing is important information. We did not see a high amount of abnormal fertilized embryos (i.e. 1PN or >2PN) in our experiment.

Q: How pick up or identify the adjusted the mutant sperm to fertilize?

A: Due to the heterozygous nature of the mutation, only half of the sperm contained the mutation, and therefore, theoretically 50% of the generated embryos will originate from a wild type sperm and 50% from a mutant sperm. Therefore, identified wild-type alleles in the investigated embryos could either correlate to wild-type sperm giving rise to the investigated embryo, or to an altered mutant allele when mutant sperm gave rise to the investigated embryo. To determine the origin (i.e. wild-type or mutant) of the sperm, we performed a short tandem repeat (STR) assay, which investigates tracks of tandemly repeated short DNA sequence motifs unique for the paternal wild-type and mutant alleles.

Q: Any data available on the quantification of plc Zeta in individual sperm?

A: In our project, we did not do individual sperm quantification of PLCζ but instead we performed some diagnostic tests (MOAT, MOCA and HOCA) with the mutated sperm to determine its activation deficiency. We performed analysis of the *PLCZ1* DNA (e.g. Sanger sequencing and Miseq) and RNA (e.g. RT-PCR and Sanger sequencing) on bulk samples of the sperm.

The quantification of PLCζ in sperm can be done by immunofluorescence studies but to my knowledge only a polyclonal antibody for PLCζ is present at this moment which does not give rise to very reliable quantification results.

Q: How do you justify the choice of PLC zeta? A treatment, AOA, exist and the genetic consequences for the offsprings are limited, no justification of PGT

A: Indeed, *PLCZ1* would not be the first gene of choice to be corrected when gene correction would be translated to a clinical setting. I recognize that other mutations with a more life-threatening or debilitating impact would probably be the first choice for gene correction. Our research was a proof-of-concept in which our focus was to investigate the DNA repair mechanisms following CRISPR/Cas9 application. We chose to work with *PLCZ1* due to our in-house knowledge about the gene. Furthermore, our lab is focused on (in)fertility and we therefore have the availability of material (e.g. mutated sperm) of patients with mutations in *PLCZ1*.

The use of gene editing instead of PGT after AOA for these patients in the future would in my opinion only be possible when gene editing is completely optimized without unwanted genetic consequences. In PGT, multiple embryos need to be created and the embryos carrying the mutation (for the *PLCZ1* mutation case, approximately 50%) are discarded. In case gene editing is 100% efficient, no embryos should be discarded in contrast to PGT.

Utilization of ultrastructural analysis and genomics of spermatozoa to better characterize subtle forms of male factor infertility - Kolbe Hancock (U.S.A.)

Q: Are thin (and short) sperm tails also a result of a mutation? If yes, which one? and what about fertilisation?

A: We described a specific phenotype, dysplasia of fibrous sheath, which are characterized by stumped tail and incomplete formation of flagellum. Indel mutations on AKAP4, SPAG16 and CATSPER1 contributes to this phenotype. Clinical outcomes were not included in this study yet.

Q: For the Globo cases, could you give us the distribution of the mutations among the different genes?

A: For consenting patients (n=3) whose spermatozoal DNA were sequenced, single nucleotide insertion on PIWIL1 were identified on all patients. All 3 men has deletions on at lease 1 gene: DPYL19L2, SPATA 16 or PICK1

Q: Is the absence of Dynein will affect the fertililization or just the motilit?

A: Absence of outer dynein arm only affects motility. Viable spermatozoa, once identified, can yield fertilization by ICSI.

Q: Did you notice a difference between the organization of microtubules from frozen / thawed and fresh sperm?

A: All specimens analyzed by TEM were fresh ejaculates since cryopreservation may alter membrane characteristics of spermatozoa. Organization of microtubules from frozen thawed spermatozoa was not yet examined but presumed to be unchanged.