ESHRE 2021 Virtual (26 June – 1 July 2021)

Questions for the speakers

PCC02: Add-ons and other debated interventions in the IVF lab

Artificial oocyte activation - Thomas Ebner (Austria)

Q: Is there a possibility the TTF to be transitory? We have faced a case of twice TFF and they had a baby in another center 2 years after

A: In such inconsistent cases I think we are rather dealing with misdiagnosis (concerning the indication for AOA) than observing a real transitory effect. It seems that we tend to overtreat patients with AOA techniques. The influence of controlled ovarian hyperstimulation and other clinical parameters should also not be underestimated.

Q: Recent literature shows more and more oocyte-related genetic causes of FF which are Ca-independent. How to resolve this?

A: In these cases calcium ionophores won´t be of help. Provided that this question does not aim for techniques such as nuclear transfer or gene editing, there is evidence that agents which can even activate oocytes in the absence of Ca^{2+} (by directly targeting MPF degradation) could be of help (e.g. Zn^{2+}chelator TPEN or puromycin). However, no human application so far to my knowledge.

Personal communication Björn Heindryckx (Ghent): Cases of Total fertilization failure showing oocyte mutations, such as WEE2, could be treated with direct injection of associated cRNA (Sang et al., 2018, Am J Hum Genet).

Q: AOA seems safe. Are we more similar to sea urchins - where calcium change is monotonic - than we could possibly believe?

A: This is a rather provocative statement since there must be a physiological reason for the existence of oscillatory Ca^{2+} pattern in mammalian (e.g., the maintenance of a signal over a very long period of time). However, it stresses that having only one single Ca^{2+} peak is sort of physiological in nature.

Q: Do you have some data in group of patients with AOA in respect of age of women?

A: In principle, the risk to end up in AOA is the higher the older a patient is. However, this is rather not related to the Ca^{2+} machinery behind it but to the number of oocytes available (ovarian reserve).

Q: AOA looks safe, but why you still concern the contraindication? Can you explain more about it?

A: Since a presentation should be well balanced contraindications have to be mentioned. Non of the contraindications listed are a “no go”. It just suggests that under these circumstances ionophores are not the best choice. In terms of safety the absence of physiological calcium oscillations should motivate us to behave cautious.

Q: Do you think that Ca2+ ionophore treatments can improve human embryo development independent of their effects on oocyte activation? Do you have data in cases of developmental arrest. Do we see Ca2+depletion during divisions and could it be restored by extra ionophore incubation?

A: Since embryonic arrest is accompanied by a reduction or even absence of calcium artificial recruitment of the same could be of benefit. This has been shown by our group (Ebner et al., 2015, Hum Reprod) and others (Darwish and Magdi, 2015, RBM Online). There is evidence that AOA as late as 44h post ICSI may be of help (Xi et al., 2020, Gynecol Endocrinol).

Q: If we use testicular sperm, do we need to do oocyte activation to increase fertilization rate, non azoospermia , in failed IVF, high DNA?

A: In our hands (and we are only allowed to apply AOA if literature is available), non-obstructive azoospermia would definitely be an indication to use AOA while obstructive cases are usually not treated with ionophore the first time. Failed IVF should be followed by ICSI and there is no evidence that cases of high DNA damage would benefit from AOA (not considered to be a severe male factor since sperm processing should accumulate better quality sperm).

Q: What is your opinion on possibility of utilization of AOA, its effect on epigenetic process?

A: No evidence so far but of course should be kept in mind and is one argument against overuse of AOA. Healthy live births are reassuring so far.

Q: Would you better use the more "physiological" PLCz instead of ionophore even without mutation in PLCz gene? or is this still not use in the clinic?

A: If available I would prefer recombinant PLC zeta. At least once the optimal concentration is published and of course if the product is CE marked! Not used in clinic so far.

Q: Do you know that there are some antihypertensive drugs that work on Ca channels and can cause TFF? Do you ask patients what they take before you advise AOA?

A: Indeed there are certain drugs affecting calcium channels and as such might influence calcium signals. If this would cause complete fertilisation failure in human IVF is not analysed as far as I know. Would be interesting and easy to follow up since hypertension (plus medication) is recorded during first consultation with clinician.

Q: For SER eggs: 1) what if it just hold in the SER and not being released? 2)do we separate SER eggs and don’t subject them to AOA?

A: The recommendation is that due to the abundance of calcium in such oocytes AOA should not be performed. If I would have a pool of 8 MII and 2 of them would show sER clusters I would only inject and subsequently treat with AOA 6 eggs. If due to the low oocyte number (e.g. n<3) sER MII are also considered for ICSI I would NOT perform AOA.

Q: What do you recommend to the majority of clinics that can't do MOAT, MOCA/HOCA tests to identify male-dependent oocyte activation deficiency?

A: Send the frozen sperm to Ghent for further testing. Ghent-Fertility And Stem cell Team (G-FaST), Department for Reproductive Medicine, Ghent University Hospital.

Q: In primate model, we use ionophore first then following DMAP to mimic calcium oscillation before. Why don't we use it in AOA failed case?

A: In human cloned embryos 6-DMAP showed activation potential but the blastocyst formation was only 20% (Yu et al. 2013, J Tissue Eng Regen Med). For human application in ART no CE certified product is available. There are probably better options in humans.

Q: How about adding calcium ionophore in ICSI sperm with previous failed fertilization?

A: In early times ionophores (A23187) were used to artificially induce acrosome reaction of spermatozoa. There could be the theoretical risk of precocious maturation of sperms. And the only activation-related thing that comes from the sperm is PLCz zeta which is not affected by ionophores. Much more the effect is on the oocyte after the sperm entered the egg.

Time-lapse embryo-culture: limits and profits - Thomas Freour (France)

Q: Would you use TLT for the patients with poor prognosis instead for everybody, although algorithms are poor developed in poor quality embryos?.

A: The clinical benefit of using TLT has not been specifically evaluated in poor prognosis patients to my knowledge. Although it could be speculated that poor quality embryos are more sensitive to suboptimal culture conditions and would therefore benefit more from the very stable culture conditions offered by TLT than high quality ones, this does not rely on scientific evidence and cannot be affirmed.

Q: If you have to renounce to any observations, because of a high workload, which ones do you maintain?

A: I would recommend day 1 for fertilization check, day 2 for abnormal cleavage check and day 5/6 for blastocyst stage

Q: Do you think that a good TL evaluation could allow for day 3 selection and transfer instead of 5-7 days culture?

A: It has been shown that early TL parameters could predict blastocyst formation. However, the current prediction ability for blastulation is not 100% and anyway it has a lower performance than day 5 evaluation / models to rank embryos within a cohort. Further research is needed before considering that early embryo evaluation outperforms blastocyst stage assessment.

ICSI for all or for some? - Gemma Arroyo Cardona (Spain)

Q: What's your opinion about mixed IVF/ICSI cycles?

A: We performed IVF / ICSI in Normozoospermia with previous AI failure, with at least 8MII,

if we don't have at least 4MII in each group, we do ICSI in all oocytes. We use to have the same fertilization results, but sometimes you avoid problems on the fertilization for too much manipulation when abnormal ZP/membrane.

However, sometimes IVF failure occurs. Then patients understand that you have the clue of their infertility problem and they thank the strategy.

Q: What is your opinion on PICSI and severe male factor/high DNA Frag? Would it help in fertilization?

A: There is no evidence from randomized controlled trials (RCTs) to show that it is effective at improving the chances of having a baby for most fertility patients.

Please see: https://www.hfea.gov.uk/search/?search=PICSI

Q: Are there any RCT for the use of combined IVF/ICSI cycle for recurrent intrauterine insemination failure?

A: I did not found anyone. It will be very interesting. If you do, please send me.

I attach a metanalysis for unexplained infertility, hope helpful

Wang R, Danhof NA, Tjon-Kon-Fat RI, Eijkemans MJ, Bossuyt PM, Mochtar MH, van der Veen F, Bhattacharya S, Mol BWJ, van Wely M. Interventions for unexplained infertility: a systematic review and network meta-analysis. Cochrane Database Syst Rev. 2019 Sep 5;9(9):CD012692. doi: 10.1002/14651858.CD012692.pub2. PMID: 31486548; PMCID: PMC6727181.

Q: Which additional sperm tests would be recommended to be more sure about sperm fertilizing capacity and to easily decide for conventional IVF?

A: Spermiograme and Hamster test (sperm penetration analysis using hamster eggs)

Q: At congresses, everybody agrees that ICSI is NOT for everybody, but the reality is very different, as shown by data from registries. Your opinion?

A: It' s important to have a good physician and an andrologist to elucidate the couple background. In egg donation, for example, it should be easy to distinguish between conventional or ICSI. Double donation should be always IVF.

Q: Do you think automated ICSI will improve the outcome of current IVF/ICSI?

A: Maybe it will minimize the risk of degeneration….

Q: Does the rescue ICSI make any difference in outcomes?

A: Not in my experience. Not statistically significative in literature….but for some patients did.

Q: If you work with the HFEA traffic light, where do you place transfer with HA and ICSI for all?

A: ICSI for all is red in some cases as I explained in my talk. In relation to Transfer with HA, I agree with:

Heymann D, Vidal L, Or Y, Shoham Z. Hyaluronic acid in embryo transfer media for assisted reproductive technologies. Cochrane Database Syst Rev. 2020 Sep 2;9:CD007421. doi: 10.1002/14651858.CD007421.pub4. PMID: 32876946.

Q: So ICSI for all is a red traffic light?

A**:** Absolutely

Q: Which your recommendation about preparing method for semen for conventional IVF.

A: Sperm density gradients. Despite only swim up will be better to avoid DNA fragmentation of centrifugation, it is necessary to reach enough concentration and final number of sperm.