ESHRE 2020 Virtual (5-8 July 2020)

Questions for the speakers

Session 48: Relating the relevance of biomarkers to infertility

Does higher level of serum folate before ovarian stimulation worsen outcomes of assisted reproductive technologies in normogonadotropic women? - Oleg Sergeyev (Russia C.I.S.)

Q: Do you have sex ratio, as folate alters maternal inflammatory response deferentially in males and females. i.e. does high folate reduce primary sex ratio?

A: Thank you for the interesting questions, we agree it can be interesting outcome. However, we did not expect that we can identify any difference due to our sample size and did not investigate the sex ratio in relation to folate levels before IVF procedure.

Q: Could you elaborate on the biological mechanism underlying the found associations?

A: It's a very interesting how the elevated folates before and during IVF cycle can interfere with folliculogenesis, hormone production etc. There are different potential ways to speculate this association but, to our knowledge, no any were confirmed. Folate can interplay with estradiol level, and we found in our study the statistically significant negative correlation between baseline folate and estradiol level. Also, *In vitro* studies have been shown that folate, as a precursor to S-adenosylmethionine, is an essential cofactor for the methylation of catechol estrogens and other derivatives. Moreover, magnesium is a cofactor of catechol-O-methyl transferase and influences the methylation and excretion of catechol estrogens. More basic research and epidemiological studies needed to identify and confirm possible biological pathways.

Q: While presenting the data: did the endometrial thickness have any influence? What is your explanation for your findings?

A: Thank you for question, we agree that endometrial thickness is important factor. In our study the embryo transfer was approved when thicknesses of endometrium was at least 8 mm. Also, the assessment of the endometrium for any pathology was provided before IVF cycle. However, we did not investigate the association between endometrial thickness (more than 8 mm) and outcomes due to different aims of parental study.

Q: Lower doses folate acid could negatively affect fetal neurodevelopment or is the lower dose still enough? Or should women increase dosage later?

A: We completely agree that folate deficiency during pregnancy can be harmful for babies. To prevent it women should consume sufficient folates with food and supplements according to WHO recommendations. However the dosage is question. In our study serum folate levels was greater than 20 ng/ml for 50% women (median in our study) and greater than 33 ng/ml for 25% (75^{th} percentile in our study). And we found that higher serum folate was associated with worse IVF outcomes. Further

studies should investigate not only a daily dose of folates and other microelements before IVF but also the total consumption of folate and microelements with food, especially fortified.

Q: Based on your findings what is your opinion on the folate supply in pre-conception period and in pregnancy?

A: We agree that current WHO recommendation of daily folate dose of 400 mkg per day seems to be safe for women and children. However, further pre-conception large-scale studies with known micro- and macronutrient status of both parents, including daily folates doses with food and supplements, as well as serum folate, Ca, Mg, homocysteine and hormone levels are needed.

Q: It would have been interesting to see vitamin D levels.

A: Sure, great suggestion, vitamin D regulates the calcium level in serum, bones and body. Further studies are needed with evaluation of consumption (diet and supplements) and serum vitamin D level.

The mechanism of action of oxytocin receptor antagonists (OTRan) in ART – a study of nolasiban on biomarkers of uterine receptivity in healthy female volunteers - Oliver Pohl (Switzerland)

Q: Could you comment nolasiban triggered gene expression differences. Was it dose dependent given the used high dose and related receptor occupancy

A: Gene expression differences were dose-dependent. At 1800mg, 10 endometrial genes were significantly differentially expressed (adjusted $p<0.05$). At 900mg, there were no differentially expressed genes.

Q: As the contractions seem to decrease over time, do you suggest to applicate Nalosiban even earlier?

A: Uterine contractions may expulse the transferred blastocyst. Thus, our aim is to administer nolasiban 4 hours before ET, thus ensuring that peak nolasiban exposures are reached (Tmax: ~4 hours) and effects on contractions are present at the time of ET. This regimen was based on published experimental regimens with oxytocin receptor antagonists in ART.

Q: How do you interpret your results and future plans in view of the negative clinical data with nolasiban thus far?

A: Nolasiban improved pregnancy and live birth rates in a dose-range finding study and a confirmatory Phase 3 study. A second Phase 3 study did not confirm nolasiban effects on pregnancy rates. Differences between studies that may explain this observation are being investigated. Also studies enabling treatment of ART patients using higher or prolonged treatment regimens are being prepared.