Lead Author Q&A – Aixia Liu

Gut bacteria and amino acid imbalance linked to higher miscarriage risk in women with PCOS

1. What inspired you to investigate the connection between gut microbiota, metabolic changes, and endometrial health in women with PCOS, particularly in relation to adverse pregnancy outcomes?

   In our clinical practice, we noticed that some women with PCOS, despite achieving pregnancy, still experienced disproportionately high rates of miscarriage and other complications, even after successful ovulation induction or embryo transfer. Interestingly, these patients often bear metabolic imbalances, such as elevated triglycerides or insulin resistance, and many reported long-term digestive issues. These observations led us to hypothesise that metabolic disturbances and gut microbial alterations might jointly contribute to poor endometrial function. To explore this, we designed a study that integrates gut microbiota, systemic metabolites, and endometrial biology, aiming to uncover potential links that could explain these adverse outcomes.

2. Were there any unexpected or surprising findings from your study?

   Yes, one of the most surprising findings was the strong link between elevated circulating isoleucine (a branched-chain amino acid) and markers of endometrial stromal cell senescence. We also found that the depletion of *Parabacteroides merdae*, a gut microbe previously linked to cardiovascular health, was associated with both metabolic dysregulation and impaired endometrial function. These findings suggest a previously underappreciated microbial-metabolic-endometrial axis in PCOS.

3. For people less familiar with the field, could you explain what endometrial senescence and impaired decidualisation mean in simple terms? And how do your findings suggest that elevated isoleucine levels might worsen these conditions in women with PCOS?

As women get older, it's normal for the uterine lining, or the endometrium, to undergo some changes in structure and function. This is known as endometrial senescence, and it's often linked to reduced fertility. However, what surprised us was that we observed clear signs of endometrial senescence even in women under 35 with PCOS, suggesting that ageing-like changes can appear much earlier in women with PCOS.

Another key process for a successful pregnancy is decidualisation, where the endometrial cells transform in response to hormones and create a supportive environment for an embryo. When this transformation is impaired, the uterus may not be able to sustain a pregnancy properly.

Our study found elevated levels of isoleucine not only in the blood but also within the endometrial tissues in women with PCOS. In lab experiments, when we added isoleucine to endometrial stromal cells undergoing decidualisation, we observed an increase in markers of cellular senescence and dysfunction, and a decrease in the molecules needed for proper decidualisation. This suggests that high isoleucine levels may worsen endometrial function. Of course, whether this fully applies to all women with PCOS still needs to be confirmed by further research.

4. **Could you clarify what the adverse pregnancy outcomes (APOs) you identified in your study were, and if possible, how frequently they occurred in women with PCOS compared to controls?**

In our study, we looked at a broad range of APOs, including early miscarriages, preterm births, macrosomia, low birth weight, perinatal deaths, and complications such as gestational diabetes mellitus and hypertensive disorders.

Although the overall pregnancy rates were similar between women with and without PCOS, those with PCOS had a significantly higher risk of experiencing at least one APO. In fact, they were nearly twice as likely (1.95 times more likely) to encounter these complications during pregnancy.

5. **Why did you focus on women under 35, and what was the rationale for using the Rotterdam criteria to diagnose PCOS in your cohort?**

We specifically chose to study women under 35 in order to minimise the impact of age-related decline in egg quality. Since advancing age can independently affect fertility and increase pregnancy risks, focusing on a younger population allowed us to more accurately examine how metabolic disturbances might influence endometrial function and pregnancy outcomes in PCOS.

For diagnosis, we applied the Rotterdam criteria, which are used internationally. This definition requires at least two of the following: irregular ovulation, clinical or biochemical signs of high androgen levels, or polycystic ovaries on ultrasound. This definition is internationally recognised and helps ensure consistency when studying PCOS across different populations.

6. You identified *Parabacteroids merdae* and branded-chain amino acids (BCAAs) as potential biomarkers for PCOS-related pregnancy risk - what are the next steps toward validating and using these findings in clinical practice?

The next steps include external validation in larger, more diverse cohorts and interventional studies to test whether modulating these biomarkers, through probiotics, dietary changes, or BCAA-restricted diets, can reduce adverse pregnancy outcomes. If validated, these markers could be incorporated into early pregnancy prediction models and targeted treatment strategies for women with PCOS.