Effect of lactate, pH and microbiomes in regulating human endometrial receptivity and pregnancy outcomes

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Background and aim: WHO 2020 estimated that 48 million couples and 186 million individuals are living with infertility globally. Recent studies suggested that the microbiome, especially the *Lactobacillus*-dominant (LD) environment in the uterus affects embryo implantation and pregnancy outcome in IVF patients. Lactobacilli produce bacteriocins, lactic acid and hydrogen peroxide to suppress the growth of some pathogenic bacteria, re-establishes the normal microbiota and normal vaginal pH. To understand the mechanism of the pH and lactates inside the uterus on embryo implantation, we conducted experiments on Ishikawa and BeWo cells which resemble endometrium and embryos.

Methods: In this study, we used various methods to investigate the role of lactate (D- & L-form) and pH on embryo implantation using the spheroid-endometrial co-culture model and studied the invasion and cytokine production of the endometrial epithelial cells. Concurrently, we employed Next-Generation Sequencing (NGS) analysis to investigate the composition and characteristics of uterine fluid and vaginal fluid samples obtained from the patients.

Results: We demonstrated that high lactate concentrations (>10mM) inhibit the cell viability and proliferation of the human endometrial epithelial Ishikawa and trophoblastic BeWo cells. Culture media at pH 6.1 (acidic), 7.2-7.4 (neutral), and 8.1 (alkaline) conditions did not affect the spheroid attachment on the treated Ishikawa cells. Moreover, high lactate concentrations (>10mM) inhibited the invasion of trophoblastic BeWo by the treated Ishikawa cells, as well as tube formation (angiogenesis) in the treated human endothelial HUVEC cells. D- & L-lactate induce the production of cytokines including TIMPs, MMPs, IL6, IL6R, and Th1 and Th2 cytokines. Taken together, our in vitro studies suggested that high lactate concentrations, but not pH6.1-8.1, did not favour spheroid attachment.

Conclusion: To sum up, our in vitro studies suggested that *Lactobacillus* and Bifidobacteriaceae is most abundant bacteria inside uterine and vaginal fluid. However, high lactate concentrations, but not pH 6.4-8.1, do not significant effect on spheroid attachment. The underlying molecular mechanism of how the LD microenvironment favour pregnancy outcome could be due to changes in cytokines and secretome that modulate endometrial receptivity and immune responses in pregnancy.

Limitations, reasons for caution: This is in vitro study using cancer cell lines. The sample size of uterine and vaginal fluid is small and limited to our IVF patients.

Wider implications of the findings: Our findings suggested lactate and pH levels did not affect spheroid attachment. The effect of lactate-derived cytokines on trophoblast development, and the whether *Lactobacillus*, and *Bifidobacterium* could be promising indicators for pregnancy outcomes prediction warrant further investigation.

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