### O-130 The influence of vaginal microbiota on frozen blastocyst implantation after transfer: a prospective study through next-generation 16S rRNA sequencing

**H. Asakura[1], Y. Nakahara[1], Y. Nagai[2], Y. Sakuraba[2]**

[1]*Ohgimachi Ladeis' Clinic, Reproductive Medicine, Osaka, Japan ;*
[2]*Varinos- Inc., DNA Laboratory, Tokyo, Japan*

**Study question:** A prospective study to investigate the relationship between the composition of vaginal microbiota through next-generation sequencing and the efficacy of single frozen blastocyst transfer in the same cycle.

**Summary answer:** Dominant presence of lactobacillus and other lactate producing microbes in the upper vagina was highly correlated with implantation of transferred blastocyst in this pilot study.

**What is known already:** Next-generation sequencing of 16S rRNA detected microbes in the uterine cavity and recent studies indicated that dominant presence of *Lactobacillus* correlated highly with successful implantation of the transferred embryos. Aberrant vaginal microbiota has been known to cause poor obstetrical outcomes, however little is known for its effect on embryo implantation in assisted reproduction.

**Study design, size, duration:** A prospective study with 25 female subjects transferring a frozen blastocyst using autologous oocyte, over 14 months period in 2019-2020.

**Participants/materials, setting, methods:** 25 female patients without tubal and uterine pathology and no history of multiple miscarriages and implantation failures were recruited with consent at a private ART clinic. Transdermal estrogen was used to prepare endometrium. Upper vaginal fluid was obtained in follicular phase of the the same cycle and analyzed through next-generation sequencing, but the result was reported after pregnancy confirmation. Single frozen blastocyst transfer and standard luteal phase support were performed. Institutional IRB approved the protocol.

**Main results and the role of chance:** The mean age was 36.2 y.o.(range 29-41 y.o.), and 14 gestational sacs (implantation rate 56%), and 3 miscarriage (21.4%) were observed. Next-generation sequencing for 16S rRNA revealed average 69.2% presence of *Lactobacillus* (0-100%) and average 78.0% (0.2-100%) lactate producing microbes (LPM: *Lactobacillus, Bifidobacterium, Streptococcus,* and *Enterococcus*) in the vaginal fluid. Using 90% as cut-off, implantation rates were 11/15 (73.3%) and 3/10 (30%) with *Lactobacillus* dominant and non-dominant, 12/16 (75%) and 2/9 (22.2%) with LPM dominant and non-dominant microbiota, respectively. The difference in each group were not statistically significant. The relative risks for pregnancy were 2.63 (95%CI 1.03-6.67, $P$=0.04)

for *Lactobacillus* and 3.11 (95%CI 1.24-7.79, *P*=0.02) for LPM. As for ROC analysis for embryo implantation and dominant microbes, AUC and associated criterion were 0.62 and 90.7% (sensitivity 78.6%, specificity 72.7%) for *Lactobacillus,* 0.69 and 96.6% (sensitivity 85.7%, specificity 72.7%) for LPM, respectively. The difference of AUC was not significant (*P*=0.24).

**Limitations, reasons for caution:** Despite prospective nature of the study, small sample size limited the analytical power of the study. Aneuploidy screening was not performed to remove confounding factor.

**Wider implications of the findings:** Our pilot study revealed possible relationship between vaginal microbiota and embryo implantation. Dominance of *Lactobacillus* or other lactate producing microbes may be advantageous for successful ART. Sampling vaginal fluid for microbe analysis is less invasive than endometrial sampling and can obtain more abundant RNA with possible higher accuracy of analysis.

**Trial registration number:** not applicable