**TOPIC:** EV (extracellular Vesicles)

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TITLE: Endometrial fluid derived EVs as low invasive diagnostic biomarkers of implantative endometrium.

ABSTRACT: In the last years, increasing embryo implantation rates has become one of the greatest challenges in assisted reproduction techniques. The implantation is a complex process, which requires a synchrony between the development of the embryo and the endometrium, but also, an adequate embryo-endometrial cross talk. The presence of extracellular vesicles (EVs) as mediators of this communication has been described in the endometrial fluid. Moreover, recent studies have shown that endometrial derived EVs are able to modulate the implantative capacity of the embryos and vice versa. Usually an endometrial biopsy is done in order to identify a receptive endometrium, which prevents embryo transfer in the same cycle, as it is detrimental for the implantation. However, the analysis of the endometrial fluid, obtained in a non-invasive manner (without the need of an incision), may constitute an alternative to determine the ideal time for embryo transfer. The hypothesis of this project is that the molecular analysis of the content of the EVs (DNA, mRNA, miRNA and proteins) from endometrial fluid could be a non-invasive method to recognize an implantative endometrium and consequently improve the implantation rates. Therefore, the main objective is to determine by means of a liquid biopsy a signature of miRNAs that allows the identification of an implantative endometrium. The first goal was to establish a robust methodology for analyzing EVs from endometrial fluid in clinical settings, where the sample is limited (5-50uL) and no sophisticated equipment is available. There were compared different sample processing protocols (pure endometrial fluid vs. diluted fluid (with DTT)) and five different methodologies to define a simple and effective strategy for detecting miRNAs associated with EVs from endometrial fluid. Then the selected methodology was applied in a set of real samples with different implantation outcome (fluid from women with successful (n=15) versus unsuccessful implantation (n=15)). The results show that with the proposed methodology, it is possible to isolate and analyze EVs from very low volume of endometrial fluid and it could be implemented in clinical practice to improve implantation rates.