TITLE: Differential Regulation of In Vitro Decidualization by Circulating Exosomes Isolated from Recurrent Pregnant Loss (RPL) Women and Normal Pregnant Women

Name of Authors: **Kanika[1]**, Shivi Khurana[1], Unnati Sharma[1], Jaganmoy Choudhury[1], Bodhana Dhole[1], Pradeep K Chaturvedi[1], Aparna K Sharma[2], Surabhi Gupta[1]

Affiliations: [1]Department of Reproductive Biology, All India Institute of Medical Sciences, New Delhi, INDIA

[2]Department of Obstetrics & Gynaecology, All India Institute of Medical Sciences, New Delhi, INDIA

Presenting Author: **KANIKA**

Email: kanika2808@gmail.com

Corresponding Author: Surabhi Gupta

Email: surabhi@aiims.edu

Proper implantation is a major determinant of successful pregnancy which requires an extensive cross-talk between the trophoblast cells of the blastocyst and the endometrial cells of the receptive uterus. Recently, shedding of exosomes and microvesicles has been identified as an important tool for intercellular communication among several different cell types. However, their importance in mediating embryo-maternal interactions during pregnancy is just beginning to be recognized. Decidualization is one of the important processes required for implantation and further sustenance of pregnancy. Hence, it can be hypothesized that exosomes mediating the maternal-fetal cross-talk influence the decidualization process also.

Previous literature suggests that dysregulation of the decidualization process can lead to recurrent pregnancy loss (RPL). Therefore, the aim of this study was to investigate whether circulating exosomes isolated from pregnant women with RPL history modulate decidualization differently than those isolated from normal pregnant women.

Blood samples were collected from pregnant women with RPL history (n=25), normal pregnant women (gestational age 8-12 weeks; n=20) and healthy non-pregnant women with regular menstrual cycle (n=20). Exosomes were isolated and their size distribution was determined using Nanoparticle Tracking Analysis. The isolated exosomes were characterized by western blotting. Decidualization was induced in human endometrial stromal cell line, THESC cells, by addition of medroxy progesterone acetate (MPA), cAMP and estrogen (EPA mix). THESC

cells were incubated with exosomes isolated from different sources. The effect of exosomes on decidualization was evaluated by observing the morphology and measuring the decidual markers such as prolactin and IGFBP-1.

Exosomes isolated from the serum samples had size in the range of 50 – 150 nm and concentration ranging from 0.5 – 4.0 x 10^{11}/ml. Presence of exosomes was confirmed by western blotting with CD9, CD81 and TSG101 antibody. THESCs treated with EPA mix showed increased levels of the decidualization marker prolactin in a time-dependent manner. THESCs incubated with exosomes isolated from RPL women showed reduced decidualization compared to those incubated with exosomes from normal pregnant women as indicated by lower levels of prolactin and IGFBP-1. Morphological parameters such as area, roundness of cells and f-actin structure were also affected differently by exosomes from the two groups.

In conclusion, our data showed that exosomes isolated from normal pregnant and RPL pregnant women had different effect on regulating the decidualization process.