Hormonal Regulation of a New Cell Model to Examine Mechanisms of Human Blastocyst Initial Attachment

D. M. Phelan[1], M. Marcolongo[1], N. Robertson[2], M. Jost[2] S. Minassian[2]
Department of Materials Science and Engineering, Drexel University[1], College of Medicine, Drexel University[2]

Introduction: It is estimated that one in three pregnancies ends in miscarriage (1). Little is understood about the underlying protein/receptor interactions that are responsible for attachment of the blastocyst to the uterine epithelial tissue. Even *in vitro* fertilization (IVF) sustains only a 30% average success rate. (2) A primary cause of implantation failure in IVF, even after twenty years of practice, is thought to be problems with the initial attachment process (3). A recently reported study (4) has shown that the *initial* attachment of the blastocyst to the uterine wall is mediated by selectin oligosacharide-based ligands. Human trophoblasts (peripheral cells of the blastocyst) were found to express the cell adhesion protein, L-selectin which binds ligand-expressing uterine luminal epithelium in tissue sections. *Further insight into these complex interactions will enable a more comprehensive understanding of the necessary condition to support the formation of human life and may lead to clinically relevant treatments for promoting fertility in vivo as well.* This study established and validated a trophoblast cell model for examining initial attachment events for the presence of L-selectin and L-selectin ligands as well as the expression of these molecules in a hormonally-regulated environment. Normal hormonal stimulation with progesterone and estrogen, as well as stimulation with dexamethasone, a steroid frequently included in the protocol for oocyte retrieval in IVF were used to challenge the cell models.

Materials and Methods: Three choriocarcinoma human trophoblast cell lines, JAR, Jeg-3 and BeWo, were used in this study. Western blotting and flow cytometry were used for L-Selectin and L-Selectin ligand detection in the cell lines. All cell lines were treated in the log growth phase for 24 hours, and lysates for Western Blotting were prepared with a lysis buffer previously described (5) Primary antibodies were Dreg-56 (L-Selectin) and MECA-79 (antibody that recognizes a sulfate and carbohydrate epitope on an important ***class*** L-selectin ligands). To model the human uterine epithelial tissue, Ishikawa cells were tested for basal and hormonal L-selectin ligand expression using Western Blot analysis.

Results: Western blots showed the basal expression of L-Selectin in all trophoblast cell lines, however, flow cytometry showed extracellular expression only in Jeg-3 and BeWo cells. (Figures 1 and 2). Hormonal challenges with estrogen and progesterone resulted in impeded L-selectin expression, while the combination of the two hormones resulted in an upregulation of L-selectin for the JEG-3 cell line. Ishikawa cells showed the presence of L-selectin ligands at basal levels. These ligands were upregulated in the presence of estrogen and progesterone, as well as dexamethasone.

Discussion and Conclusions

Characterization of three trophoblast cell lines for expression and hormonal regulation of L-Selectin has been shown. Jeg-3 and BeWo cells possess the required expression response profiles to be considered an appropriate trophoblast cell models for further studies in attachment and implantation. Ishikawa cells express L-selectin ligands which are hormonally regulated. The combination of trophoblast and human uterine epithelial cell models create a unique cellular environment for exploring initial attachment mechanisms, critical for the formation of human life.

References 1.http://www.healthsquare.com/fgwh/wh1ch27 .htm PDR Fam. Guide Women's Health. 2. Croxatto H, et al. (1978). Am. J.Obstet. Gyn. **132**: 629-634. 3. Carson D., et al. (2000). Dev. Biol. **223**: 217-237. 4. Genbacev, O et al. (2003). Sci. **299**: 405-408. 5. Lessey, BA, et al, (1996) J. Steroid.Biochem.Molec.Biol. **51:** 31-39

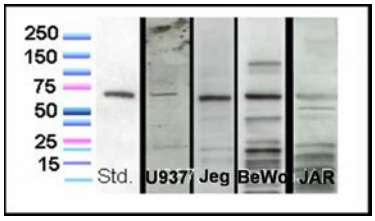

Figure 1: Basal expression of L-selectin is shown in Western Blot of JEG-3, BeWo and JAR cells.

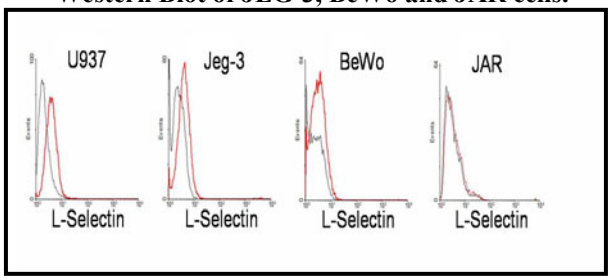

Figure 2: Flow cytometry results demonstrate that JEG-3 and BeWo cells (but not JARs) express L-selectin on the extracellular membrane.

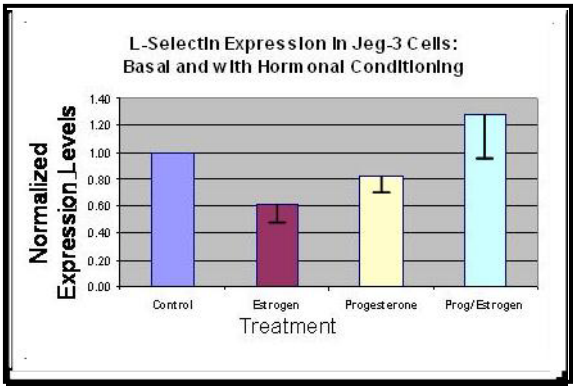

Figure 3: L-Selectin in JEG-3 cells is regulated by hormonal conditioning representative of the luteal and follicular menstrual phases.