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Modulating the endometrial epithelial proteome and secretome in preparation for pregnancy: The role of ovarian steroid and pregnancy hormones

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ABSTRACT

Dialogue between an appropriately developed embryo and hormonally-primed endometrium is essential to achieve implantation and establish pregnancy. Importantly, the point-of-first-contact between the embryo and the maternal endometrium occurs at the endometrial luminal epithelium (LE). Implantation events occur within the uterine cavity microenvironment regulated by local factors. Defects in embryo-endometrial communication likely underlie unexplained infertility; enhanced knowledge of this communication, specifically at initial maternal-fetal contact may reveal targets to improve fertility. Using a human endometrial luminal-epithelial (LE) cell line (ECC1), this targeted proteomic study reveals unique protein changes in both cellular (98% unique identifications) and secreted (96% unique identifications) proteins in the transition to the progesterone-dominated secretory (receptive) phase and subsequently to pregnancy, mediated by embryo-derived human chorionic gonadotropin (hCG). This analysis identified 157 progesterone-regulated cellular proteins, with further 193 significantly altered in response to hCG. Cellular changes were associated with metabolism, basement membrane and cell connectivity, proliferation and differentiation. Secretome analysis identified 1059 proteins; 123 significantly altered by progesterone, and 43 proteins altered by hCG, including proteins associated with cellular adhesion, extracellular-matrix organization, developmental growth, growth factor regulation, and cell signaling. Collectively, our findings reveal dynamic intracellular and secreted protein changes in the endometrium that may modulate successful establishment of pregnancy.

Biological significance: This study provides unique insights into the developmental biology of embryo implantation using targeted proteomics by identifying endometrial epithelial cellular and secreted protein changes in response to ovarian steroid hormones and pregnancy hormones that are essential for receptivity and implantation.

1. Introduction

The endometrium is a highly dynamic tissue that undergoes cyclical remodeling and differentiation each menstrual cycle throughout a women's reproductive life, under the influence of the ovarian steroid hormones. Estrogen dominates the proliferative phase, when the endometrium is regenerated after menses, while progesterone is essential for the cellular differentiation that defines the secretory phase. This differentiation promotes preparation of an endometrium receptive for embryo implantation (Fig. 1). Successful implantation and pregnancy are achieved only when the endometrium and the fertilized embryo develop synchronously [1,2]. The endometrium is receptive to an embryo only during a brief 4-day period in the mid-secretory phase spanning day 20–24 of a normalized menstrual cycle (Fig. 1) when significant molecular and cellular remodeling of the endometrium, and embryo-endometrial interactions via secreted proteins within the uterine cavity, are optimal [1,3,4]. Understanding the changes associated with 'receptivity' and their regulation in the endometrial luminal cells, the first point of interaction between the mother and the embryo, is critical if we are to improve fertility in certain infertile women and/or develop new contraceptives that target the endometrium.

Abbreviations: CI, cell index; CL, cell lysate; CM, culture media; DMEM, Dulbecco's Modified Eagle's Medium; E, estrogen; ECM, extracellular matrix; EP, estrogen plus progesterone; hCG, human chorionic gonadotropin; ITS, insulin-transferrin-selenium MS/MS; NC, nitrocellulose; Nsc, significant normalized spectral count; pEEC, primary endometrial epithelial cells; Rsc, ratio (fold change) of normalized spectral counts; SS, soluble-secreted; SS/Ex, soluble secreted/extracellular vesicles; TF, transferrin; TTPBS, Tween-Tris Phosphate Buffered Saline.

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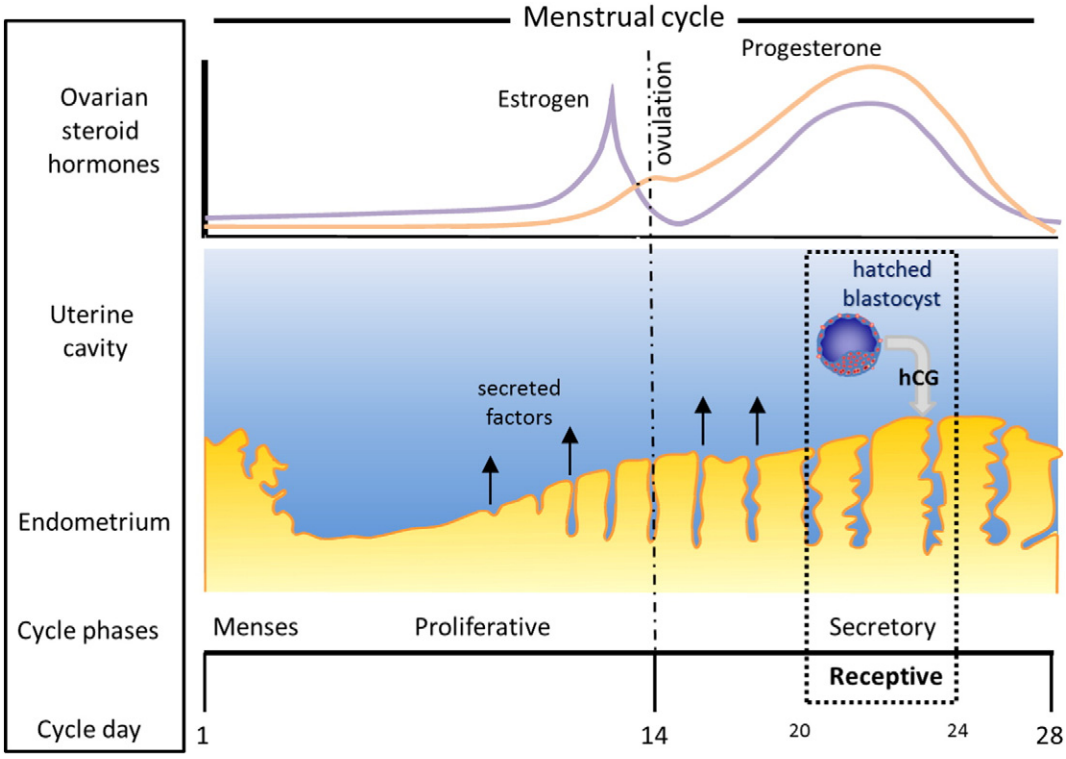

Fig. 1. The menstrual cycle of human endometrium. The endometrial menstrual cycle (normalized as of 28 day duration) is divided into three main phases; menses, proliferative and secretory. The dynamic changes are regulated by the changing levels of ovarian hormones estrogen and progesterone (upper panel). After menstruation, when most of the functional endometrium is shed, endometrial repair and restoration of endometrial thickness occur (driven largely by estrogen action) during the proliferative phase. Following ovulation at mid-cycle, all the cells enter a phase of differentiation, driven by progesterone in the continuing presence of estrogen. Successful embryo implantation can occur only during a brief period in the mid-secretory phase, known as the receptive phase (lower panel). In a conception cycle, when an embryo at blastocyst stage is present, the hatched blastocyst secretes human chorionic gonadotrophin (hCG), that signals to the endometrium to further promote receptivity, enabling implantation and establishment of pregnancy.

Numerous expression profiling studies of the uterine endometrium throughout the menstrual cycle, using discovery-driven methods, have revealed that proliferative and secretory phases can be distinguished at a global level by transcript, microRNA, and proteomic profiling [5–9]. However, the few proteomic studies have used now outdated technologies that detect predominantly high abundance proteins. Both cellular and secreted proteins are differentially regulated between the receptive and non-receptive phases of the menstrual cycle [9–19]. Previous studies identifying 'cellular' changes have analyzed tissue biopsy material which is comprised of multiple cell types; teasing out specific alterations within the endometrial luminal epithelial cells (eLEs), that provide the first contact with the blastocyst, from these studies is therefore impossible. Secreted proteins have been identified in aspirate, lavage and primary endometrial epithelial cell cultures [13,14]. Importantly, such secreted proteins contribute to the extracellular embryo–maternal interactions [20] that are important during the implantation process [1,18,21,22]. Human chorionic gonadotrophin (hCG), one of the earliest proteins secreted by the pre-implantation embryo [23–26] (Fig. 1) is known as a critical signaling hormone for establishment and maintenance of pregnancy [24,27,28]. While effects of hCG on secretion of a specific subset of cytokine and growth factors from endometrial epithelial cells have been determined [29–32], the global eLE protein changes in response to hCG have not been examined.

In this study, using the human endometrial ECC1 cell line which is best representative of eLE, we have specifically analyzed both the cellular and secreted proteins in response to estrogen, (characteristic of the proliferative phase) combined estrogen and progesterone (representing the secretory phase) and these hormones together with hCG mimicking the presence of an embryo. This targeted approach has identified numerous proteins, many previously unknown, associated with endometrial remodeling and particularly those associated with the receptive state. This study provides new understanding of the protein changes induced by both maternal and embryonic hormones, essential for successful implantation and establishment of pregnancy.

2. Materials and methods

2.1. Endometrial epithelial cell line and primary cell isolation and culture

Primary endometrial epithelial cells are difficult to obtain in sufficient quantity for extensive study. Furthermore, most epithelial cells in primary tissues cultures are derived from endometrial glandular epithelium. Since the first point of contact of the embryo is with the endometrial luminal epithelium, the human ECC1 cell line was used as a model for this study. This is an endometrial adenocarcinoma epithelial cell line [33] that closely resembles the luminal epithelium. These ECC1 cells were validated by Karyotype analysis [33,34] according to the ATCC guidelines [35], with allele match in STR profile of 100%. They were cultured and maintained in a 1:1 mix of DMEM and Hams F-12 medium (DMEM/F-12) (Invitrogen-GIBCO, Carlsbad, USA) supplemented with 10% FCS (Invitrogen-GIBCO), 1% (v/v) Penicillin Streptomycin (Pen/Strep) (Invitrogen-GIBCO), and incubated at 37 °C with 5% CO_2 [36]. Validation was performed also with primary human endometrial epithelial cultures.

Ethical approval was obtained for all human sample collections from Human Ethics Committees at Southern Health (#03066B) and Monash Surgical Private Hospital (#04056) and written informed consent was obtained from all women. Endometrial tissue was obtained by dilatation and curettage from women with no known endometrial abnormalities, undergoing minor gynaecological surgical procedures, such as laparoscopic sterilization or investigation of tubal patency or first trimester termination of pregnancy [29]. All women had regular menstrual cycles and no contraceptive or steroid treatment for at least 3 months prior to surgery (mean age 34.6, mean BMI 28.5). Primary endometrial