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Editorial

Proteomic Analysis to Unravel Endometrial Function/Dysfunction

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Infertility is defined as a couple unable to conceive or maintain pregnancy after adequately trying for 12 months. Infertility affects about 10% of the population of reproductive age worldwide and is not only related with health issues but also associated with social and mental stigma, especially for women in developing countries although men and women are both affected equally. Maintaining the focus of this article towards understanding female infertility and proteomics advances for the same. Women of reproductive age undergo cyclic changes termed as menstrual cycle, wherein the endometrium which is the innermost layer of uterus undergo dynamic changes such as growth, differentiation and degradation under the influence of sex hormones.

Function of endometrium is to provide space for implantation to the embryo and subsequently provide nutrition, but embryo implantation is a very time limiting process and endometrium remains hostile towards embryo except during "window of receptivity". This "window of receptivity" is achieved by synchronization of various spatiotemporally regulated functions by different cell types such as luminal epithelium, glandular epithelium, stroma and their secretions [1]. Inability to attain the 'receptivity' renders endometrium incompetent for embryo attachment or implantation and has been postulated as one of the major causes of embryonic loss in women undergoing repeated *in vitro* fertilization failures [2]. Implantation has also been known as a limiting factor in the success of natural reproductive outcome.

To date, the 'gold standard' of evaluating endometrial receptivity has been the histological analysis of the secretory phase endometrial biopsy [3]. The drawback for this method is, it is invasive and thus renders that cycle unusable for embryo transfer and it cannot be used as a predictive tool for endometrium function in future cycles. Moreover, the morphologically similar endometrium can be "out of phase" biochemically. Microarray analyses of human endometrium have led to the identification of several genes, which are expressed differentially during the receptive phase [4]. However, there has not been a consensus on the number and pattern of differentially expressed genes due to various reasons.

The uterine fluid is a biological fluid that bathes the uterine cavity and contains an array of proteins and proteolytic enzymes secreted from the endometrial glands, fallopian tubes and plasma transudates. It lubricates the endometrium, acts as a line of defense against pathogens, signals fertility and aids sperm migration and pregnancy. The collection procedure for uterine fluid is minimally invasive, thus making it an ideal sample for assessment of endometrial function/dysfunction. With the advances in proteomics technologies, few investigations have been undertaken to develop the human uterine fluid proteome and also to identify the quantitative changes in its protein repertoire during the receptive phase.

MacLaughlin and Richardson were the first investigators to use two dimensional gel electrophoresis (2D) technique for the identification of proteins present in human uterine fluid in proliferative and secretory phases [5]. The study revealed significant differences in the protein profiles of proliferative and secretory phase uterine fluid samples. Several proteins were found to appear specifically during the secretory phase in the pI range of 5.9 - 6.4 and in the molecular weight range of 60 - 67 kDa. Parmar., *et al*. [6] generated 2D proteome maps of proliferative and mid-secretory or receptive phase human uterine fluid samples, where she could find identities for several

proteins. A comprehensive catalogue of proteins present in the secretory phase uterine fluid has been developed [7]. To achieve this objective, three different but complementary strategies were used: First, in-solution trypsin digestion followed by reverse phase high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS); second, protein separation by denaturing one-dimensional electrophoresis (SDS-PAGE) followed by HPLC- 33 MS/MS analysis, and finally two dimensional polyacrylamide gel electrophoresis (2DPAGE) followed by MALDI-TOF/TOF analysis. The three different strategies followed for endometrial fluid aspirate analysis led to identification of 391, 489, and 191 proteins, respectively. Combining the three strategies, they successfully identified 803 proteins having different International Protein Index (IPI) accession numbers. However, this study did not focus on the identification of proteins which display differential abundance during the receptive phase. In another study 2D DiGE was employed for the identification of differentially abundant uterine fluid proteins [8]. The study design involved comparison of mid-proliferative and receptive phase uterine fluid protein samples. Seven proteins (three different isoforms of alpha-2-macroglobulin, serum albumin, activin receptor type-2B, alpha-1-antitrypsin, interalpha-trypsin inhibitor family heavy chain-related protein) were found to be differentially abundant in the receptive phase as compared to the mid-proliferative phase uterine fluid. Results from our data indicates, 27 proteins to be differentially expressed during pre-receptive and receptive human uterine fluid using iTRAQ labeling. One of the interesting proteins identified was HMGB1 which on further validation showed that higher levels of HMGB1 during receptivity during receptivity is detrimental for successful pregnancy outcome in rats [9].

The major challenges or road block towards proteomic analysis of uterine fluid may be attributed to difficulties in obtaining phase specific uterine fluid samples from healthy fertile women. Uterine fluid samples have few very abundant proteins masking the identification of low abundant proteins therefore require depletion strategies. The overall protein amount obtained from uterine flushing's or lavage is very low for successful identification of large number of proteins post-depletion.

At present there are very few studies targeted towards identification of biomarker for receptivity in uterine fluid therefore more studies will help to facilitate the development of less invasive marker of endometrial receptivity, which may be of clinical value, especially for women undergoing IVF procedures or for women suffering from recurrent pregnancy loss.

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