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Orchestrating the Menstrual Cycle: Discerning the Music from the Noise

During the menstrual cycle, the uterine endometrium undergoes a remarkable series of structural, cellular, and biochemical changes that ultimately render it capable of receiving an implantation-competent blastocyst (Fig. 1). In the absence of a developmentally normal blastocyst, the endometrium undergoes shedding and regeneration in preparation for yet another round of potential embryo implantation. The successive phases of endometrial tissue growth, differentiation, and remodeling occur in close synchrony with preimplantation development of the embryo. Indeed, infertility largely arises as a consequence of developmental asynchrony between the uterus and the embryo (1, 2).

The now-classic histological evaluations of endometrium from normal cycling women underscored the molecular complexities of this tissue as well as identified distinct stages of endometrial development (3), predominantly orchestrated by the changing levels of circulating estrogen and progesterone (Fig. 1). Perhaps more significantly, these early studies helped establish a foundation for the subsequent development and implementation of *in vitro* fertilization and embryo transfer techniques to alleviate infertility in couples, a blending of basic and translational research. Although reproductive technologies have made a large impact on the problem of human infertility, they suffer from relatively low success rates due, in part, to our current inability to properly distinguish receptive from nonreceptive states of the recipient's endometrium (4). This knowledge gap has stimulated much recent effort to identify individual genes and the transcriptome that underpin cyclic changes in human endometrium and embryo-receptive state. The paper by Talbi *et al.* (5) in the current issue of *Endocrinology* has raised the bar in this active area of research.

Earlier microarray studies that mainly examined receptive *vs.* prereceptive human endometrium were published over the last several years (6–12). These studies showed that relatively large numbers of endometrial genes are induced or repressed in their mRNA expression because the uterus attains the embryo-receptive state. Moreover, some regulated genes were found among multiple studies, whereas others were not consistently identified. Some of the disparities between studies undoubtedly arose from sample heterogeneity and differences in the genomics and bioinformatics platforms used, a major point discussed in the present study.

Strengths of the findings reported here by Taibi *et al.* (5) result from the relatively large number of well-documented subjects (28 normoovulatory women with accompanying careful endometrial staging by multiple pathologists); consistency in endometrial tissue sampling; examination of the entire menstrual cycle; comprehensive microarray platform; and extensive gene and functional annotation. RNA transcripts for 54,600 genes and expressed sequence tags were examined. Using clustering algorithms, the authors found that their microarray data clearly defined four phases of the menstrual cycle and mirrored known histological changes: the proliferative, early secretory, midsecretory (receptive), and late secretory phases. Importantly, the authors were able to classify histologically ambiguous endometrium by application of microarrays, thus potentially solving a major problem confronting reproductive technologists (5).

Each phase of the cycle was distinguished by a unique genetic signature or expression profile consisting of more than a thousand different gene transcripts. Genes whose mRNA abundance differed (>1.5-fold) between successive phases were identified and these when summed across the cycle totaled 7231 genes. These numbers easily dwarfed previous estimates for gene expression differences in this tissue. Interestingly, each cycle phase transition had roughly similar numbers of genes with altered expression. In addition, nearly equal numbers of transcripts were induced or repressed at each transition. Although the physiological significance of the comparable numbers of gene changes remains to be explored, the results confirmed the stunningly complex nature of endometrial cyclicity as driven by seemingly small fluctuations in two circulating hormones, estrogen and progesterone (Fig. 1).

The Talbi *et al.* (5) data set represents the largest addition to a growing collection of microarray data for human and nonhuman primate endometrium and cycling and pregnant rodent uterus (6–18). Many more studies of single genes whose uterine transcripts exhibit estrous cycle-, steroid hormone-, or pregnancy stage-dependence have been published. Perusal of such studies is enlightening, albeit anxiety producing. On the one hand, it is reassuring that gene expression signatures correlate with the classical morphological transitions of cyclic endometrium. However, the sheer numbers of genes that exhibit temporal changes in their expression across the cycle or during early pregnancy are daunting (6–18). The embryo modulates genes within the endometrium to facilitate its attachment and implantation (1, 2); hence, a molecular basis for the required developmental synchrony of uterus and embryo must be factored in, further increasing the repertoire of players and the complexity of the overall process. The degree of overlap in different microarray final gene lists, although less than ideal, nonetheless identifies numerous genes for which corroborating single gene data are available. However, for some genes, the corresponding female knockout mouse is fertile, consistent with multiple levels of functional redundancy built into endometrial gene networks to ensure survival of the species. Clearly the

Abbreviation: PR, Progesterone receptor.

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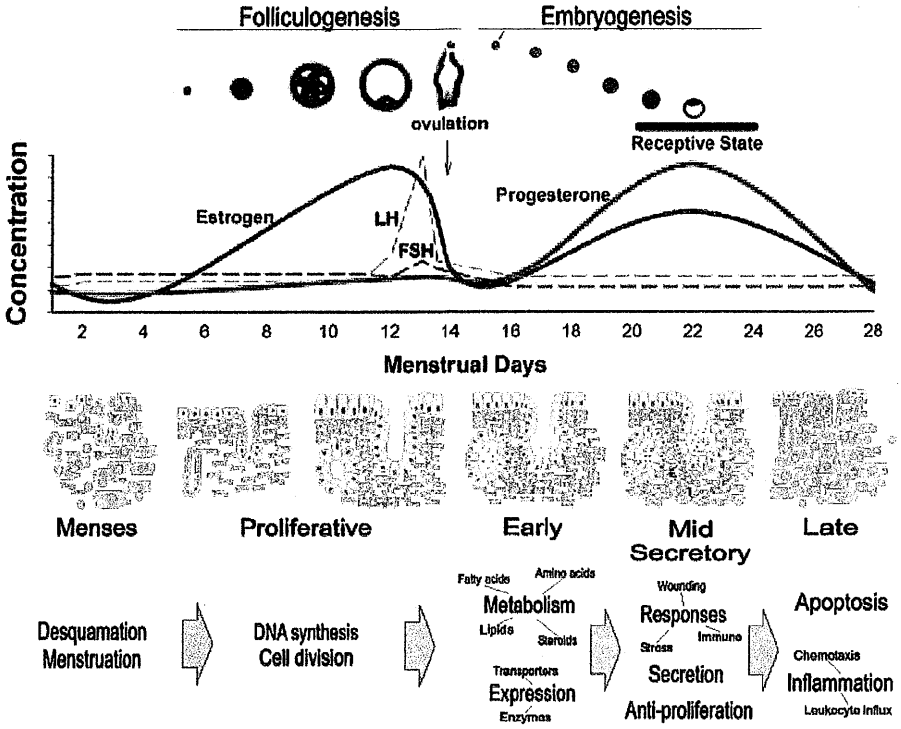

FIG. 1. The human menstrual cycle. After menstruation and desquamation of the endometrium, developing ovarian follicles generate a rise in serum estrogen, which leads to increased cell proliferation in the endometrium. Surges of LH and FSH induce ovulation, thereby releasing an egg capable of fertilization and embryogenesis. The blastocyst implants in a receptive endometrium, attained by transformation from a proliferative/metabolic state to a less proliferative and highly secretory state. Implantation does not occur after this window of receptivity is passed because the endometrium is already destined for apoptosis and tissue remodeling. *Yellow*, Endometrial glandular and luminal epithelium; *brown*, endometrial stroma; *blue*, leukocytes; *red*, spiral arterioles.

endometrium is ripe for examination by the next generation of systems biologists.

What do the Talbi *et al.* (5) and earlier data tell us about this unique tissue? Not surprisingly, the proliferative phase has heightened expression of many genes that drive DNA synthesis and cell division, synthesis and deposition of extracellular matrix, and steroid hormone actions. The early secretory phase, in contrast, is characterized by induction of genes that subserve metabolism and encode various molecular transporters and enzymes. The midsecretory (receptive) phase is antiproliferative and highly metabolically active and exhibits increased expression of genes that govern immune, stress, and wounding responses. Such genes nicely fit the premise of an immune-privileged, implantation-facilitative uterus (10). The late secretory phase exhibits marked apoptosis, inflammatory responses, matrix protein cleavage, chemotaxis, and influx of leukocytes, all of which demarcate the end of the receptive phase and preparing the endometrium for desquamation and menstruation (5).

Dispersed among the most recent and predecessor gene lists for human and model species are several old friends of the uterine biology community: members of the IGF/IGF binding protein, epidermal growth factor and TGFβ families; leukemia inhibitory factor; secretory leukocyte protease inhibitor; several Hox genes; estrogen receptor-α and progesterone receptor (PR); and enzymes of polyamine metabolism. New regulated genes in the uterus include a variety of chemokines and immune response genes; receptor tyrosine kinases such as Axl receptor tyrosine kinase; ligands, inhibitors, and coreceptors of Wnt signaling; nuclear receptors; leptin receptor; and interestingly, classical gut hormones such as gastrin. Some of these genes (AXL, Leptin Receptor, Wnt pathway inhibitors) are induced or repressed in endometrial tumors *vs.* normal endometrium (19), providing insights into how growth control pathways, when gone awry, can lead to endocarcinoma. Surprising was the lack of noted changes in gene expression of nuclear receptor coactivators, whose functions define the direction and magnitude of PR and estrogen receptor trans activity in endometrial cells (20–22). The paucity of microarray data supporting menstrual cycle-dependent changes in these genes suggests their constitutive synthesis, although this should be further evaluated.

Where do we go from here? The gene lists are obvious starting points for unraveling important genetic pathways, abnormal expression of which contributes to infertility, endometrial carcinoma, endometriosis, intrauterine growth retardation, and other disorders of the uterus (5, 15, 19). A more complete understanding of uterine receptivity, uterine stroma decidualization, and molecular mechanisms of progesterone action will enable translational research in fertility, contraception, and premature or delayed delivery. Many menstrual cycle-dependent genes are likely to be regulated, directly or indirectly, by estrogen, progesterone, or the combination. To date, only a few uterine genes have been identified to be direct targets of hormone-bound PRs. The new gene catalogs should facilitate progress in this area.

Related work is identifying the functional roles of growth factors, cytokines, and other soluble mediators that work in concert with estrogen and progesterone and their receptors during uterine cyclicity and implantation (23). The Wnt system clearly warrants further attention in this regard and is poised to become the next favorite of reproductive biologists (5, 23–25). The identification of menstrual cycle-dependent uterine genes implies their importance in human and possibly mouse embryo implantation. The latter can be con-

firmed by careful study of fertility phenotypes of female mice with null mutations in these genes. Recently several elegant studies combined microarray methodology with knockout mouse models to unravel uterine pathways regulated by estrogen and progesterone (26, 27). This combination of approaches may become the standard for probing functionality of uterine-regulatory genes. Embryo-maternal signaling has moved to the forefront of implantation research but is difficult, if not impossible, to study in the human. The further examination, in model organisms, of such relationships and as framed by the new information will surely contribute to our understanding of the endometrium and its interactions with the embryo. Lastly, combining chromatin immunoprecipitation with microarrays (ChIP-CHIP) will be useful for illuminating the connections between uterine nuclear regulatory proteins and their genetic readouts.

The uterus remains a fascinating experimental subject. The microarray data sets of Talbi *et al.* (5) and contemporaries have revealed many new directions for study and highlighted the genetic complexities and biological redundancies of the functional uterus. Undoubtedly biologists pursuing leads such as those presented here will unravel new pathways applicable to pregnancy, cancer, and uterine disorders, including those associated with obesity and use of hormone-replacement therapy. Talbi *et al.* have presented us an entire molecular orchestra: the challenge is to recognize the key players relevant to uterine physiology.

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