Expression of complement system regulatory molecules in the endometrium of normal ovulatory and hyperstimulated women correlate with menstrual cycle phase

The present study describes the temporal expression of complement regulatory molecules membrane cofactor protein (MCP), decay accelerating factor (DAF), CR1, and CD59 in the human endometrium throughout the normal menstrual cycle and in patients submitted to ovarian hyperstimulation. During its proliferative phase, the endometrium expresses MCP, with increased expression during the secretory phase. Phase-dependent expression also was observed for DAF and CD59, mainly in the secretory phase. Expression of CR1 was not detected. These results suggest the presence of complement system activity during the menstrual cycle, with greater expression of regulatory molecules during the secretory phase to protect the epithelial integrity of human endometrium. (Fertil Steril® 2006;86:758–61. ©2006 by American Society for Reproductive Medicine.)

The human endometrium undergoes monthly cycles of proliferation, cellular differentiation, and secretory activity. In the absence of blastocyst implantation, the endometrium collapses, and menstruation occurs (1). These events are regulated mainly by endocrine and neuroendocrine mechanisms, especially hypothalamic hormones, gonadotropins, and steroids secreted by the ovarium. Female steroid hormones, such as estrogen and P, control production of cytokines and growth factors in the uterus (2). It also has been reported that secretion of the C3 complement system protein is controlled by sexual steroids (3).

Regarding complement system regulatory proteins, there are a few controversial studies on their role and expression levels in the endometrium throughout the normal menstrual cycle (4–6), and there are not descriptions of the profile of these molecules in the endometrium of hyperstimulated women. The deleterious effects of supraphysiological levels of estrogen in the endometrium during IVF-ET have been the objects of numerous studies. It has been shown that high levels of estrogen may interfere with endometrial development (7) and with embryo implantation (8). Thus, the use of hormones for treating infertility in IVF-ET procedures may affect the expression pattern of molecules that regulate the complement system.

The goal of the present study is to evaluate the expression of complement system regulatory molecules (membrane cofactor protein [MCP], decay accelerating factor [DAF], CR1, and CD59) in the normal human endometrium throughout the menstrual cycle and to compare this expression with that observed in patients who are submitted to ovarian hyperstimulation.

This study is in accordance with the ethical standards of the committee responsible for human experimentation at the University Hospital of the School of Medicine of Ribeirao Preto, University of São Paulo (Ribeirao Preto, Brazil). Endometrial tissue was collected from 32 ovulating women (average age, 36 years) who had undergone tubal ligation and were not undergoing sex-hormone therapy or treatment with any other medications, at different phases of the menstrual cycle, determined by anatomic–pathological analysis, as described by Noyes et al. (9). Endometrial tissue also was collected from 10 women (average age, 31 years) submitted to ovarian hyperstimulation for IVF-ET. Tissue samples from women submitted to IVF-ET were obtained during the intermediate secretory phase (physiological period in which embryo implantation occurs, on the 7th or 8th day after oocyte retrieval) in patients who did not have embryos transferred. The day on which oocytes were retrieved was considered to be the ovulating day.

The long protocol was applied to ovarian hyperstimulation, starting at the final secretory phase of the previous menstrual cycle, by using leuprolide acetate (Lupron, Abbott, São Paulo, Brazil; 0.2 mL = 1 mg) as GnRH analogue for suppression of the pituitary gland. Ovulation was induced with FSH (Metrodin HP; Serono Laboratories, Randolph, MA). Follicular growth was monitored by transvaginal ultrasonography until administration of hCG (Profasi HP; Serono Laboratories; 1 ampoule = 10,000 UI). Oocyte capture was performed under vaginal ultrasonography 34 to 36 hours after administration of hCG. In these women,

Received October 19, 2005; revised and accepted February 11, 2006.
Supported by Fundação de Apoio ao Ensino, Pesquisa e Assistência do Hospital das Clínicas de Ribeirão Preto (FAEPA, Ribeirão Preto, Brazil).
Reprint requests: José Elpídio Barbosa, Ph.D., University of São Paulo, Department of Biochemistry and Immunology, Avenue Bandeirantes, 3900, Ribeirao Preto, São Paulo, 14049-900, Brazil (FAX: 55-015-16-6336840; E-mail: jebarbos@fmrp.usp.br).

0015-0282/06/$32.00
doi:10.1016/j.fertnstert.2006.02.095

oocytes were not fertilized, and luteal phase complementation was not performed.

The immunohistochemical reaction included the tissue incubation with the respective anti–complement regulatory molecule monoclonal antibodies at 4°C (anti-MCP and anti-CD59 were purchased from Serotec; anti-CR1 was purchased from Dakopatts [Glostrup, Denmark]; and anti-DAF was provided by a colleague). After incubation with primary antibodies, the slides were incubated with biotinylated mouse anti-IgG antibody (Vector Laboratories, Burlingame, CA). Subsequently, the slides were incubated with avidin-biotin-peroxidase complex (Vector Laboratories) for 45 minutes, at room temperature. Reactions were developed with 3-amino-9-ethylcarbazol solution (Sigma, St. Louis, MO) and were visualized under microscopy. Tissue sections were counterstained with Mayer's hematoxylin. Sections of normal human kidney were used as positive control for CR1, and normal placenta was used as control for MCP, DAF, and CD59. As negative control, primary antibodies were omitted in the assays. Intensity of tissue staining was classified by histological score with values 0, 1, 2, 3, and 4 (negative, weak, weak to strong, and strong, respectively).

Data obtained from each subphase were converted into histological score and were analyzed by analysis of variance (Kruskal-Wallis test). Data from each phase, proliferative and secretory, of the menstrual cycle were grouped and compared by the Dunn test. Intraphase analysis (initial, intermediate, and final) also was performed by the same test. To evaluate differences in the expression of complement regulatory molecules in the endometrium from normal women and from women who were submitted to ovarian hyperstimulation during the intermediate secretory phase, the Mann-Whitney nonparametric test was used.

Expression of MCP was observed in glandular epithelial cells but not in stromal cells, and it was observed in vascular endothelium from normal endometrium or from endometrium from women submitted to ovarian hyperstimulation. Differences in MCP expression in glandular epithelial cells were observed between the proliferative and the secretory phases of the menstrual cycle in endometrium samples from women with normal menstrual cycle. However, no differences were observed between the proliferative and secretory subphases (Fig. 1). There was no difference in MCP expression in endometrium from women submitted to ovarian hyperstimulation and from control-group women during the intermediate secretory subphase.

Previous studies did not demonstrate differences in MCP expression throughout the menstrual cycle (4–6). However, most results either are qualitative observations or observations that are restricted to specific subphases of the menstrual cycle and do not adequately represent the phenomenon in a temporal manner. We are assuming that the role of MCP is to protect endometrial cells in a normal situation, in the presence of few active complement system molecules, but this regulatory molecule can be influenced directly or indirectly by hormonal variations. With an increase of C3 in the endometrium during the secretory phase (3), an increase in MCP expression is necessary to protect cells of complement activation. However, such increase in MCP expression does not appear to be enough to protect the endometrium in the secretory phase. Therefore, as observed in this study, expression of other regulatory molecules is required.

Decay accelerating factor was predominant in glandular epithelial cells ($n = 8$), compared with blood vessels ($n = 2$). Stromal cells did not show reactivity for DAF. When proliferative and secretory phases were compared, DAF expression showed differences in glandular epithelial cells (Fig. 1). No difference in reactivity was observed within the subphases, although reactivity was more evident in the intermediate and final secretory subphases.

All samples of endometrium that were obtained from women submitted to ovarian hyperstimulation showed strong reactivity for DAF in the glandular epithelial cells. Expression of DAF was not detected in the stromal cells. No difference was observed in DAF expression between control endometrium and endometrium that was obtained during the intermediate secretory subphase from women who were submitted to ovarian hyperstimulation (Fig. 1).

This finding corroborates with the idea of a defense mechanism against activation of the complement system in the endometrium. Expression of DAF during the secretory phase of the menstrual cycle may be responsible for optimizing protection of endometrial cells during this phase. These results are in agreement with those described by Hasty et al. (4), who described P-dependent expression of DAF in the apical surface of endometrial cells. Recently, Young et al. (10) suggested that DAF expression in the midsecretory phase is stimulated directly by heparin-binding epidermal growth factor (HB-EGF) or by other members of the EGF family that are expressed in the uterus at the time of implantation. These investigators also assumed that the function of up-regulation of DAF is to protect the epithelial integrity of human endometrium against increased complement expression.

Similarly to the case with DAF, CD59 predominantly was detected during the secretory phase. Expression of CD59 was observed mainly on the free surface of glandular cells and also in the vascular endothelium. Differences in CD59 expression were observed between the proliferative and secretory phases. No differences were found between the proliferative subphases, but differences were observed between the initial and intermediate secretory subphases. However, there was no difference in CD59 expression in vascular endothelium between all phases and subphases of the normal menstrual cycle (Fig. 1). Expression of CD59 was not detected in the stromal cells.