Leukemia Inhibitory Factor Expression in Different Endometrial Locations Between Fertile and Infertile Women Throughout Different Menstrual Phases

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Purpose: *The purpose was to demonstrate the leukemia inhibitory factor (LIF) expression in different endometrial locations between fertile and infertile women throughout different menstrual phases. The relationship between progesterone level and LIF expression were evaluated.*
Methods: *Endometrial biopsies were performed on idiopathic infertile and normal fertile women accepted the in follicular, periovulatory, and luteal phases. The luteal progesterone level was measured. Endometrial LIF immunostaining of luminal epithelium, glandular epithelium, and stroma were detected. The relationship between luteal LIF expression and progesterone level was evaluated.*
Results: *Significant LIF expression was noted in the endometrium of fertile women rather than that of infertile women. The LIF expression was highest in the luminal epithelium, moderate in the glandular epithelium, and lowest in the stroma. The luminal and glandular epithelial staining were lowest in follicular phase, moderate in periovulatory phase, and strongest in luteal phase. The stromal LIF presented with a noncyclical manner. The LIF expression is not related with the progesterone level.*
Conclusions: *Endometrial LIF expression is related to human fertility. Endometrial LIF expression is dependent on cellular localizations and menstrual stages. Stronger LIF expression presents in the endometrial epithelium during luteal phase.*

KEY WORDS: Endometrium; leukemia inhibitory factor.

INTRODUCTION

Leukemia inhibitory factor (LIF) plays a role in human reproduction (1,2). This glycoprotein secreted by the endometrium during the implantation window is related to signaling between the endometrium and the blastocyst (3). Vogiagis et al. (4) demonstrated cyclic LIF expression in endometrium. Chen et al. (5) demonstrated that LIF expression is dependent on endometrial cell type. Laird et al. (6) demonstrated that the decreased LIF concentrations occur in women with unexplained infertility. However, few investigators demonstrated the longitudinal and cross-surveys about the endometrial LIF expression in the different menstrual phases and different endometrial locations and its relationship with fecundity. In this series, we compared endometrial LIF expression of different endometrial locations and phases between fertile and infertile women. To our knowledge, this article is among the first reports of this aspect.

MATERIALS AND METHODS

Between January 1998 and December 1998, all idiopathic infertile women in China Medical College Hospital were included in the study group. Normal fertile women were recruited for the control group. Older women (>35-year-old) with oligomenorrhea, polymenorrhea, endometrial polyp, submucosal myoma, and the male infertility (according to World Health Organization criteria) were excluded from this series. No women had used oral contraceptives or an intrauterine device prior to this study. Informed consent was signed by all couples who were recruited. The protocol was approved by the Ethical Committee of the China Medical College Hospital. Approval from the Institutional Review Board had been obtained before this series.

According to the individual menstrual interval (*n*), endometrial biopsy was performed on all patients three times: follicular (menstrual day *n*-22 to day *n*-17),

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periovulatory (menstrual day *n*-16 to day *n*-11), and luteal phases (menstrual day *n*-10 to day *n*-5). The endometrial biopsies were obtained from the uterine fundus using a disposable endometrial curettage (Zinnanti Co., Chatsworth, CA). All specimens were washed with phosphate- buffererd saline (PBS) and stored in 30% sucrose solution (Sigma Co., St. Louis, MO) at 4°C. The glass slide were rinsed with coating buffer (1% $CrK_2(SO_4)_2$, 0.1% gelatin, J.T. Baker), which contain RNase free diethyl pyrocarbonate (DEPC, Sigma Co., St. Louis, MO). The specimens were mounted in an embedding medium (OCT compound; Miles Inc, Elkhart, IN) and sectioned at 6 μm. Then the sections were placed on glass slides and stored at −70°C.

The frozen specimen was warmed at 45°C and washed in 0.1 M PBS. The specimen was put in 0.02 N HCl and rinsed in the 0.1% (vol/vol) Triton-X 100/0.1 M PBS pH 7.4. Then the specimens were divided into three groups: controlled group 1 (DEPC water), controlled group 2 (β-actin 600 pg/ml, Sigma Co., St. Louis, MO), and experiment group (^{35}S-UTP-labeled PBS hLIF 600 pg/ml). The LIF pBluscript cDNA was prepared according to Hsu and Heath (7). A control specimen was included as a positive control. The specimens of experiment group were washed with 0.1 M PBS and rinsed with 0.1% Triton-X100/0.1 M PBS (pH 7.4) and added with 0.01 U proteinase K/1 M PBS (pH 7.4). After the addition of the ^{35}S-UTP-labeled pBluscript human LIF (5 × 106 CPM/ml), the specimen was dehydrated with 70% alcohol and washed with emulsion (Amersham RPN40). After the addition of D-19 solution (Kodak), fixer solution (Kodak), and 2% methyl green (Sigma Co., St. Louis, MO), the specimen was observed under microscopy.

Three locations of LIF presentation were detected: luminal epithelium, glandular epithelium, and stroma. The immunostaining levels for LIF mRNA were divided: absent (0% staining; score 0), weak (1–30% staining; score 1), moderate (31–60% staining; score 2), or strong (61–100% staining; score 3).

The LIF expression in different locations and phases between both groups were compared. All cases accepted the progesterone measurement during the luteal phase. Two groups were divided according to the progesterone level: ≥10 or <10 ng/ml. The relationship between the luteal LIF expression (mean of three locations) and progesterone level was evaluated. Further statistic analyses were performed by using the SAS statistic package with *t*-test. A *P*-value of <0.05 was considered statistically significant.

RESULTS

A total of 41 infertile women and 35 fertile women were enrolled in this study. Ages of both groups were comparable (Table I). Positive immunostaining for LIF was observed in all the specimens between different endometrial locations and phases. In the follicular phases, endometrial LIF expressions of different endometrial locations between fertile and infertile women were nonsignificantly different. In the periovulatory and luteal phase, the LIF expression of fertile women was significantly higher than that of infertile women (Table I).

In the fertile women, the LIF immunostainings of endometrial luminal and glandular epithelium were relatively low in the follicular phase, moderate in the periovulatory phase, and strongest in the luteal phase. In contrast, in the infertile women, the LIF immunostainings of luminal and glandular epithelium were expressed in a noncyclic pattern (Table I). Stromal cells of all patients were expressed in a noncyclical pattern. In the follicular phase, the LIF expressions in different locations were not dfferent in all patients. In the periovulatory and luteal phases of all patients, the LIF expression was highest in the luminal epithelium,

Table I. The Score of Endometrial LIF Immunostaining in Different Locations Between Fertile and Infertile Women During Different Menstrual Phases[a]

	Fertile	Infertile
Patients No.	35	41
Age[b]	33.8 ± 4.1	34.6 ± 3.9
Follicular phase		
Luminal epithelium[b]	1.7 ± 0.7[d,f]	1.6 ± 0.7[e,f]
Glandular epithelium[b]	1.5 ± 0.7[d,e]	1.5 ± 0.7[e,f]
Stroma[b]	1.4 ± 0.6[e,f]	1.3 ± 0.5[e,f]
Periovulatory phase		
Luminal epithelium[c]	2.4 ± 0.7[d,g]	1.7 ± 0.8[e,f]
Glandular epithelium[c]	1.9 ± 0.7[d,g]	1.4 ± 0.7[e,f]
Stroma[c]	1.5 ± 0.7[e,g]	1.2 ± 0.5[e,f]
Luteal phase		
Luminal epithelium[c]	2.7 ± 0.6[d,g]	1.8 ± 0.8[e,g]
Glandular epithelium[c]	2.3 ± 0.7[d,g]	1.4 ± 0.7[e,g]
Stroma[c]	1.6 ± 0.6[e,g]	1.2 ± 0.5[e,g]

[a] Score of LIF immunostaining: score 0 (0% staining); score 1 (1–30% staining); score 2 (31–60% staining); score 3 (61–100% staining).
[b] No difference between fertile and infertile women.
[c] Higher LIF score of the fertile women than that of the infertile women.
[d] Different score between different phase.
[e] No difference between different phase.
[f] No difference between different locations.
[g] Highest LIF score in the luminal epithelium, moderate in the glandular epithelium, and lowest in the stroma.

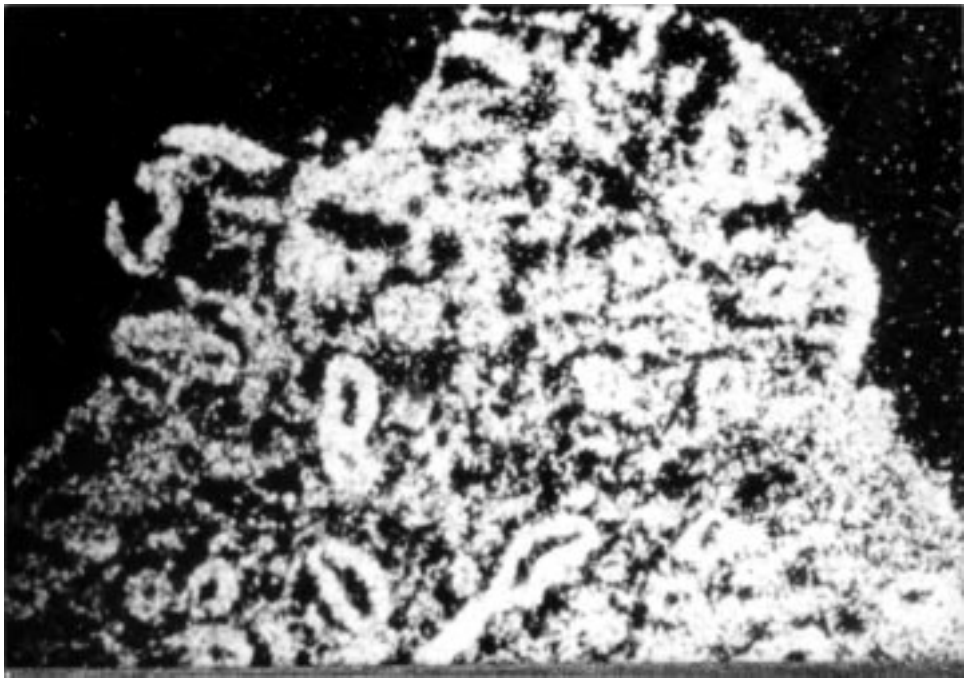

Fig. 1. Endometrial LIF immunostaining of luminal epithelium, glandular epithelium, and stroma.

moderate in the glandular epithelium, and lowest in the stroma (Table I).

During the luteal phase, the LIF expressions of the patients with high or low progesterone level were nonsignificant. The values of the high and low progesterone were 14.3 ± 2.8 and 6.7 ± 1.9, respectively. The LIF scores in both groups were nonsignificant (2.0 ± 0.7 vs. 1.9 ± 0.5).

DISCUSSION

Leukemia inhibitory factor LIF, a pleiotropic cytokine, is essential for blastocyst implantation (1,2). The expression of endometrial LIF mRNA and protein has been described (3,5). The transcript of endometrial LIF is highest around the time of implantation (4). The LIF staining appeared stronger in the mid- and late luteal phase than immediately after ovulation (8). The amount of LIF RNA was low in the follicular phase but increased by approximately six times in the mid- to late luteal phase (3). Glandular epithelial cells of the mid-luteal phase secreted significantly more LIF than at other menstrual stages (9).

Charnock-Jones et al. (3) demonstrated that LIF is present solely in the epithelium during the luteal phase. Cullinan et al. (10) demonstrated that the LIF expression is restricted to the endometrial glands during the luteal phase but is not present in the endometrium during the follicular phase. In contrast, in this series, the LIF protein was present throughout the entire menstrual cycle in luminal epithelium, glandular epithelium, and stroma. Kojima et al. (9) demonstrated that LIF mRNA expression in endometrial glandular epithelium was greater than that in stromal cells. We observed the higher LIF expression in the luminal and glandular epithelium than that of stroma. The noncyclic LIF expression of stroma during different menstrual phases was comparable with the previous report (9).

In this series, LIF expressions in the follicular phase between both groups were comparable. In periovulatory and luteal phases, the LIF expressions of fertile women were statistically higher than those of infertile women. We also observed the maximal LIF level in the mid-luteal phase, which coincided with the time of human implantation. The cyclical changes of LIF in the fertile women suggest a autocrine–paracrine role of LIF in endometrial epithelium. The LIF is essential for providing an ideal environment for embryo implantation.

In conclusion, endometrial LIF expression is related to human fertility. Endometrial LIF expression was dependent on menstrual stage and cellular localization. Endometrial epithelium expresses LIF in a cyclical manner. Epithelial LIF presence is highest at the luteal

phase and lowest at the follicular phase. The stroma LIF presented noncyclical change during different menstrual phases. During the luteal phase, the LIF expression is not related with progesterone level. However, the relationship between endometrial LIF expression and infertility merits further analysis.

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