

Prolactin

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Comparison of Expression of Endometrial Prolactin in Infertile Women with Luteal Phase Defect According to Clomiphene Citrate Administration

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Objective: Clomiphene citrate is one of the most commonly used drugs in the treatment of infertility, but the pregnancy rate achieved with clomiphene citrate is significantly lower than the ovulation rate due to its antiestrogenic effect on the endometrium. Endometrial prolactin is considered to be a marker and an inducer of predecidualization that is characteristic of secretory endometrium. The purpose of this study was to evaluate the association of clomiphene citrate and unsatisfactory endometrial differentiation to secretory endometrium by examining the endometrial expression of prolactin in clomiphene citrate-treated infertile women with luteal phase defect.

Methods: The endometrial samples from infertile women with luteal phase defect (n=27) were examined. Five cases during secretory phase and six cases during proliferative phase were obtained by biopsy. Sixteen cases were obtained by biopsy during secretory phase after clomiphene citrate treatment. By immunohistochemical staining for prolactin, all obtained endometrial tissues were examined. The differences in the endometrial expression of prolactin were evaluated between proliferative phase and secretory phase, and between clomiphene citrate treated group and no treatment group during secretory phase.

Results: The staining of endometrial prolactin was significantly more intense in the glandular epithelial cells and stromal cells in the secretory endometrium than in the proliferative endometrium. The glandular expression of prolactin in the secretory endometrium was not significantly different between the clomiphene citrate-treated group and no treatment group (p=0.719), but the staining of prolactin in the stromal cells was significantly less intense in the clomiphene citrate-treated group than no treatment group (p=0.019).

Conclusion: In this investigation, we demonstrated that the endometrial stromal expression of prolactin in the secretory phase was significantly lower in the clomiphene citrate-treated group compared with no

treatment group in infertile women with luteal phase defect. And our finding suggests that clomiphene citrate may have an adverse effect on the endometrial predecidualization in infertile women.

Key Words: Endometrial prolactin, Clomiphene citrate, Luteal phase defect

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Table 1. Clinical characteristics

	Proliferative phase without CC (n=6)	Secretory phase without CC (n=5)	Secretory phase with CC (n=16)
Age (years)	29.9 ±3.3	31.6 ±3.7	29.0 ±4.6
Parity	0.3 ±0.7	0.1 ±0.3	0.1 ±0.3
Duration*	2.7 ±1.5	3.3 ±1.3	3.3 ±2.2
Biopsy date**	5.4 ±1.9	26.7 ±1.8	25.6 ±2.8
Pathologic date	-	22.7 ±1.1	23.4 ±2.7

CC: clomiphene citrate, Duration*: duration of infertility (years), Biopsy date**: menstrual cycle day when endometrial biopsy was performed

Table 2. Mean expressions of prolactin in endometrium

	Proliferative phase without CC (n=6)	p. value*	Secretory phase without CC (n=5)	p. value**	Secretory phase with CC (n=16)	p. value***
Gland	1.17	p=0.009	2.6	p=0.001	2.4	NS (p=0.719)
Stroma	0.0	p=0.004	2.0	p=0.006	0.9	p=0.019

CC: clomiphene citrate, p. value*: compared with proliferative phase without CC and secretory phase without CC, p. value**: compared with proliferative phase without CC and secretory phase with CC, p. value***: compared with secretory phase without CC and secretory phase with CC

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Figure 2
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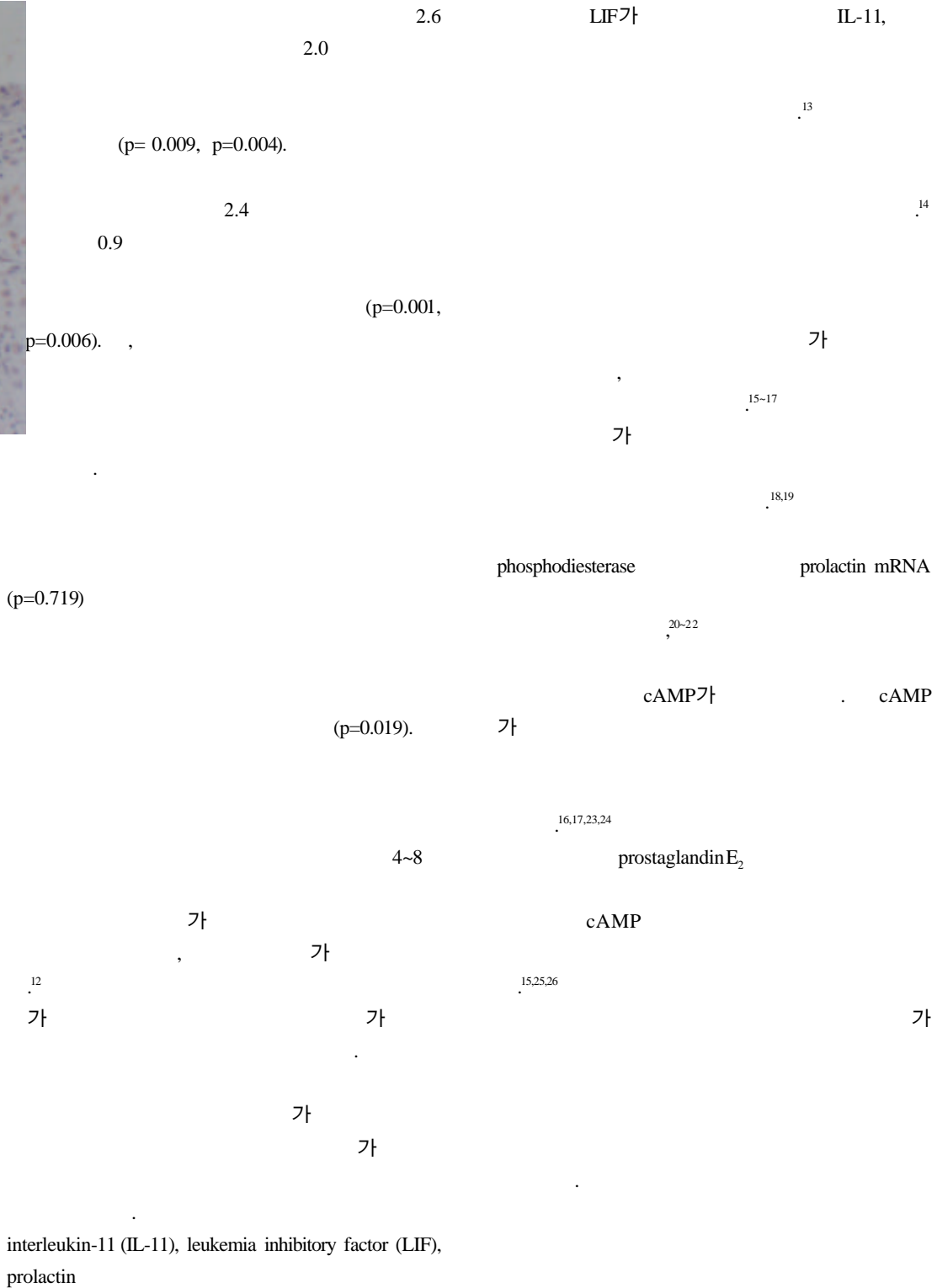
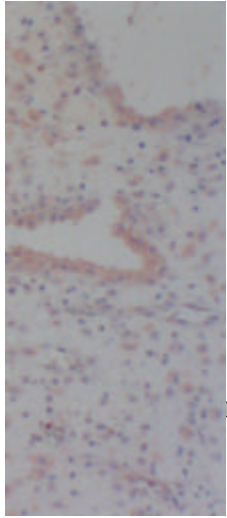


Figure 1. The prolactin expression in endometrium during secretory phase with clomiphene citrate. The stromal cells were weakly stained and the glandular epithelial cells were intensively stained by immunohistochemical staining for prolactin (×100).

Figure 2. The prolactin expression in endometrium during secretory phase without clomiphene citrate. The stromal cells and the glandular epithelial cells were intensively stained by immunohistochemical staining for prolactin (×100).

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1. Blankstein J. Use of clomiphene citrate for ovulation induction. In Collins RL, editor *Ovulation Induction*. New York: Springer, 1991: p.62-8.
2. Fritz MA, Holmes RT, Keenan EJ. Effect of clomiphene citrate treatment on endometrial estrogen and progesterone receptor induction in women. *Am J Obstet Gynecol* 1991; 165: 177-85.
3. Adashi EY. Ovulation induction: clomiphene citrate. In: Adashi EY, Rock JA, Rosenwaks Z, editors. *Reproductive endocrinology, surgery and technology*. Philadelphia: Lippincott-Raven Publishers, 1996: p. 1182-206.
4. Nelson LM, Hershlag A, Kurl RS, Hall JL, Stillman RJ. Clomiphene citrate directly impairs endometrial receptivity in the mouse. *Fertil Steril* 1990; 53: 727-

- 31.
5. Li TC, Warren MA, Murphy C, Sargeant S, Cooke ID. A prospective, randomised, cross-over study comparing the effects of clomiphene citrate and cyclofenil on endometrial morphology in the luteal phase of normal fertile women. *Br J Obstet Gynaecol* 1992; 99: 1008-13.
 6. Gelety TJ, Buyalos RP. The effect of clomiphene citrate and menopausal gonadotropins on cervical mucus in ovulatory cycles. *Fertil Steril* 1993; 60: 471-6.
 7. Fazlebas AT, Yeko TR, Connelly KM. Effect of clomiphene citrate on the synthesis and release of the human b-lactoglobulin homologue, pregnancy associated endometrial a₂-globulin, by the uterine endometrium. *Hum Reprod* 1991; 6: 783-90.
 8. Yeko TR, Nicosia SM, Maroulis GB. Histology of mid-luteal corpus luteum an endometrium from clomiphene citrate-induced cycles. *Fertil Steril* 1992; 57: 28-32.
 9. Bonhoff A, Naether O, Johansson E, Bohnet HG. Morphometric characteristics of endometrial biopsies after different types of ovarian stimulation for infertility treatment. *Fertil Steril* 1993; 59: 560-6.
 10. Massai MR, de Ziegler D, Lesobre V. Clomiphene citrate affects cervical mucus and endometrial morphology independently of the changes in plasma hormonal levels induced by multiple recruitment. *Fertil Steril* 1993; 59: 1179-86.
 11. Miller RT, Hapke MR, Greene GL. Immunohistochemical assay for estrogen receptor with monoclonal antibody D753P in routinely processed formaldehyde-fixed breast tissue. *Cancer* 1993; 71: 3541-6.
 12. Carpenter SE. Implantation. In: Wallach EE, Zacur HA, editors. *Reproductive medicine and surgery*. St. Louis (MO): Mosby, 1995: p.158-65.
 13. Dimitriadis E, Salamonsen LA, Robb L. Expression of interleukin-11 during the human menstrual cycle: coincidence with stromal cell decidualization and relationship to leukemia inhibitory factor and prolactin. *Mol Hum Reprod* 2000; 6: 907-14.
 14. Braverman MB, Bagni A, de Ziegler D, Den T, Gurside E. Isolation of prolactin-producing cells from first and second trimester decidua. *J Clin Endocrinol Metab* 1984; 58: 521-5.
 15. Tang B, Gurside E. Direct effect of gonadotropins on decidualization of human endometrial stroma cells. *J Steroid Biochem Mol Biol* 1993; 47: 115-21.
 16. Tabanelli S, Tang B, Gurside E. In vitro decidualization of human endometrial stromal cells. *J Steroid Biochem Mol Biol* 1992; 42: 337-44.
 17. Benedetto MT, Tabanelli S, Gurside E. Estrone sulfate sulfatase activity is increased during in vitro decidualization of stromal cells from human endometrium. *J Clin Endocrinol Metab* 1990; 70: 342-5.
 18. Zhu HH, Huang JR, Mazella J, Rosenberg M, Tseng L. Differential effects of progestin and relaxin on the synthesis and secretion of immunoreactive prolactin in long term culture of human endometrial stromal cells. *J Clin Endocrinol Metab* 1990; 71: 889-99.
 19. Tseng L, Gao J-G, Chen R, Zhu HH, Mazella J, Powell DR. Effect of progestin, antiprogestin, and relaxin on the accumulation of prolactin and insulin-like growth factor-binding protein-1 messenger ribonucleic acid in human endometrial stromal cells. *Biol Reprod* 1992; 47: 441-50.
 20. Kofinas AD, Rose JC, Meis PJ. Changes in cyclic adenosine monophosphate-phosphodiesterase activity in pregnant and non-pregnant human myometrium. *Am J Obstet Gynecol* 1987; 157: 733-8.
 21. Shapiro DJ, Blume JE, Nielsen DA. Regulation of messenger RNA stability in eukaryotic cells. *Bio-Essays* 1987; 6: 221-6.
 22. Baez M, Sargan DR, Elbrecht A, Kulomaa MA, Zaruchi-Schulz T, Tsai MJ, O'Malley BW. Steroid hormone regulation of gene encoding the chicken heat shock protein Hsp 108. *J Biol Chem* 1987; 262: 6582-8.
 23. Tang B, Guller S, Gurside E. Cyclic adenosine 3', 5'-monophosphate induces prolactin expression in stromal cells isolated from human proliferative endometrium. *Endocrinology* 1993; 133: 197-203.

24. Richards RG, Brar AK, Frank GR, Hartman SM, Jikihara H. Fibroblast cells from term decidua closely resemble endometrial stromal cells: induction of prolactin and insulin-like growth factor binding protein-1 expression. *Biol Reprod* 1995; 52: 609-15.
25. Yong EL, Baird DT, Hillier SG. Transduction of gonadotropin effects on human granulosa cells by cyclic AMP: on molecule, two messages. In: Sjoberg NO, Hamberger L, Janso PO, Owman C, Coelin GH, Bennink HJT, editor. *Local Regulation of Ovarian Function*. Parthenon: Lancs, 1992: p.123-7.
26. Frank GR, Brar A, Cedars M, Handwerger S. Prostaglandin E2 dramatically enhances endometrial stromal cell differentiation. 75th Annual Meeting of The Endocrine Society, Las Vegas NV, 1993: p.350 (Abstract 1197).
27. Bonhoff AJ, Naether OGJ, Johannisson E. Effects of clomiphene citrate stimulation on endometrial structure in infertile women. *Jum Reprod* 1996; 11: 844-9.
28. Nakamura Y, Ono M, Yoshida Y, Sugino N, Ueda K, Kato H. Effects of clomiphene citrate on the endometrial thickness and echogenic pattern of the endometrium. *Fertil Steril* 1997; 67: 256-60.
29. Grunfeld L. Physiology of the menstrual cycle. *Ultrasonography in reproductive medicine. Infertil Reprod Med CLin North Am* 1991; 2: 637-97.
30. Johannisson E, Parker RA, Landgren B-M, Diczfalusy E. Morphometric analysis of the human endometrium in relation to peripheral hormone levels. *Fertil Steril* 1982; 38: 564-71.
31. Hiramata Y, Ochiai K. Estrogen and progesterone receptors of the out-of-phase endometrium in female infertile patients. *Fertil Steril* 1995; 63: 984-8.