

Luteinizing Hormone(LH)

Expression of Luteinizing Hormone(LH) Gene in Human Uterus

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ABSTRACT

Recent studies, including our own, demonstrated that the novel expression of LH gene in rat gonads and uterus, indicating that the local production and action of the LH-like molecule. In the present study, we investigated whether human uterus also expresses the LH gene. Reverse transcription-polymerase chain reaction(RT-PCR) amplified the cDNA fragments coding LH β polypeptide from human endometrium but not from myometrium. Presence of the transcripts for the α -subunit in human endometrium was also confirmed by RT-PCR. Transcripts for LH β subunit were detected in endometrial samples from women with endometriosis. The gene for LH/hCG receptor was expressed in both endometrium and myometrium, showing good agreement with previous studies. Increased level of LH β transcript was determined in the endometrium from follicular phase compared to that from luteal phase. Taken together, our findings demonstrated that 1) the genes for LH subunits and LH/hCG receptor are expressed in human uterus, 2) the uterine LH expression was changed during menstrual cycle, suggesting that the uterine LH may play a local role in the control of uterine physiology and function(s).

follicle stimulating hormone(FSH) luteinizing hormone (LH) .
 gonadotropin-releasing hormone(GnRH) ,
 .

. LH FSH thyroid stimulating hormone(TSH) chorionic gonadotropin(CG) glycoprotein
 hormone family ¹. -subunit -subunit
 heterodimer ². LH
 human Chorionic Gonadotropin(hCG)
 LH LH-like molecule . 가 .
 , LH 가 ³⁻⁶.
 LH/hCG 가 ^{7,8}, LH가
 LH CG .
 LH가 , LH/hCG
^{9,10}. LH 가 가
 (endometrium) (myometrium) RNA reverse transcription-polymerase chain
 reaction(RT-PCR) .

1.

Phosphate buffered saline(PBS)

RNA

2. RNA DNA

(1) Total RNA

total RNA Trizol (GIBCO-BRL)¹¹
RNA pellet 75% ethanol 1 0.1% DEPC-water UV
spectrophotometer $A_{260} : A_{280}$

(2) Reverse transcription-polymerase chain reaction(RT-PCR)

RNA reverse transcriptase(SuperScript RT RNase H⁻; GIBCO-BRL) cDNA
PCR 가 exon 20 base pair(bp) 5' 3' primer (
, cDNA *Taq* DNA polymerase(Takara) PCR PCR
94°C 2 denaturation 1 denaturation(94°C, 30), annealing(54°C, 30),
elongation(72°C, 1) 35 1 extension(72°C, 10)
PCR touchdown , annealing primer $T_m + 2^\circ\text{C}$
1 cycle 0.3°C 40 cycle
(2% agarose gel) ethidium bromide LH subunit PCR primer
exon 2 5'-AGCCGCTTCGGCCATGGTGC-3'(sense) exon 3
5'-GTCCACAGCGACAGCTGAGA-3'(antisense)¹², -subunit PCR primer
5'-GCTCCTGATGTGCAGGATTG-3'(sense) 5'-ACAAGTACTGCAGTGGCACG-3' (antisense)¹³.
LH/hCG PCR primer 5'-ATGCACAGTGGAGCCTTCC-3'(sense)
5'-CTCCAGTGAAGTCAGTGTCG-3' (antisense)¹⁴.

(3) PCR DNA sequencing

RT-PCR cDNA 가 DNA extraction
kit(Quigen) dideoxy chain termination-PCR PCR sequencing kit(
)

LH subunit RT-PCR RNA 261 bp -subunit
268 bp -subunit cDNA fragment가 (Fig. 1A). LH subunit 500 bp band
RNA genomic DNA RNA 1
PCR cDNA PCR-sequencing
-subunit (data not shown).

LH PCR

(Fig. 1B).

(Fig. 1C). LH PCR luteal phase follicular phase
LH/hCG PCR 1306 bp cDNA

(Fig. 2).

GnRH 가 LH 가 가 LH

가 , LH , LH 가 , LH agonist hCG

LH , CG CG corpus luteum 가

^{15,16,6} LH/hCG 가 ⁷ LH/hCG

¹⁷ LH/hCG 가 ^{9,18} alternative splicing isoform 가

cDNA LH/hCG 가

(growth factors)

cytokine ¹⁹ GnRH LH가

(apoptosis) LH

^{20,21} Follicular pahse luteal phase

LH LH 10-15%

LH 가 , 가 GnRH

analog LH LH

LH/hCG LH LH hCG

가 , LH LH가 가

1. Albanese C, Colin IM, Crowley WF, Ito M, Oestell RG, Weiss J, Jameson JL. The gonadotropin genes : evolution of distinct mechanisms for hormonal control. *Recent Prog Horm Res* 1996; 51: 23-58.
2. Ryan RJ, Chalesworth MC, McCormick DJ, Milius RP, Keutmann HT. The glycoprotein hormones : recent studies of structure-function relationships. *FASEB J* 1988; 2: 2661-9.
3. Zhang FP, Markkula M, Toppari J, Huhtaniemi I. Novel expression of luteinizing hormone subunit genes in the rat testis. *Endocrinology* 1995; 136: 2904-12.
4. Zhang FP, Rannikko A, Huhtaniemi I. Isolation and characterization of testis-specific cDNAs for luteinizing hormone -subunit in the rat. *Biochem Biophys Res Commun* 1995; 210: 858-65.
5. Lee SH. Expression of luteinizing hormone(LH) subunit genes in the rat ovary. *Kor J Fertil Steril* 1998; 25: 199-205.
6. Lee SH, YK Lee. Expression of luteinizing hormone(LH) gene in rat uterus and epididymis. *Kor J Fertil Steril* 1999; 26: 157-61.
7. Ziecik AJ, Derecka-Reszka K, Rzedzido SJ. Extragonadal gonadotropin receptors, their distribution and function. *J Physiol Pharmacol* 1992; 43(4 Suppl 1): 33-49.
8. Lin J, Lei ZM, Lojun S, Rao CV, Satyaswaroop PG, Day TG. Increased expression of luteinizing hormone/human chorionic gonadotropin receptor gene in human endometrial carcinomas. *J Clin Endocrinol Metab* 1994; 79: 1483-91.
9. Stewart EA, Sahakian M, Rhoades A, Van Voorhis BJ, Nowak RA Messenger ribonucleic acid for the gonadal luteinizing hormone/human chorionic gonadotropin receptor is not present in human endometrium. *Fertil Steril* 1999; 71:368-72.
10. Rao CV. No mRNAs for the LH/hCG receptor in human endometrium? *Fertil Steril* 1999; 72: 374-5.
11. Chomzynski P, Sacchi N. Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 1987; 162: 156-9.
12. Talmadge K, Vamvakopoulos NC, Fiddes JC. Evolution of the genes for the beta subunits of human chorionic gonadotropin and luteinizing hormone. *Nature* 1984; 307: 37-40.
13. Fiddes JC, Goodman HM. Isolation, cloning and sequence analysis of the cDNA for the alpha-subunit of human chorionic gonadotropin. *Nature* 1979; 281: 351-6.
14. Minegishi T, Nakamura K, Takakura Y, Miyamoto K, Hasegawa Y, Ibuki Y, Igarashi M. Cloning and sequencing of human LH/hCG receptor cDNA. *Biochem Biophys Res Commun* 1990; 172: 1049-54.
15. Dong KW, Marcelin K, Hsu MI, Chiang CM, Hoffman G, Roberts JL Expression of gonadotropin-releasing hormone (GnRH) gene in human uterine endometrial tissue. *Mol Hum Reprod* 1998; 4: 893-8.
16. Ikeda M, Taga M, Sakakibara H, Minaguchi H, Ginsburg E, Vonderhaar BK. Gene expression of gonadotropin-releasing hormone in early pregnant rat and steroid hormone exposed mouse uteri. *J*

Endocrinol Invest 1996; 19: 708-13.

17. Ojeda SR, Female reproductive function. In: Griffin JE, Ojeda SR. editor. Textbook of endocrinology. 3rd ed. UK: Oxford University Press; 1995. p. 164-200.
18. Tsai-Morris CH, Geng Y, Buczko E, Dufau ML. A novel human luteinizing hormone receptor gene. *J Clin Endocrinol Metab* 1998; 83: 288-91.
19. Simmen FA, Simmen RCM. Peptide growth factors and proto-oncogenes in mammalian conceptus development. *Biol Reprod* 1991; 44: 1-5.
20. Billig H, Furuta I, Hsueh AJW. Gonadotropin-releasing hormone directly induces apoptotic cell death in rat ovary : Biochemical and in situ detection of deoxyribonucleic acid fragmentation in granulosa cells. *Endocrinology* 1994; 134: 245-52.
21. Billig H, Furuta I, Rivier C, Tapanainen J, Parvinen M, Hsueh AJW. Apoptosis in testis germ cells : Developmental changes in gonadotropin dependence and localization to selective tubule stages. *Endocrinology* 1995; 136:5-12.

Figure legends

Figure 1. Detection of the transcripts for LH subunits in human endometrium. **A**; PCRs for amplification of α -subunit and LH β cDNA. m, DNA size marker, 1, RT using single extracted RNA sample; 2, RT using double extracted RNA; (-), negative control. **B & C**: human LH β PCR. m, DNA size marker; E, endometrium; M, myometrium; Es, endometriosis; F1 and F2, samples form follicular phase; L1 and L2, samples from luteal phase;

Figure 2. Amplification of LH/hCG receptor cDNA by RT-PCR using human uterine RNAs.

m, DNA size marker; E, endometrium; M, myometrium; Es, endometriosis; (-), negative control.