

# Luteinizing Hormone(LH)

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## Expression of Luteinizing Hormone(LH) Gene in Rat Uterus and Epididymis

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## ABSTRACT

Recent studies clearly demonstrated that the novel expression of LH gene in the rat testis, and suggested the local action of the LH-like molecule. The present study was performed to analyze the expression of LH genes in the rat accessory reproductive organs. Expression of LH subunit genes in the rat uterus and epididymis was demonstrated by reverse transcription-polymerase chain reaction(RT-PCR) and specific LH radioimmunoassay(RIA). The LH $\beta$  transcripts in these organs contained the published cDNA structure, the pituitary type exons 1-3, which encoded the entire LH $\beta$  polypeptide. Presence of the transcripts for the  $\alpha$ -subunit in the rat reproductive tissues were also confirmed by RT-PCR. In the LH RIA, significant levels of LH were detected in crude extracts from the rat ovary, uterus and epididymis. The competition curves with increasing amount of tissue extracts were parallel with those of standard peptide, indicating that the immunoreactive LH-like materials in these tissues are similar to authentic pituitary LH molecule. In rat epididymis, the highest amount of immunoreactive LH were detected in corpus area. Our findings demonstrated that the genes for LH subunits are expressed in the rat accessory reproductive organs, and suggested that these extrapituitary LH may act as a local regulator with auto and/or paracrine manner.

**Key words : LH, Gene expression, Uterus, Epididymis, Local regulator**

gonadotropin-releasing hormone(GnRH)  
gonadotropin, follicle stimulating hormone(FSH) luteinizing  
hormone(LH) . . FSH LH 가  
, LH 가  
(Zhang *et al.*, 1995a,b; Lee, 1998).  
LH LH가 steroidogenesis  
(El-Gehani *et al.*, 1998), LH가 가  
가 LH  
human Chorionic Gonadotropin(hCG) progesterone .  
(Griffin and Ojeda, 1995),  
LH-like molecule . 가 .  
. LH 가 .

1.

25

(Sprague-Dawley strain)

pregnant mare's serum gonadotropin(PMSG; 15 IU/rat, Sigma)

(Lee *et al.*, 1994).

( 4 )

RNA

LH

-70°C

2.

### (Radioimmunoassay, RIA)

LH 1 N acetic acid(10 vol)

1 N HCl acidify . Chloramine-T I<sup>125</sup>(NEN)

LH reference peptide(NIDDK, Bethesda, USA) anti-rat LH antiserum( 1:200,000)

(72 hour protocol)

(Lee *et al.*, 1994).

### 3. RNA DNA

#### (1) Total RNA

RNA acid phenol- guanidium isothiocyanate- chloroform (Chomzynski and Sacchi, 1987) Trizol (GIBCO-BRL)

pellet 75% ethanol 0.1% DEPC- water

UV spectrophotometer

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

RNA reverse transcriptase(SuperScript RT RNase H<sup>-</sup>; GIBCO-BRL)

cDNA . LH exon 20 base pair

5' 3' primer oligomer ( ), 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 ) .

(2% agarose gel) ethidium bromide . PCR

primer LH subunit 5'- GT GCCGGCCT GT CAACGCAAC -3'(sense)

5'-CAGCTCATTGGTTGAGTCCTG-3'(antisense) , -subunit 5'-ATACTTCTCCAAG

CTGGGTGC-3'(sense) 5'-CGACACTCAGTGCCATCGCAG-3'(antisense) (Godine *et al.*,

1982; Zhang *et al.*, 1995b).

#### (3) PCR DNA sequencing

RT-PCR cDNA

가

DNA fragment

dideoxy chain termination-PCR

PCR sequencing kit

( )

LH

transcript RT-PCR , RNA LH subunit  
 bp (Fig. 1A). - subunit transcript cDNA 306  
 294 bp cDNA가 PCR (Fig. 1B). PCR cDNA  
 PCR-sequencing - subunit  
 (data not shown). LH ,  
 LH RIA , LH standard curve  
 competition curve immunoreactive LH-like  
 가 (Fig. 2). LH 1/10  
 , homogenate LH RIA  
 corpus LH 가  
 (41.5 pg/mg), caudal(27.8 pg/mg) caput(24.2 pg/mg) (Fig. 3).

LH transcript

(Zhang *et al.*, 1995a; Lee, 1998),

LH RIA LH - subunit  
 subunit 가 . LH가  
 LH , LH  
 LH  
 10 ,  
 10 GnRH 가  
 GnRH .  
 gonadotropin

(i) steroidogenesis , (ii) , (iii) (oocyte maturation) , (iv) , (v) (apoptosis)  
(Hsueh *et al.*, 1994; Richards, 1994; Gnessi *et al.*, 1997).

가 , (seminal vesicle), GnRH가  
(Khodr and Siler-Khodr, 1980; Izumi *et al.*, 1985; Ikeda *et al.*, 1996),  
GnRH chorionic gonadotropin 가 (Siler-Khodr *et al.*,  
1986). GnRH-LH 가 .

GnRH가 LH가  
- GnRH LH (endocrine)  
GnRH LH autocrine paracrine 가  
. LH GnRH  
(circuitry) , LH  
GnRH  
. LH ,  
LH가 (excretion) 가 .

LH FSH thyroid stimulating hormone(TSH) chorionic  
gonadotropin glycoprotein hormone family (Albanese *et al.*, 1996).  
- subunit , - subunit가  
- subunit가 (Ryan *et al.*, 1988).  
LH subunit 3  
5' untranslated region(UTR) (3.2, 2.4, 0.86 kb)가  
(0.8 kb) (Zhang *et al.*, 1995b). LH  
가 ,  
(round type spermatid) (spermiogenesis)  
(Zhang *et al.*, 1995b).  
LH pregnant mare's serum gonadotropin(PMSG)  
(Lee, 1998), LH .  
LH LH

LH

LH subunit (RT-PCR)

LH

LH $\beta$  subunit exon primer RT-PCR

306 bp band가 , LH, FSH, TSH

hCG -subunit PCR

LH standard curve , curve가

sigmoid immunoreactive LH가

LH가

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## Figure legends

**Figure 1.** Detection of the transcripts for rat LH subunits in the rat reproductive tissues by RT-PCR. PCR and electrophoresis were carried out as described in Materials and Methods. **A**; Amplification of cDNA fragments coding the LH $\beta$  regions commonly found in the rat pituitary and testis. **B**; Amplification of cDNA fragments from the  $\beta$ -subunit transcripts. M, DNA size marker; P, pituitary; T, testis; E, epididymis; O, ovary; U, uterus; (-), negative control.

**Figure 2.** LH RIA parallelism with increasing amounts of LH standard and the tissue extracts, indicating the presence of immunoreactive LH-like molecules in the rat ovary. In this experiment, ovaries and uteri from immature rats(Day 25) and epididymis from adult rats(4 months old) were used.

**Figure 3.** LH RIA for measuring the rat epididymal LH contents. Caput, corpus and caudal part of epididymis from adult rats(4 months old) were homogenized, centrifuged and the resulting supernatant were used in RIA.