

Localization of Luteinizing Hormone (LH) Subunits Like Substances in the Mouse Placenta

Eun Hye Ko, [†]Sung-Ho Lee

Department of Biotechnology, Sangmyung University, Seoul 03016, Korea



Received: April 22, 2026
 Revised: May 30, 2026
 Accepted: June 8, 2026

[†]Corresponding author

Sung-Ho Lee
 Department of Biotechnology,
 Sangmyung University, Seoul 03016,
 Korea.
 Tel: +82-2-2287-5139
 E-mail: shlee@smu.ac.kr

Copyright © 2026 The Korean Society of Developmental Biology.
 This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

ORCID

Eun Hye Ko
<https://orcid.org/0009-0006-5335-1922>
 Sung-Ho Lee
<https://orcid.org/0000-0003-2866-3642>

Conflict of interests

The authors declare no potential conflict of interest.

Acknowledgements

Not applicable.

Authors' contributions

Conceptualization: Lee SH.
 Data curation: Lee SH.
 Formal analysis: Lee SH.
 Methodology: Ko EH, Lee SH.
 Validation: Lee SH.
 Investigation: Ko EH, Lee SH.
 Writing-original draft: Lee SH.
 Writing-review & editing: Ko EH, Lee SH.

Ethics approval

This article does passed Sangmyung University IACUC (approval number: E-1302-3). All animal experiments were conducted in accordance with the Guide

Abstract

Luteinizing hormone (LH) is a vital reproductive hormone produced by the anterior pituitary gland, essential for both fertility and sexual development. Interestingly, there is evidence indicates that rodent placenta is extrapituitary site of LH synthesis. On this basis, we performed immunohistochemistry (IHC) using specific antisera for LH subunits to investigate the tissue distribution of the LH subunits like molecules in mice placentae. Pregnant mice on gestation day 17 were sacrificed and the placentae were obtained. Glycogen rich trophoblast cells (GlyT) in the junctional zone (JZ) were positive for CG α immunohistochemical staining, and the LH- β positive staining was found in all cell layers in both JZ and labyrinth zone (LZ). Immunoreactive LH-receptor (LH-R) staining was observed on the membrane of GlyT in JZ and partial syncytiotrophoblast cells layer in LZ. The present study demonstrated that immunoreactive LH subunits like substances were localized differentially in the microstructures of mice placentae. This finding that the substances exhibiting immune responses to these and their receptors exist in the same type of cells and/or adjacent cells, suggesting the possibility of local regulation through these substances. Further studies will be helpful for understanding the biochemical nature and the role(s) of local LH-like substances in sophisticated placental physiology.

Keywords: Rat, Placenta, Luteinizing Hormone (LH), Subunit like, Substances, Localization

INTRODUCTION

Luteinizing hormone (LH) is secreted by the anterior pituitary and plays a role in reproductive functions such as ovulation in females and synthesis of androgens in males (Ezcurra & Humaidan, 2014). Furthermore, LH is a versatile molecule that might play a various role and has been reported to be expressed in extrapituitary regions (Harvey et al., 2012). For example, LH is found in the fetal brain and persists in the adult rat and human (Hojvat et al., 1982; Bowen et al., 2004), in rat testis (Zhang et al., 1995a; Kim & Lee, 2017), in hamster testis (Choi, 2022), in ovine pineal gland (Noteborn et al., 1992), and in cycling rat uterus (Kim & Lee, 1999).

Concerning the placenta, which exists temporarily during pregnancy and connects the mother and fetus, it is well-known that placenta secretes a large amount of chorionic gonadotropin (CG) which has a structure very similar to LH in some mammalian species including rat (Blank & Dufau, 1983; Herkert et al., 2022). In this regard, it is interesting that, even though CG exists as a substitute for LH,

transcripts identical to the LH subunits found in the pituitary gland are also present in rat placenta (Shinozaki et al., 1997).

Research on the placental LH and/or LH-like substance is potentially important as it is a physiological signaling molecule with great potential to play a crucial role in rapid tissue development and differentiation after implantation. As on the first try, we performed immunohistochemistry (IHC) to investigate the tissue distribution of the mice placental LH like substances, a common alpha subunit (Cg α) of glycoprotein hormones, LH beta subunit (LH- β) and LH receptor (LH-R).

MATERIALS AND METHODS

1. Animals

All animal experiments were approved by the Institutional Animal Care and Use Committees (IACUC; approval number: E-1302-3). Timely pregnant mice on gestation day (GD) 12 were supplied by the DBL (Eumseong, Korea) and raised for 5 days at the Sangmyung University animal facility under a 12 hours light/dark cycle (lights on at 07:00 hour), with food and water supplied *ad libitum* at constant temperature of $22\pm1^\circ\text{C}$. The animal was sacrificed on GD 17.

2. Placenta collection

After sacrificing the mouse, the placental-fetal pairs were rapidly removed. For tissue section preparation, placentae were immediately immersed in 4% paraformaldehyde (PFA, Bio PURE, Hampshire, UK) in phosphate buffered saline (PBS, pH 7.4, Bio PURE) and stored at 4°C for 24 hours.

3. Tissue section

Fixed placentae in 4% PFA were dehydrated in graded ethanol (70%, 80%, 90%, 95%, 100%; Duksan, Ansan, Korea) each for an hour and a half using gentle agitation (Green SSeriker, Gwangju, Gyeonggi-do, Korea) and then in absolute ethanol (Merck, Darmstadt, Germany) overnight. After that, the samples were immersed in xylene (Samchun, Pyeongtaek, Korea) at room temperature 3 times at intervals of 40 minutes and into paraffin (Merck) at 60°C 3 times at intervals of 20 minutes. Tissue was embedded (Microm, Walldorf, Germany) in paraffin using the cassette (Hyunil LAB-MATE, Seoul, Korea). The block was sectioned using microtome (Microm) to a thickness of $6\ \mu\text{m}$ and specimens were attached to microscope slides (Marienfeld, Lauda-Königshofen, Germany) using water bath (Microm). Heat was applied to the specimens at 63°C in the slide warmer (Jisico, Seoul, Korea) for 12 hours.

4. Immunohistochemistry

The slides were dewaxed in xylene for 5 minutes, 3 times and hydrated in 100%, 95%, 90%, 80%, and 70% ethanol for 5 minutes each. The specimens were washed in PBS for 5 minutes, 4 times and immersed in hydrogen peroxide solution [10% H_2O_2 (Daejung, Siheung, Korea) and 10% Methanol (Merck)] in PBS for 20 minutes, and washed in PBS 2 times. The slides were incubated with blocking serum [2% BSA (Bio PURE) and 2% normal goat serum (VECTOR, Newark, CA, USA)] in PBS for 1 hour and incubated overnight with diluted primary antibody (dilution of 1:50, 1:500 and 1:1,000, respectively, for Cg α , LH- β , and LH-R). Antisera for Cg α and LH- β were purchased from National Hormone & Pituitary Program (NHPP, Torrance, CA, USA) and antiserum for LH-R was purchased from Mybiosource (MBS244081, San Diego, CA, USA). The slides were washed 2 times and incubated with biotinylated goat anti-rabbit IgG secondary

antibody (VECTOR) for 1 hour (in the case of Cg α , overnight) and washed in PBS 2 times. The samples were incubated with avidin-biotin-peroxidase complex using the ABC kit (VECTOR) for 40 minutes and washed 2 times. The chromogenic reaction was progressed using the DAB kit (VECTOR) and stopped by washing in tap water. The slides were stained in hematoxylin (VECTOR) for 7 minutes and dehydrated in graded ethanol (70%, 80%, 90%, 95%, and 100%) for 3 minutes each and then cleaned in xylene for 5 minutes, 3 times. The slides were mounted with Permount (Fisher Scientific, Waltham, MA, USA). The images were captured with the BX51 Microscope & DP70 Digital Camera System (Olympus, Tokyo, Japan).

RESULTS

Localization of Cg α , LH- β , and LH-R in the mouse placenta was investigated by specific IHCs. Cg α staining were detected on the nuclei of glycogen rich trophoblast cells (GlyT, red arrows) in a junctional zone (JZ; Fig. 1, upper panel). In a labyrinth zone (LZ), weaker signals were found in mononucleate trophoblast cell, and nuclei of fetal blood cell (fbc) (Fig. 1, lower panel).

LH- β staining was observed in all cell layers. Especially, immunoreactive LH- β was detected

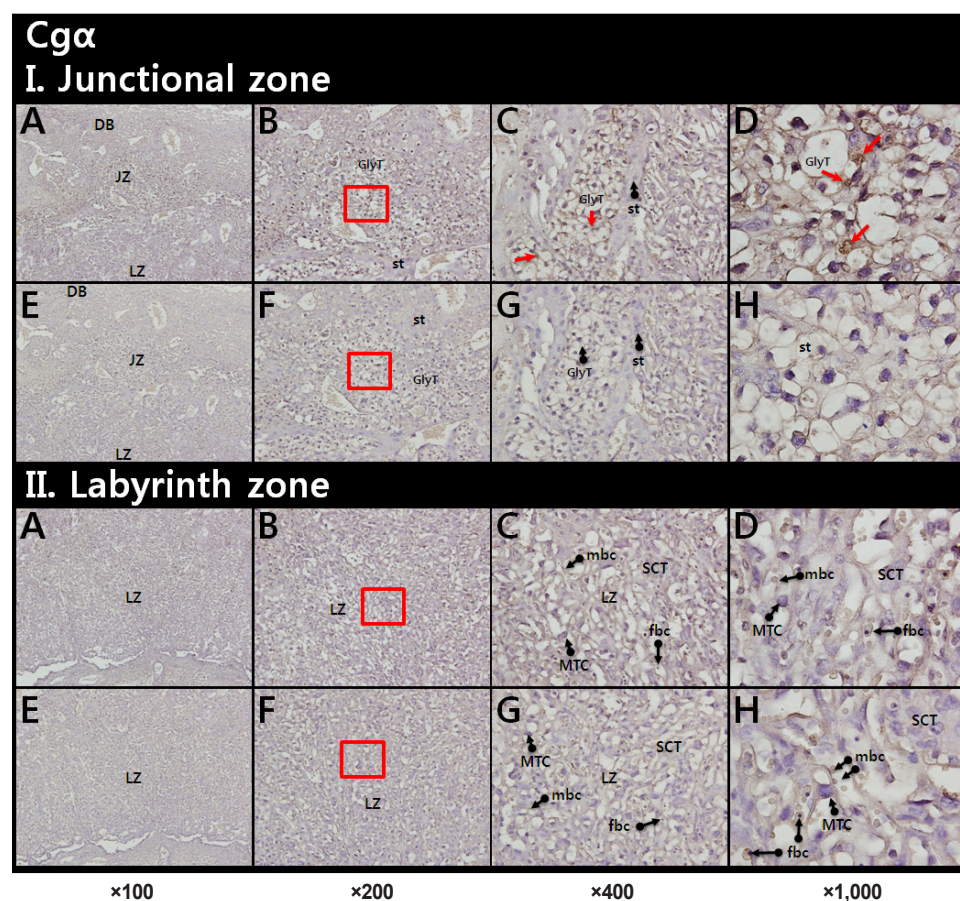


Fig. 1. Immunohistochemical detections of the Cg α in mouse placenta. A–D were shown in the immunohistochemically stained location of Cg α in the mouse placenta. E–H were sections showing negative control without the primary antibody. The red arrows are stained site. The black arrows are non-stained site. The red quadrangle of B and F is approximate position of D and H, respectively. Magnification, $\times 100$ (A, E), $\times 200$ (B, F), $\times 400$ (C, G), $\times 1,000$ (D, H). DB, decidua basalis; JZ, junctional zone; LZ, labyrinth zone; GlyT, glycogen rich trophoblast cells; st, syncytiotrophoblast; mbc, maternal blood channels; SCT, syncytiotrophoblast cells; MTC, mononucleate trophoblast cell; fbc, nuclei of fetal blood cell.

in and around GlyT in the JZ (Fig. 2, upper panel) and on cytoplasm of syncytiotrophoblast cells (SCT), fetal capillary endothelial cells (EC), and basement membrane (bm) in the LZ (Fig. 2, lower panel).

Our IHC also revealed that the immunoreactive LH-R located on the membrane and in a part of cytoplasm of GlyT in the JZ (Fig. 3, upper panel). Relatively weak LH-R staining was also shown in the SCT layer of the LZ (Fig. 3, lower panel).

DISCUSSION

Research on LH expression outside the pituitary gland has been ongoing for decades, but little amount of information is available so far. Extrapituitary production of LH has been reported primarily in reproductive organs like gonads, as well as the accessory sex organs (Harvey et al., 2012); The local LH has been identified in the rat testis (Zhang et al., 1995a; Kim & Lee, 2017), mouse testis (Kim & Lee, 2017), hamster testis (Choi, 2022), rat ovary (Schirman-Hildesheim et al., 2008), rat uterus and epididymis (Kim & Lee, 1999; Lee & Lee, 1999), and rat placenta

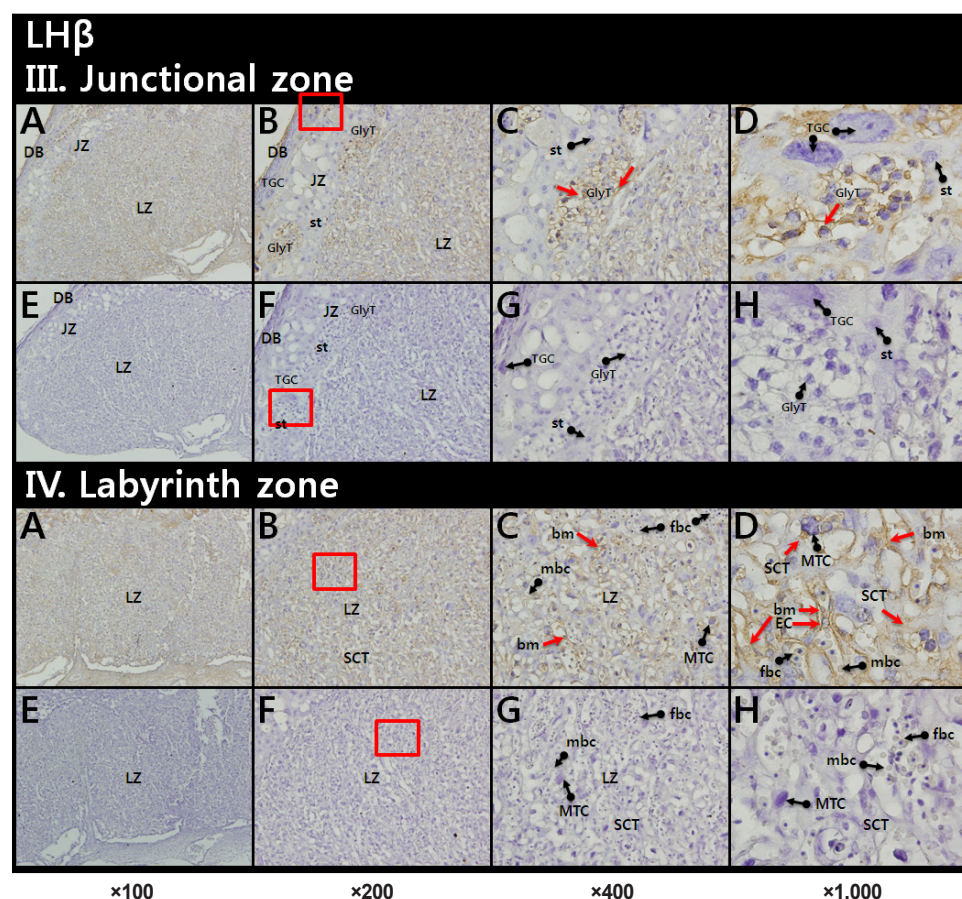


Fig. 2. Immunohistochemical detections of the LH-β in mouse placenta. A–D were shown in the immunohistochemically stained location of LH-β in the mouse placenta. E–H were sections showing negative control without the primary antibody. The red arrows are stained site. The black arrows are non-stained site. The red quadrangle of B and F is approximate position of D and H, respectively. Magnification, ×100 (A, E), ×200 (B, F), ×400 (C, G), ×1,000 (D, H). DB, decidua basalis; JZ, junctional zone; LZ, labyrinth zone; GlyT, glycogen rich trophoblast cells; st, syncytiotrophoblast; mbc, maternal blood channels; SCT, syncytiotrophoblast cells; MTC, mononucleate trophoblast cell; fbc, nuclei of fetal blood cell; EC, fetal capillary endothelial cells; bm, basement membrane; LH, Luteinizing hormone.

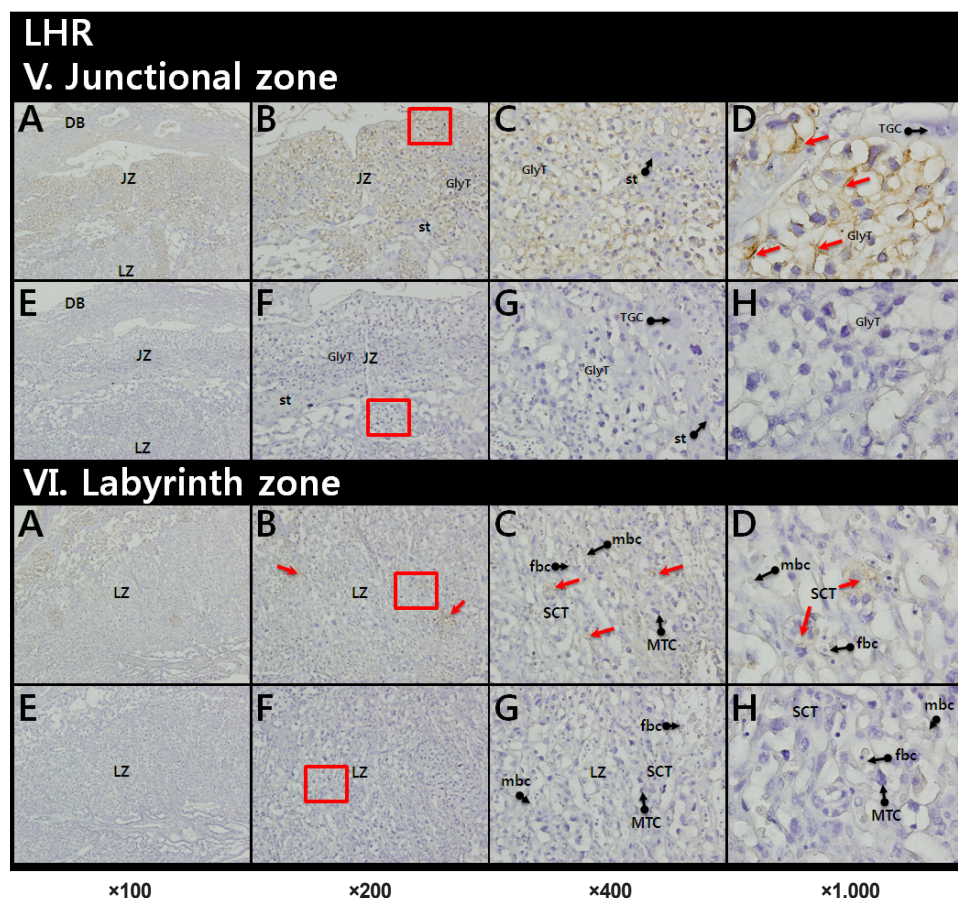


Fig. 3. Immunohistochemical detections of the LH-R in mouse placenta. A–D were shown in the immunohistochemically stained location of LH-R in the mouse placenta. E–H were sections showing negative control without the primary antibody. The red arrows are stained site. The black arrows are non-stained site. The red quadrangle of B and F is approximate position of D and H, respectively. Magnification, $\times 100$ (A, E), $\times 200$ (B, F), $\times 400$ (C, G), $\times 1,000$ (D, H). DB, decidua basalis; JZ, junctional zone; LZ, labyrinth zone; GlyT, glycogen rich trophoblast cells; st, syncytiotrophoblast; mbc, maternal blood channels; SCT, syncytiotrophoblast cells; MTC, mononucleate trophoblast cell; fbc, nuclei of fetal blood cell; LH, Luteinizing hormone.

(Shinozaki et al., 1997). This local production, particularly in gonads, might acts in a paracrine or autocrine manner to modulate gametogenesis, steroidogenesis and other reproductive-related phenomena. For example, while the pituitary provides the main endocrine surge for ovulation, the local LH might act as a ‘fine-tuning’ mediator of reproductive signals.

The placenta is a temporary organ that forms inside the uterus during pregnancy to connect the mother and the fetus. It performs respiration, nutrient supply, and excretion functions essential for the survival and growth of the fetus, and is expelled from the body after childbirth (Wide & Wide, 1979; Makrigiannakis et al., 2017; Herkert et al., 2022). HCG is a hormone secreted by the placenta in early pregnancy that maintains corpus luteum function and helps produce progesterone to maintain pregnancy (Cindrova-Davies & Sferruzzi-Perri, 2022). Interestingly, HCG has structure and function which are very similar to LH, therefore, one could wonder why pituitary type LH subunits are expressed in the placenta (Shinozaki et al., 1997).

The gonadotropin (LH, FSH, and hCG) is noncovalently joined by the common α subunit and specific β subunit (Ryan et al., 1988; Alevizaki & Huhtaniemi, 2002). The Cg α gene is coded by a single gene that consists of 4 exons and 3 introns (Gharib et al., 1990). The authentic LH- β gene is composed of 3 exons and 2 introns in the mouse (Kumar & Matzuk, 1995). LH is well known to be

synthesized and secreted by the anterior pituitary (Steelman et al., 1959; Hartree, 1966; Lynch et al., 1988). In contrast, Zhang et al. found the expression of testis-specific mRNA variants of the LH- β gene (Zhang et al., 1995a). There are three testicular transcripts in the rat of lengths of 3.2, 2.4, and 0.86 kb (Zhang et al., 1995b). Follow-up studies, including our own, indicates that the presence of tissue-specific expression of LH- β gene in mouse testis (Kim & Lee, 2017), hamster testis (Choi, 2022), and rat ovary (Schirman-Hildesheim et al., 2008).

The presence of transcripts for placental LH subunits in rat was initially proven by reverse transcription-polymerase chain reactions (RT-PCR), Southern blot assay and partial sequencing of the PCR products (Shinozaki et al., 1997). We recently found that the transcripts for mice placental LH- β were almost identical to those found in rat testis including all known exons of authentic pituitary type transcripts (data not shown). So far, the physiological significance of the placental LH subunit substances has not been addressed. Regarding the gonadal and placental LH, research on their molecular structure(s) and characteristics must be conducted first. In particular, it is necessary to investigate molecular properties, such as the molecular weight of partially purified tissue extracts, by adopting classical methodologies such as column chromatography, and measure physiological effects through *in vivo* injection or *in vitro* treatment.

The present study demonstrated that immunoreactive LH subunits like substances were localized differentially in the microstructures of mice placentae. The fact that substances exhibiting immune responses exist in the same cells or adjacent cells suggests the possibility of local regulation between these substances. Further studies will be helpful for understanding the biochemical nature and the role(s) of local LH-like substances in sophisticated placental physiology.

REFERENCES

- Alevizaki M, Huhtaniemi I (2002) Structure-function relationships of glycoprotein hormones; lessons from mutations and polymorphisms of the thyrotrophin and gonadotrophin subunit genes. *Hormones* 1:224-232.
- Blank MS, Dufau ML (1983) Rat chorionic gonadotropin: Augmentation of bioactivity in the absence of the pituitary. *Endocrinology* 112:2200-2202.
- Bowen RL, Verdile G, Liu T, Parlow AF, Perry G, Smith MA, Martins RN, Atwood CS (2004) Luteinizing hormone, a reproductive regulator that modulates the processing of amyloid- β precursor protein and amyloid- β deposition. *J Biol Chem* 279:20539-20545.
- Choi D (2022) Expressions of gonadotropin subunit genes in the reproductively inactive golden hamsters. *Dev Reprod* 26:37-47.
- Cindrova-Davies T, Sferruzzi-Perri AN (2022) Human placental development and function. *Semin Cell Dev Biol* 131:66-77.
- Ezcurra D, Humaidan P (2014) A review of luteinising hormone and human chorionic gonadotropin when used in assisted reproductive technology. *Reprod Biol Endocrinol* 12:95.
- Gharib SD, Leung PCK, Carroll RS, Chin WW (1990) Androgens positively regulate follicle-stimulating hormone β -subunit mRNA levels in rat pituitary cells. *Mol Endocrinol* 4:1620-1626.
- Hartree AS (1966) Separation and partial purification of the protein hormones from human pituitary glands. *Biochem J* 100:754-761.
- Harvey S, Arámburo C, Sanders EJ (2012) Extrapituitary production of anterior pituitary hormones: An overview. *Endocrine* 41:19-30.
- Herkert D, Meljen V, Muasher L, Price TM, Kuller JA, Dotters-Katz S (2022) Human chorionic gonadotropin: A review of the literature. *Obstet Gynecol Surv* 77:539-546.
- Hojvat S, Emanuele N, Baker G, Connick E, Kirsteins L, Lawrence AM (1982) Growth hormone

- (GH), thyroid-stimulating hormone (TSH), and luteinizing hormone (LH)-like peptides in the rodent brain: Non-parallel ontogenetic development with pituitary counterparts. *Dev Brain Res* 4:427-434.
- Kim HS, Lee SH (2017) Expression of luteinizing hormone (LH) subunit genes in mouse testis. *Dev Reprod* 21:327-333.
- Kim SR, Lee SH (1999) Expression of luteinizing hormone (LH) and its receptor gene in uterus from cycling rats. *Korean J Fertil Steril* 26:383-388.
- Kumar TR, Matzuk MM (1995) Cloning of the mouse gonadotropin β -subunit-encoding genes, II. Structure of the luteinizing hormone β -subunit-encoding genes. *Gene* 166:335-336.
- Lee SH, Lee Y (1999) Expression of luteinizing hormone (LH) gene in rat uterus and epididymis. *Korean J Fertil Steril* 26:157-161.
- Lynch SS, Reay P, Bluck M (1988) The extraction and purification of human pituitary follicle-stimulating hormone and luteinizing hormone. *Acta Endocrinol Suppl* 288:12-19.
- Makrigiannakis A, Vrekoussis T, Zoumakis E, Kalantaridou SN, Jeschke U (2017) The role of hCG in implantation: A mini-review of molecular and clinical evidence. *Int J Mol Sci* 18:1305.
- Noteborn HPJM, de Koning J, de Jong FH, Ebels I, Saleminck CA (1992) Identification of luteinizing hormone-like proteins in the ovine pineal gland. *J Pineal Res* 12:118-127.
- Ryan RJ, Charlesworth MC, McCormick DJ, Milius RP, Keutmann HT (1988) The glycoprotein hormones: Recent studies of structure-function relationships. *FASEB J* 2:2661-2669.
- Schirman-Hildesheim TD, Gershon E, Litichever N, Galiani D, Ben-Aroya N, Dekel N, Koch Y (2008) Local production of the gonadotropic hormones in the rat ovary. *Mol Cell Endocrinol* 282:32-38.
- Shinozaki M, Uchida H, Ikeda S, Min KS, Shiota K, Ogawa T (1997) Expression of LH- α and - β subunit mRNAs in the rat placenta. *Endocr J* 44:79-87.
- Steelman SL, Segaloff A, Andersen RN (1959) Purification of human pituitary follicle stimulating (FSH) and luteinizing (LH) hormones. *Proc Soc Exp Biol Med* 101:452-454.
- Wide L, Wide M (1979) Chorionic gonadotrophin in the mouse from implantation to term. *J Reprod Fertil* 57:5-9.
- Zhang FP, Markkula M, Toppari J, Huhtaniemi I (1995a) Novel expression of luteinizing hormone subunit genes in the rat testis. *Endocrinology* 136:2904-2912.
- Zhang FP, Rannikko A, Huhtaniemi I (1995b) Isolation and characterization of testis-specific cDNAs for luteinizing hormone β -subunit in the rat. *Biochem Biophys Res Commun* 210:858-865.