Serum kisspeptin levels are mainly regulated by ovarian expression of *Kiss1* mRNA in female rats

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"다만 이 뿐 아니라 우리가 환난중에도 즐거워하나니 이는 환난은 인내를, 인내는 연단을, 연단은 소망을 이루는 줄 앎이로다." 로마서 5장 3절-4절

앞이 보이지 않고 막막할 때에도 언제나 제 앞길을 예비하시고 상상하지 못한 방법과 길로 인도하시는 하나님, 내 삶을 계획하시고, 동행해 주셔서 감사드립니다. 박사과정이 물론 바울이 말하는 환난에 빗댈 수는 없지만, 어려운 과정에 좋은 사람들을 예비하여 주셔서 인내하여 이겨낼 수 있게 하시고, 그 과정을 통해 나를 연단하시고, 결국 소망을 이루게 해주심을 감사합니다. 하나님의 사람으로서 부끄럽지 않도록 늘 인내하고 연단할 수 있기를 소망합니다. 그리고, 나를 모태신앙으로 믿음을 가질 수 있게 해주신 나의 믿음의 조상, 외할머니. 외할머니의 기도로 제 길이 평탄한 것 같습니다. 그리고 지금도 천국에서 엄마와 함께 저를 위해 간구하고 계신다는 것에 감사드립니다.

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드디어, 저는, 박사가 되었습니다.

2021년 12월
권아름 올림

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ABSTRACT

Serum kisspeptin levels are mainly regulated by ovarian expression of *Kiss1* mRNA in female rats

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The hypothalamic kisspeptin/KISS1 receptor system is essential for puberty onset and reproductive development. Although serum kisspeptin might be associated with puberty, its levels, according to developmental stage, and its origin still remain unclear. This study evaluated the changes in serum kisspeptin levels during puberty, and the corresponding *Kiss1* mRNA and protein expression in various organs of female rats to identify the source of serum kisspeptin. Tissues from several organs, including the ovaries and anteroventral

periventricular nucleus (AVPV) and arcuate nucleus (ARC) in the hypothalamus, were obtained for assessing *Kiss1* mRNA and protein expressions. Serum kisspeptin levels progressively increased with developmental stages until the peripubertal stage. The ovaries showed the highest *Kiss1* expression among the organs examined. Next, we explored the changes in serum kisspeptin levels and hypothalamic *Kiss1* expression in ovariectomized and estradiol-treated ovariectomized rats. After ovariectomy, serum kisspeptin levels decreased regardless of estradiol treatment, while *Kiss1* expression was not affected by ovariectomy in the ARC and that was enhanced by estradiol in the AVPV, suggesting that serum kisspeptin may be associated with pubertal development and is mainly regulated by ovarian *Kiss1* expression. Thus, serum kisspeptin levels are associated with puberty and may serve as a downstream marker of ovarian reproductive function.

Key words : kisspeptins; ovary; female; sexual maturation; rats; RNA, messenger

Serum kisspeptin levels are mainly regulated by ovarian expression of *Kiss1* messenger RNA in female rats

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I. INTRODUCTION

Puberty is a highly orchestrated and regulated process which occurs by activating the hypothalamic–pituitary–gonadal (HPG) axis. The HPG axis is a complex biological system that is not yet fully characterized. Kisspeptin, produced by *KISS1*, acts as gatekeeper for puberty onset via its cognate receptor GPR54 (also known as the KISS1 receptor [KISS1R])[1,2]. The kisspeptin/KISS1R system has been established as a regulator of puberty based on studies showing that inactivating and activating mutations in either *KISS1* or *KISS1R* were associated with hypogonadotropic hypogonadism and precocious puberty[1,3-5]. In addition, *KISS1* is expressed within the hypothalamus, especially in the anteroventral periventricular nucleus (AVPV) and arcuate nucleus (ARC)[6,7], and *KISS1* expression increases as puberty progresses[7,8]. In animal studies, central infusion of a kisspeptin antagonist suppresses gonadotropin-releasing hormone (GnRH) pulses and reduces

luteinizing hormone (LH) pulse frequency[9-11], while central administration of kisspeptin induces precocious activation of the HPG axis and results in precocious puberty[12]. Therefore, the kisspeptin/KISS1R system is an upstream regulator of GnRH release and has been proposed to play an important role in the onset of puberty.

Since hypothalamic kisspeptin affects puberty onset, serum kisspeptin has been suggested as an attractive marker for puberty onset. Evaluating the serum kisspeptin level, according to pubertal stage, may help determine whether it can be used as a biomarker for puberty. To date, several studies[13-17] have shown conflicting results regarding serum kisspeptin levels during puberty. Serum kisspeptin levels increase with age, and peak around puberty[13]; they are significantly higher in girls with central precocious puberty than pre-pubertal girls of the same age[14,15]. In contrast, there were no differences in serum kisspeptin levels between pre-pubertal and pubertal groups[16] or between a central precocious pubertal and normal group[17]. Therefore, a detailed evaluation of the change in serum kisspeptin levels according to developmental stage is urgently needed.

Identifying the origin of serum kisspeptin might elucidate the association between serum kisspeptin levels and puberty. *KISS1* is widely distributed not only in the hypothalamus but also other organs, such as the pituitary gland, placenta, kidneys, pancreas, adrenal gland, adipose tissue, testes, and ovaries[18-20]. Thus, it is not clear whether changes in *Kiss1* expression in the hypothalamus reflect changes in serum kisspeptin, or whether these changes are brought about by some other organ. Emerging evidence has indicated potential physiological roles of extra-hypothalamic kisspeptins in modulating puberty and the reproductive system[21,22]. In particular, ovaries express *Kiss1*[20,21,23], which seems to be controlled by LH[23]. Furthermore, local administration of a high dose kisspeptin antagonist to an ovary exerts a negative influence on puberty onset[24]. Therefore, the ovary is also suspected to be a candidate organ for the main

source of serum kisspeptin. Investigating the association between serum kisspeptin levels and changes in *Kiss1* expression in various organs, including the hypothalamus and ovary at different developmental stages, will help determine which organ expressing *Kiss1* is the main source of serum kisspeptin during puberty. This analysis might help in understanding the biological significance of serum kisspeptin levels during puberty.

In this study, we explored changes in serum kisspeptin levels in female rats from the neonatal stage to puberty. We also evaluated *Kiss1* mRNA and protein expression in several organs during development to explore the source of serum kisspeptin. In addition, we investigated serum kisspeptin levels and hypothalamic *Kiss1* mRNA and protein expression in ovariectomized (OVX) rats, with and without estradiol replacement, to explore the role of the ovaries and hypothalamus in regulating serum kisspeptin levels.

II. MATERIALS AND METHODS

1. Animals

The experimental procedures used in this study were approved by the Institutional Animal Care and Use Committee of Yonsei University College of Medicine (approval number 2016-0046) and conducted in accordance with the Guide for the Care and Use of Laboratory Animals (1996 [7th ed.] Washington, DC: National Research Council, National Academies Press). To ensure that all the female rats used were of the same conditions, timed-pregnant Sprague–Dawley rats (17th day of pregnancy, n = 8) were purchased from Japan SLC, Inc. (Shizuoka, Japan). Each pregnant rat bore six to eight female littermates, and a total of 56 littermates were used in this study. The current study was designed to minimize the number of animals used. Female littermate rats born before 1000 h were considered to be one day old. The animals were maintained under standard conditions of a 12:12 light: dark cycle (lights on at 0800 h) and a temperature of 22 °C. The rats were weaned on day 21 and housed, three per

cage, with free access to pellet food and tap water. Body weights were checked every morning at 1000 h. To confirm the completion of puberty, vaginal opening (VO), which was defined by the vagina being pink, wrinkled, and completely canalized[25], was also checked every morning at 1000 h.

2. Experimental procedures

A. Experiment I

The developmental stages of the female rats were defined as follows: neonate (days 1–7), infant (days 8–21), peripubertal (days 22–31), and pubertal completion (day of VO)[25]. To evaluate serum kisspeptin patterns at differential development stages, serum samples were obtained at onset and half-way through each developmental stage; that is, on day 4 (P4, middle of neonate stage), day 8 (P8, onset of infancy), day 14 (P14, mid-infancy), day 23 (P23, onset of peripuberty), day 27 (P27, mid-peripuberty), and the day of VO (completion of puberty). Each day, samples were taken from four to eight female rats. To evaluate *Kiss1* mRNA and protein expression in the hypothalamus, pituitary gland, ovaries, uterus, adrenal glands, and pancreatic tissues, tissue samples from these organs were obtained at the same time points. The rats were euthanized by decapitation between 1000 h and 1100 h. The hypothalamus was removed according to the rat brain atlas[26], using a micro knife to make a 2 mm-deep horizontal cut that began 1 mm away (in the anterior direction) from the optic chiasm and continued to the posterior borders of the mammillary bodies and the hypothalamic fissures. The AVPV and ARC were compartmentalized according to the rat brain atlas [26]. The anterior and posterior ends of the AVPV tissue sections were approximately 0.84 mm and 0.60 mm posterior to the bregma, respectively. The anterior and posterior ends of the ARC tissues were approximately 1.80 and 4.08 mm posterior to the bregma, respectively. Tissues from several rat organs were removed

immediately following decapitation, frozen in liquid nitrogen, and stored at –80℃ until processing for mRNA analyses.

B. Experiment II

Next, to evaluate the role of ovarian *Kiss1* expression on serum kisspeptin levels, we measured serum kisspeptin levels and hypothalamic *Kiss1* mRNA and protein expression in OVX rats. Rats were subjected to ovariectomies at P14, and serum samples and hypothalamic tissues were obtained on P23, P27, and P34. Because OVX rats had a delayed pubertal onset, samples were obtained at P34 (mean day of VO in intact female rats), instead of the day of VO.

C. Experiment III

We also measured serum kisspeptin levels and *Kiss1* mRNA and protein expression in the hypothalamus in OVX rats after estradiol replacement to explore the regulatory effect of estradiol on serum kisspeptin levels. We subcutaneously administered estradiol (E-8875, Sigma Chemical Co., St. Louis, MO) at a dose of 25 μg/kg/day from P14 to the day of VO. Serum samples and hypothalamic tissues were obtained on P23, P27, and the day of VO.

3. Measuring serum kisspeptin levels

Plasma samples were collected by immediate centrifugation and stored at -70°C before determining serum kisspeptin levels. Serum kisspeptin levels were measured using a highly sensitive enzyme-linked immunosorbent assay kit (E-EL-R2530, Elabscience Biotech, Wuhan, China) with a detection range of 78.13–5,000 pg/mL. Serum kisspeptin concentration was measured by diluting the blood of rats to one-fiftieth.

4. RNA extraction and RT-qPCR analysis

To determine *Kiss1* mRNA expression in tissues at each developmental stage, RNA was extracted and analyzed by RT-PCR. Tissues from several rat organs were removed immediately following decapitation, frozen in liquid nitrogen, and stored at -80 °C until processing for mRNA analyses. Total RNA was isolated using the Trizol reagent (Invitrogen, Carlsbad, CA, USA). A total of 2 μg RNA was synthesized using LaboPass cDNA kit (Cosmo Gentech, Seoul, Korea) according to the manufacturer's instructions. Primer sequences used for RT-PCR were obtained from the published sequence of *Kiss1* (GenBank accession number AY196983.1; Table 1) and *Gapdh* was used as the reference gene. PCR was carried out at an initial denaturation cycle at 95 °C for 5 min, followed by a variable number of amplification cycles defined by denaturation at 94 °C for 40 s, annealing 55 °C for 40 s, and 30 cycles of extension at 72 °C for 50 s. A final extension cycle of 72 °C for 10 min (Takara, Japan) was also included. PCR products were separated on 1.5% agarose gels and visualized by ethidium bromide staining.

Table 1. Real-Time Quantitative RT-PCR Primer Pair Sequences

Gene	Forward Primer	Reverse Primer
Gapdh	5`-GGTGATGCTGGTGCTGAGTA-3`	5`-ACTGTGGTCATGAGCCCTTC-3`
Kiss1	5`-ACTCGTTAATGCCTGGCAAA-3`	5`-AGGCCAAAGGAGTTCCAGTT-3`

5. mRNA analyses by quantitative real-time PCR

The *Kiss1* mRNA levels in representative samples from female rats in different developmental stages were quantified by qPCR. qPCR was performed with 20 μl of PCR amplification reaction mixture containing 900 ng of complementary DNA, hydrolysis probe (*Kiss1*: Rn00710914_m1, *Gapdh*: Rn01775763_g1), and TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA, USA). Amplification was performed in duplicate with the following thermocycling profile: 50 °C for 2 min, 95 °C for 10 min,

and 40 cycles of 95 °C for 15 s and 60 °C for 1 min. A StepOnePlus instrument (Applied Biosystems) was used for the reactions. The relative expression of mRNA was calculated using the $2^{-\Delta\Delta Ct}$ method[27]. The reference samples were arbitrarily taken from each organ from 4-day-old female rats. qPCR analyses were performed in triplicate.

6. Western immunoblotting analysis

To determine the protein expression levels of Kiss1, we performed western immunoblotting analysis. Frozen hypothalamus, ovary, pituitary gland, adrenal gland, and uterus tissues were homogenized and lysed on ice in RIPA lysis buffer (WSE-7420, ATTO, Tokyo, Japan.) Protein extracts were centrifuged at 17,000 × *g* for 30 min. Then, the protein concentrations were determined using the BCA protein assay (Applygen Technologies Inc., Beijing, China). Equal amounts of proteins (150 μg) were boiled for 5 min at 100 °C with loading buffer (containing 8% β-mercaptoethanol) for denaturation. Gapdh was tested in the same way using protein 20 μg. The denatured proteins were loaded on a 12.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel for 1 h. The proteins were separated by SDS-PAGE and transferred to a 0.25-μm polyvinylidene fluoride membrane for 30 min. The membranes were blocked with phosphate-buffered saline (PBS) containing 5% skim milk for 30 min at room temperature (as around 20–22 °C). Then, the membranes were incubated separately with primary antibodies against kisspeptin (1:500, catalog number GTX130503, GeneTex, Irvine, California, USA) and Gapdh (1:3000, catalog number #2118, Cell Signaling Technology, Danvers, Massachusetts, USA) at 4 °C overnight. After washing thrice with PBS containing 0.2% Tween 20 (PBS-T) for 10 min, the membranes were incubated with a horseradish peroxidase-conjugated secondary goat anti-rabbit antibody (1:5000) for 30 min at room temperature, followed by four washes in PBS-T for 10 min. Then, the membranes were exposed to enhanced chemiluminescence. Blots were exposed

to medical X-ray film and quantification of protein band intensities was performed using image j software.

7. Statistical analyses

Quantitative RNA data are presented as the mean ± standard error of the mean. One-way ANOVA followed by Tukey's test was performed to compare changes in serum kisspeptin levels at different developmental stages. To compare changes in serum kisspeptin levels in OVX and estradiol-treated OVX female rats at different developmental stages, two-way (age and OVX/estradiol-treated OVX) ANOVA was performed. In addition, if age-by-OVX/estradiol-treated OVX interaction effects were significant with *P*-value <0.05, then an independent *t*-test was performed to confirm the treatment effect on each group by post-hoc analysis. The Bonferroni multiple comparison test was used for multiple comparisons. All analyses were performed using SAS software (version 9.2; SAS Inc., Cary, NC, USA). *P* values <0.05 were considered to reflect statistically significant differences.

III. RESULTS

1. Developmental profile of serum kisspeptin levels in intact female rats

Serum kisspeptin levels were analyzed at different developmental stages, and each group contained four to eight animals. Serum kisspeptin levels were moderate during the neonatal period, slightly decreased in early infancy, and significantly increased during successive developmental stages (compared to just before stages, all $P < 0.01$ or 0.001), peaking at peripuberty (P27) (Figure 1a). However, the levels began to decrease at the completion of puberty ($P < 0.001$) (Figure 1a).

Figure 1

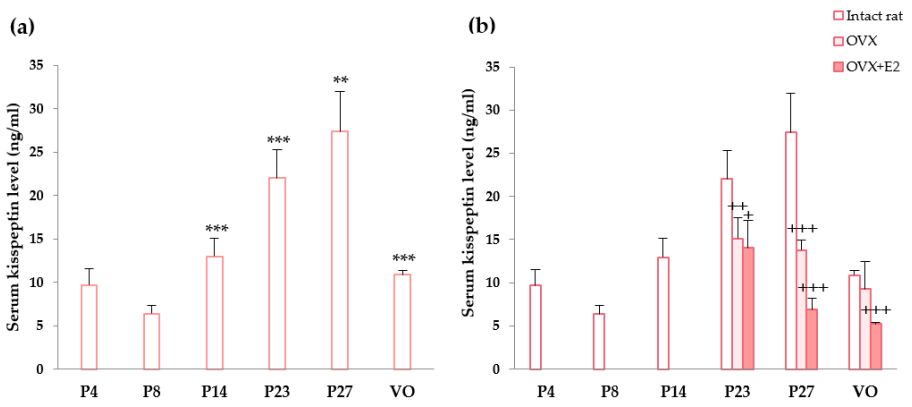


Figure 1: Changes in serum kisspeptin levels at different developmental stages. (a) Serum kisspeptin levels in intact female rats. Specimens were collected on days 4, 8, 14, 23, and 27, and on the day of vaginal opening (VO). (b) Changes n serum kisspeptin levels in ovariectomized (OVX) female rats and estradiol-treated OVX female rats (OVX + E2). Ovariectomy was conducted on day 14 and specimens were collected on days 23, 27, and on the day of vaginal opening (VO) or day 34. Each group had four to eight animals. Data are presented as the mean ± standard error of mean. $^{*}P < 0.05$ vs. just before the stage; $^{**}P < 0.01$ vs. just before the stage; $^{***}P < 0.001$ vs. just before the stage; $^{+}P < 0.05$ vs. intact female rats; $^{++}P < 0.01$ vs. intact female rats; $^{+++}P < 0.001$ vs. intact female rats

2. *Kiss1* mRNA and protein expressions in various organs in intact female rats according to the developmental stage

Kiss1 mRNA and protein expression were analyzed in several organs, including the AVPV and ARC in the hypothalamus, pituitary gland, ovaries, uterus, adrenal glands, and pancreas. The expression of *Kiss1* mRNA in each organ was evaluated based on *Kiss1* mRNA in the ovaries of 4-day-old female rats. The ovaries expressed the highest levels of *Kiss1* mRNA near the onset of

puberty, and the pituitary gland and hypothalamus expressed the second highest levels (Figure 2a). *Kiss1* mRNA was barely expressed in the uterus, adrenal glands, and pancreas (Figure 2a). In the ovaries, the *Kiss1* mRNA levels were lowest from the neonatal to infant periods, but significantly rapidly increased at the peripubertal stage ($p < 0.001$), peaking on P23, and then gradually decreasing until puberty completed (Figure 2a). Changes in *Kiss1* mRNA expression in the ovaries corresponded to changes in serum kisspeptin levels, although they were one developmental step ahead of the corresponding serum kisspeptin levels. That is, changes in mRNA expression corresponding to the developmental stage were observed prior to the changes observed in serum levels. *Kiss1* mRNA expression in the AVPV was low from the neonatal period until early infancy, then increased during the peripubertal stages and peaked at P23 (compared to just before the stage at P14, $P < 0.05$) (Figure 2a). Similarly, *Kiss1* mRNA levels in the ARC were moderate during the neonatal period, gradually increased during developmental stages until P23, and slightly decreased at P27 and VO (Figure 2a). However, there were no significant differences between developmental stages in the ARC. Although *Kiss1* mRNA expression in the pituitary gland was persistent and increased at P23 and at VO (compared to just before the stage, both $P < 0.01$), no specific pattern was observed during developmental stages (Figure 2a).

We then performed western immunoblotting analysis to determine changes in the protein expression of Kiss1 in various organs at P4, P23, P27, and VO. Similar to the *Kiss1* mRNA expression pattern, the ovaries expressed the highest levels of Kiss1 protein at P23, and P27 (Figure 2b). The level of Kiss1 protein in the AVPV also reached a peak at P23, and that in the ARC increased along with developmental stage as did *Kiss1* mRNA expression (Figure 2b). The expression of Kiss1 protein was barely detected in the uterus, adrenal glands, and pancreas (Figure 2b). These patterns were similar to those of *Kiss1* mRNA expression.

Figure 2

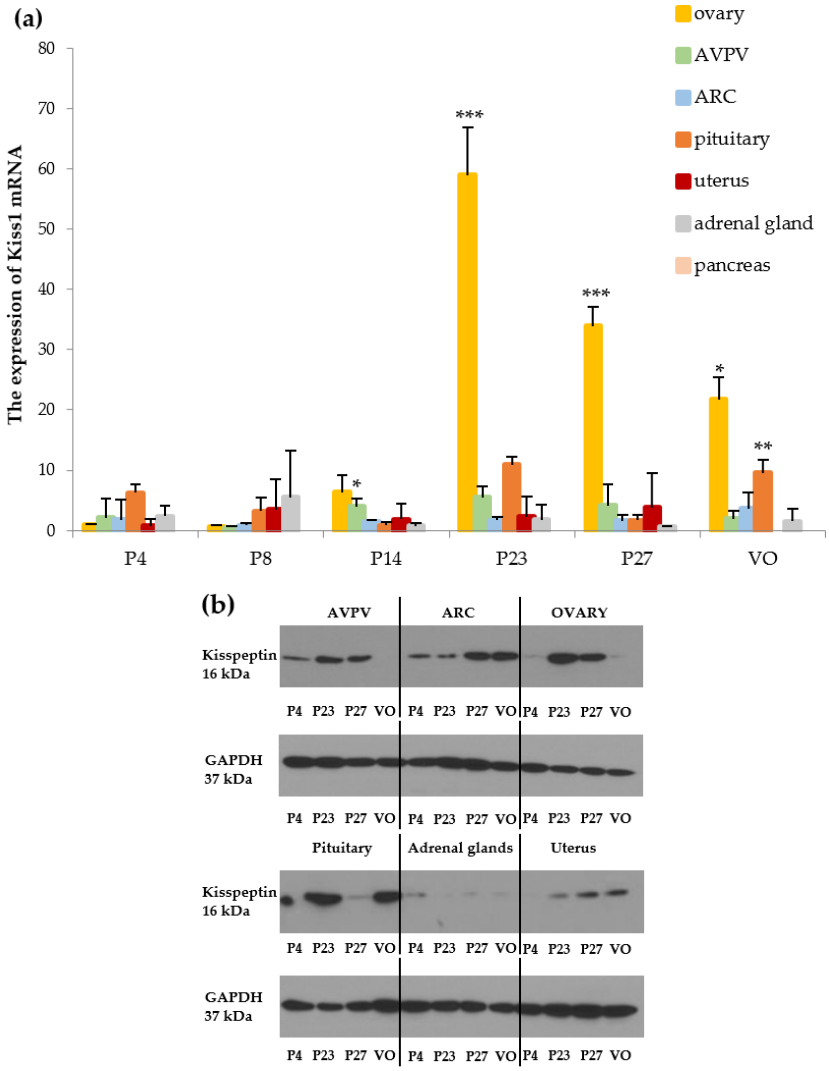


Figure 2: (a) Developmental profiles of *Kiss1* mRNA expression in several organs, such as the anteroventral periventricular nucleus (AVPV) and arcuate nucleus (ARC) in the hypothalamus, and the pituitary gland, ovaries, uterus, adrenal glands, and pancreas. Specimens were collected on days 4, 8, 14, 23, and 27, and on the day of vaginal opening (VO). *Kiss1* mRNA expression were quantified by qPCR in triplicate. Each value was quantified on the basis of the value of *Kiss1* mRNA-expression in the ovary on day 4. (b) Protein expression

levels of Kiss1 were quantified by western immunoblotting in triplicate. Each group had four to eight animals. Data are presented as the mean ± standard error of mean. *$P < 0.05$ vs. just before the stage; **$P < 0.01$ vs. just before the stage; ***$P < 0.001$ vs. just before the stage

3. Changes of serum kisspeptin levels in OVX rats and estradiol-treated OVX (OVX + E2) female rats at different developmental stages

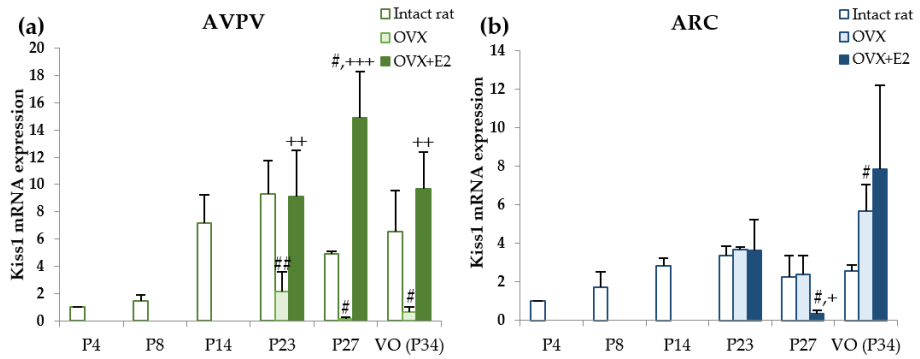
We performed ovariectomy and measured the serum kisspeptin level in OVX female rats to elucidate the effects of the ovaries on changes in serum kisspeptin levels. In addition, the serum kisspeptin levels in OVX female rats were compared with those in estradiol-treated OVX female rats to determine whether the change in serum kisspeptin level was due to the ovaries or estradiol. Since there was a significant interaction effect between developmental stage and OVX/estradiol-treated OVX for serum kisspeptin ($P < 0.001$), we confirmed the change in serum kisspeptin according to OVX or estradiol-treated OVX at each developmental stage. The serum kisspeptin levels in OVX female rats were significantly lower than those in intact female rats at P23 ($P < 0.01$) and P27 ($P < 0.001$), and serum kisspeptin levels in OVX female rats did not recover after estradiol treatment (compared with those in intact female rats; P23, $P < 0.01$; P27 and VO, $P < 0.001$) (Figure 1b).

4. Changes in hypothalamic *Kiss1* mRNA and protein expression in OVX and estradiol-treated OVX female rats

To determine the effect of *Kiss1* expression in the AVPV and ARC on serum kisspeptin levels, *Kiss1* mRNA and protein expression in the AVPV and ARC were measured in both OVX and estradiol-treated OVX rats and compared to the serum kisspeptin pattern. In the AVPV, there was a significant interaction effect between developmental stage and OVX/estradiol-treated OVX ($P<0.05$).

We confirmed the change in *Kiss1* mRNA and protein expression in the AVPV according to OVX/estradiol-treated OVX at developmental stages. The *Kiss1* mRNA of the AVPV was significantly lower in OVX female rats than in intact rats at all developmental stages (P23, P <0.01; P27, P < 0.05; P34, P < 0.05) (Figure 3a). However, these levels normalized after estradiol treatment, implying that *Kiss1* mRNA expression was increased by estradiol (compared with those in OVX rats, P23, P <0.01; P27, P <0.001; VO, P <0.01) (Figure 3a). In contrast, there was no significant interaction between age and OVX/estradiol-treated OVX in *Kiss1* mRNA expression in the ARC. *Kiss1* mRNA expression in the ARC was not decreased in OVX rats, compared to that of intact female rats, and rather increased at VO (P <0.05) (Figure 3b). After estradiol administration, *Kiss1* mRNA expression was similar to those of intact female rats and OVX rats at P23, but significantly decreased at P27 (P <0.05) (Figure 3a). Kiss1 protein expression measured by western immunoblotting analysis showed a pattern similar to *Kiss1* mRNA expression in the AVPV and ARC (Figure 3c and Figure 3d).

Figure 3



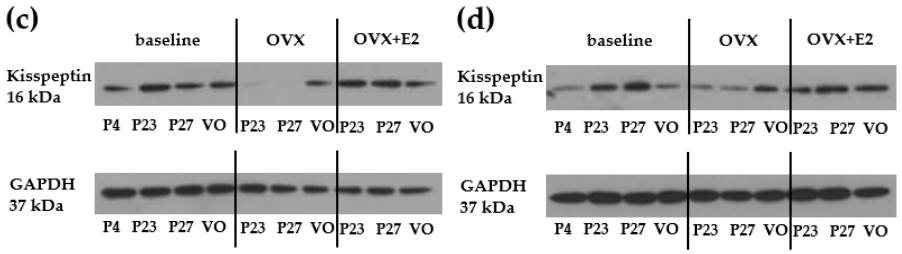


Figure 3: Developmental profiles of *Kiss1* mRNA and protein expression in the anteroventral periventricular nucleus (AVPV) and arcuate nucleus (ARC) in intact female, ovariectomized (OVX), and estradiol-treated OVX rats (OVX+E2) from the neonatal stage to the onset of puberty. Ovariectomy was conducted on day 14 and specimens were collected on days 23, 27, and on the day of vaginal opening (VO) or day 34. Each group contained four to eight animals. (a) *Kiss1* mRNA expression of the AVPV was quantified by qPCR. Each value was quantified on the basis of the value of *Kiss1* mRNA expression in the AVPV on day 4. (b) *Kiss1* mRNA-expression of the ARC was quantified by qPCR. Each value was quantified on the basis of the value of *Kiss1* mRNA expression in the ARC on day 4. (c) Kiss1 protein levels in the AVPV were quantified by western immunoblotting. (d) Kiss1 protein levels in the ARC were quantified by western immunoblotting. Each group contained four to eight animals. qPCR analyses and western immunoblotting analyses were performed in triplicate. Data are presented as the mean ± standard error of mean. $^{\#}P < 0.05$ vs. intact female rats; $^{\#\#}P < 0.01$ vs. intact female rats; $^{\#\#\#}P < 0.001$ vs. intact female rats; $^{+}P < 0.05$ vs. OVX rats; $^{++}P < 0.01$ vs. OVX rats

IV. DISCUSSION

This study evaluated patterns in serum kisspeptin levels in different developmental stages and investigated the main source of serum kisspeptin in female rats. Serum kisspeptin level increased progressively in accordance with developmental stage until the peripuberty stage, suggesting that it may be an

associated marker of puberty. In addition, we also evaluated *Kiss1* expression in various organs at similar stages, and demonstrated that the pattern of *Kiss1* expression in the ovary, AVPV, and ARC was similar to that of serum kisspeptin. Especially, the ovaries expressed the highest levels of *Kiss1* mRNA and Kiss1 protein near the onset of puberty. Because the highest expression of *Kiss1* mRNA and Kiss1 protein during development was observed in the ovary, we evaluated the changes in serum kisspeptin level, and the *Kiss1* mRNA and protein expression in the AVPV and ARC in OVX and estradiol-treated OVX rats. Serum kisspeptin level decreased in OVX rats independent of estradiol treatment, unlike the changes in *Kiss1* mRNA expression in the AVPV and ARC. These results suggest that the ovaries are the main source of serum kisspeptin and an increase in serum kisspeptin levels along with the progression of puberty is not related to *Kiss1* expression in the AVPV and ARC.

Previous findings have clearly shown that the kisspeptin/KISS1R system regulates puberty onset and development. Just as kisspeptin in the AVPV and ARC in the hypothalamus control pubertal development[1,2], peripheral serum kisspeptin might also have significant implications in pubertal development[13-15,17,28,29]. Serum kisspeptin concentrations increased with the subsequent stages of puberty[13,17] and were higher in girls with central precocious puberty than pre-pubertal girls[14,15]. In addition, daily injections of kisspeptin peripherally advanced puberty onset in mouse and humans[28,29]. These data suggest that serum kisspeptin levels may reflect the onset of puberty and that serum kisspeptin plays an important role in pubertal development. However, due to conflicting results[16,17], reports on changes in serum kisspeptin levels during puberty are controversial, and studies on the patterns of serum kisspeptin levels in successive developmental stages are required to clarify the pattern of serum kisspeptin. To the best of our knowledge, this is the first study to evaluate the patterns of serum kisspeptin levels at different developmental stages in female rats. We found that serum kisspeptin levels increased progressively during the

sequential developmental stages until the peri-pubertal stage, suggesting that serum kisspeptin may reflect puberty or serve as a marker associated with puberty.

However, it remains unclear whether the increase in serum kisspeptin levels during successive developmental stages reflects the changes in hypothalamic *Kiss1* expression that occur at puberty onset. A recent report showed that serum kisspeptin levels were elevated in girls compared to boys, suggesting that the higher levels may be due to greater hypothalamic kisspeptin signaling in girls than boys[13]. However, since kisspeptin is expressed in several other organs[18-20], these organs may also be candidates that control the serum kisspeptin levels during developmental stages. Kanasaki *et al.*[30] suggested that only local kisspeptin, which is produced within the hypothalamic neuron, may exert its stimulatory effect on GnRH via a paracrine mechanism, and that circulating kisspeptin may originate from other peripheral organs. Little effort has been directed towards determining the source of serum kisspeptin, and only a limited number of studies have been performed to analyze the significance of *Kiss1* expression in various organs according to the developmental stage. Therefore, to better identify the origin of serum kisspeptin, we evaluated changes of *Kiss1* mRNA and protein expression in several organs known to express *Kiss1* according to the developmental stage in intact female rats. In this study, while the other organs sparsely expressed *Kiss1* mRNA, *Kiss1* mRNA expression prominently increased with the progression of puberty in the ovary and the AVPV and ARC, similar to the changes in serum kisspeptin levels.

To test this possibility more specifically, we investigated the serum kisspeptin levels and *Kiss1* expression in the AVPV and ARC in both OVX and estradiol-treated OVX rats. Serum kisspeptin levels decreased significantly in OVX rats, suggesting that the ovaries are the main organs that contribute to serum kisspeptin or that estrogen exclusion, due to ovariectomy, decreased *Kiss1* expression in the AVPV, thereby decreasing serum kisspeptin levels.

Previous studies[6,31-34] have shown that *Kiss1* expression decreased after ovariectomy and increased with estradiol treatment in the AVPV, whereas an opposing trend was observed in the ARC. These results led to the hypothesis that estradiol may play a positive-feedback role in the AVPV in regards to kisspeptin, whereas in the ARC, it may serve a negative-feedback role[6,32]. In this study, changes in *Kiss1* mRNA and protein expression in the AVPV in OVX and estradiol-treated OVX rats were consistent with the results of previous studies[6,33,34]. However, the decrease in the serum kisspeptin levels in OVX rats, which was similar to the reduced *Kiss1* expression in the AVPV, was not restored to those observed in intact female rats even after estradiol treatment. In addition, although the change in *Kiss1* expression in the ARC in OVX and estradiol-treated OVX rats did not show consistent findings with previous studies, the expression did not decrease after OVX, then this finding confirmed that change of *Kiss1* expression in ARC was different from the change in serum kisspeptin. These findings suggest that *Kiss1* expression in the ovaries, rather than in the hypothalamus, mainly regulates serum kisspeptin levels.

Terao *et al*.[20] was the first to report that *Kiss1* was expressed in rat ovaries, which was then confirmed in several studies[21,23,35]. Although different expression patterns were observed among these studies because of age discrepancies and samples obtained having been at different points in the estrous cycle, ovarian *Kiss1* expression has been associated with follicle growth, oocyte maturation, and ovulation[21,23,35]. In addition, ovarian *Kiss1* expression is directly stimulated by LH surge through the LH receptor[21] or by the injection of human chorionic gonadotropin[20], which is blocked by prevention of the preovulatory gonadotropin surge[23]. These suggest that ovarian *Kiss1* expression might be regulated by LH[21,23,36]. To summarize, ovarian kisspeptin might play a role in follicular development and ovulation, which is associated with LH secretion and not hypothalamic kisspeptin. However, hypothalamic *Kiss1* expression increases with puberty, leading to HPG axis activation and increased

LH levels, thereby increasing ovarian kisspeptin expression. As a result, serum kisspeptin levels increase according to pubertal development. Therefore, although hypothalamic *Kiss1* expression does not directly affect serum kisspeptin, it indirectly affects its pattern during puberty. Thus, although serum kisspeptin could not be a direct biomarker for the onset of puberty or the activation of HPG axis, it can be an indirect indicator for puberty by judging the maturation of reproductive function according to puberty development.

V. CONCLUSION

In the present study, we evaluated patterns in serum kisspeptin levels and *Kiss1* mRNA and protein expression in accordance with developmental stage in various organs. Serum kisspeptin levels increased as puberty progressed, and among the organs studied, the ovaries expressed the highest level of *Kiss1* in a pattern similar to the serum kisspeptin levels. In addition, serum kisspeptin levels decreased in OVX rats independent of estradiol treatment. Our findings support the conclusion that serum kisspeptin levels are mainly regulated by *Kiss1* expression in the ovaries, suggesting that the ovaries are the main source of serum kisspeptin. Therefore, we indicate that serum kisspeptin may be a downstream marker for ovarian activity but do not represent the degree of activation of the HPG axis during puberty. However, since LH stimulates *Kiss1* expression in the ovary, the serum kisspeptin level might serve as an indirect marker for puberty. Nevertheless, further studies are needed to evaluate the roles of serum kisspeptin in the development of the reproductive system.

References

1. Seminara, S. B. *et al.* The GPR54 gene as a regulator of puberty. *N. Engl. J. Med.* **349**, 1614–1627 (2003).

2. Ohtaki, T. *et al.* Metastasis suppressor gene KiSS-1 encodes peptide ligand of a G-protein-coupled receptor. *Nature* **411**, 613–617 (2001).

3. de Roux, N. *et al.* Hypogonadotropic hypogonadism due to loss of function of the KiSS1-derived peptide receptor GPR54. *Proc. Natl. Acad. Sci. U. S. A.* **100**, 10972–10976 (2003).

4. Topaloglu, A. K. *et al.* Inactivating KISS1 mutation and hypogonadotropic hypogonadism. *N. Engl. J. Med.* **366**, 629–635 (2012).

5. Novaira, H. J. *et al.* Disrupted kisspeptin signaling in GnRH neurons leads to hypogonadotrophic hypogonadism. *Mol. Endocrinol.* **28**, 225–238 (2014).

6. Smith J. T. *et al.* Cunningham MJ, Rissman EF, Clifton DK, Steiner RA. Regulation of Kiss1 gene expression in the brain of the female mouse. Endocrinology. **146**, 3686–3692 (2005)

7. Clarkson J. *et al*. Postnatal development of kisspeptin neurons in mouse hypothalamus; sexual dimorphism and projections to gonadotropin-releasing hormone neurons. Endocrinology. **147**, 5817–5825 (2006).

8. Semaan, S. J. & Kauffman, A. S. Daily successive changes in reproductive gene expression and neuronal activation in the brains of pubertal female mice. *Mol. Cell. Endocrinol.* **401**, 84–97 (2015).

9. Li, X.-F. *et al.* Kisspeptin signalling in the hypothalamic arcuate nucleus regulates GnRH pulse generator frequency in the rat. *PLoS One* **4**, e8334 (2009).

10. Roseweir, A. K. *et al.* Discovery of potent kisspeptin antagonists delineate physiological mechanisms of gonadotropin regulation. *J. Neurosci.* **29**, 3920–3929 (2009).

11. Adachi S. *et al*. Involvement of anteroventral periventricular metastin/kisspeptin neurons in estrogen positive feedback action on luteinizing hormone release in female rats. *J Reprod Dev.* **53**, 367–378 (2007).

12. Navarro, V. M. *et al.* Advanced vaginal opening and precocious activation of the reproductive axis by KiSS-1 peptide, the endogenous ligand of GPR54. *J. Physiol.* **561**, 379–386 (2004).

13. Jayasena, C. N. *et al.* Age-dependent elevations in plasma kisspeptin are observed in boys and girls when compared with adults. *Ann. Clin. Biochem.* **51**, 89–96 (2014).

14. Vries, L. D., De Vries, L., Shtaif, B., Phillip, M. & Gat-Yablonski, G. Kisspeptin serum levels in girls with central precocious puberty. *Clinical Endocrinology* vol. 71 524–528 (2009).

15. Rhie, Y. J. *et al.* Serum kisspeptin levels in Korean girls with central precocious puberty. *J. Korean Med. Sci.* **26**, 927–931 (2011).

16. Pita, J. *et al.* Circulating kisspeptin levels exhibit sexual dimorphism in adults, are increased in obese prepubertal girls and do not suffer modifications in girls with idiopathic central precocious puberty. *Peptides* **32**, 1781–1786 (2011).

17. Xue, J. *et al.* Serum Kisspeptin and AMH Levels Are Good References for Precocious Puberty Progression. *Int. J. Endocrinol.* **2020**, 3126309 (2020).

18. Gaytán, F. *et al.* KiSS-1 in the mammalian ovary: distribution of kisspeptin in human and marmoset and alterations in KiSS-1 mRNA levels in a rat model of ovulatory dysfunction. *Am. J. Physiol. Endocrinol. Metab.* **296**, E520–31 (2009).

19. Lee, J. H. *et al.* KiSS-1, a novel human malignant melanoma metastasis-suppressor gene. *J. Natl. Cancer Inst.* **88**, 1731–1737 (1996).

20. Terao, Y. *et al.* Expression of KiSS-1, a metastasis suppressor gene, in trophoblast giant cells of the rat placenta. *Biochim. Biophys. Acta* **1678**, 102–110 (2004).

21. Laoharatchatathanin, T., Terashima, R., Yonezawa, T., Kurusu, S. & Kawaminami, M. Augmentation of Metastin/Kisspeptin mRNA Expression by

the Proestrous Luteinizing Hormone Surge in Granulosa Cells of Rats: Implications for Luteinization. *Biol. Reprod.* **93**, 15 (2015).

22. Uenoyama, Y., Pheng, V., Tsukamura, H. & Maeda, K.-I. The roles of kisspeptin revisited: inside and outside the hypothalamus. *J. Reprod. Dev.* **62**, 537–545 (2016).

23. Castellano, J. M. *et al.* Expression of KiSS-1 in rat ovary: putative local regulator of ovulation? *Endocrinology* **147**, 4852–4862 (2006).

24. Ricu, M. A., Ramirez, V. D., Paredes, A. H. & Lara, H. E. Evidence for a celiac ganglion-ovarian kisspeptin neural network in the rat: intraovarian anti-kisspeptin delays vaginal opening and alters estrous cyclicity. *Endocrinology* **153**, 4966–4977 (2012).

25. Ojeda, S. R. & Urbanski, H. F. Puberty in the rat. In 'The Physiology of Reproduction'.(Eds E. Knobil and J. Neill.) pp. 1699--737. (1988).

26. Khazipov, R. *et al.* Atlas of the Postnatal Rat Brain in Stereotaxic Coordinates. *Front. Neuroanat.* **9**, 161 (2015).

27. Rao, X. *et al*. An Improvement of the 2ˆ(-Delta Delta CT) Method for Quantitative Real-Time Polymerase Chain Reaction Data Analysis. *Biostat Bioinform Biomath*. **3**, 71–85 (2013).

28. Decourt, C. *et al.* A synthetic kisspeptin analog that triggers ovulation and advances puberty. *Sci. Rep.* **6**, 26908 (2016).

29. Young, J. *et al.* Kisspeptin restores pulsatile LH secretion in patients with neurokinin B signaling deficiencies: physiological, pathophysiological and therapeutic implications. *Neuroendocrinology* **97**, 193–202 (2013).

30. Kanasaki, H. *et al.* Circulating kisspeptin and pituitary adenylate cyclase-activating polypeptide (PACAP) do not correlate with gonadotropin serum levels. *Gynecol. Endocrinol.* **29**, 583–587 (2013).

31. Smith, J. T., Popa, S. M., Clifton, D. K., Hoffman, G. E. & Steiner, R. A. Kiss1 neurons in the forebrain as central processors for generating the preovulatory luteinizing hormone surge. *J. Neurosci.* **26**, 6687–6694 (2006).

32. Roa, J. *et al.* Hypothalamic expression of KiSS-1 system and gonadotropin-releasing effects of kisspeptin in different reproductive states of the female Rat. *Endocrinology* **147**, 2864–2878 (2006).

33. Kauffman, A. S. *et al.* Sexual differentiation of Kiss1 gene expression in the brain of the rat. *Endocrinology* **148**, 1774–1783 (2007).

34. Takase, K. *et al.* Possible role of oestrogen in pubertal increase of Kiss1/kisspeptin expression in discrete hypothalamic areas of female rats. *J. Neuroendocrinol.* **21**, 527–537 (2009).

35. Fernandois, D. *et al.* Kisspeptin is involved in ovarian follicular development during aging in rats. *J. Endocrinol.* **228**, 161–170 (2016).

36. Peng, J. *et al.* Kisspeptin stimulates progesterone secretion via the Erk1/2 mitogen-activated protein kinase signaling pathway in rat luteal cells. *Fertil. Steril.* **99**, 1436–1443.e1 (2013).

ABSTRACT (IN KOREAN)

사춘기 발달에 따른 혈청 kisspeptin의 변화 및 혈청 kisspeptin의 기원이 난소임을 밝힌 연구

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권 아 름

사춘기는 시상하부-뇌하수체-생식샘 축의 활성으로 인해 시작되는 정교하고 복잡한 생물학적 과정으로, 사춘기의 시작 기전은 아직 충분히 밝혀지지 않았지만, 시상하부의 Kisspeptin/KISS1R 시스템이 시상하부-뇌하수체-생식샘 축을 활성화시켜 사춘기의 시작을 유도하는 것으로 생각되고 있다. 시상하부의 Kisspeptin/KISS1R 시스템이 사춘기 시작에 중요한 역할을 하기 때문에 혈청 kisspeptin 또한 사춘기와 관련이 있는 것으로 생각되지만, 아직까지 성장 단계에 따라 혈청 kisspeptin 농도가 어떻게 변하는지, 어느 장기가 혈청 kisspeptin의 주요 기원인지에 대해서는 연구가 되지 않았다. 본 연구는 암컷 쥐의 발달 단계에 따라 혈청 kisspeptin 농도가 어떻게 변하는지를 확인하고, 혈청 kisspeptin이 어느 장기에서 유래하는지 확인하고자 하였다. 본 연구에서는 암컷 쥐를 신생아기-영유아기-사춘기-사춘기완성시기로 분류하여 각각의 단계의 초기와

중기 시기에 Kisspeptin의 혈청 농도를 측정하고, 같은 시기에 *Kiss1*을 발현하는 것으로 알려진 여러 장기에서의 *Kiss1* mRNA 발현의 변화를 확인하였다. 이 연구에서 *Kiss1* 발현을 확인한 장기는 시상하부의 앞배쪽뇌실주위핵과 궁상핵, 뇌하수체, 난소, 자궁, 부신, 췌장이었다. 혈청 kisspeptin 농도는 사춘기가 진행됨에 따라 증가하여, 사춘기 시기에 최고치에 도달하였으나, 사춘기 완성시기에 다시 감소하였다. *Kiss1* mRNA 발현을 확인한 여러 장기 중, 난소가 *Kiss1* 발현이 가장 높았고, 그 패턴은 혈청 kisspeptin 과 비슷하였으나 한 단계 앞서 있었다. 또한 시상하부의 앞배쪽뇌실주위핵과 궁상핵에서도 사춘기 발달에 따라 *Kiss1* 발현이 증가였으며, 혈청 kisspeptin 과 비슷한 변화 패턴을 보였다.

혈청 kisspeptin이 난소의 *Kiss1*에서 유래하는지, 시상하부의 Kisspeptin/KISS1 시스템의 후속 반응인지를 확인하기 위하여, 난소를 제거한 암컷쥐와 난소 제거 후 여성호르몬을 투여한 암컷쥐에서의 혈청 kisspeptin의 농도가 어떻게 변하는지를 확인하였고, 함께 시상하부의 앞배쪽뇌실주위핵과 궁상핵에서의 *Kiss1* 발현의 변화도 확인하였다. 기존의 연구에서 앞배쪽뇌실주위핵에서의 *Kiss1* 발현은 여성호르몬에 대하여 양성되먹임 반응, 즉, 여성호르몬이 증가하면 *Kiss1* 발현 또한 증가하였고, 궁상핵에서는 이와는 반대로 여성호르몬에 대하여 음성되먹임 반응, 즉, 여성호르몬이 증가하면 *Kiss1* 발현이 감소하였다. 따라서 이 연구 결과를 통해 혈청 kisspeptin이 어느 장기의 *Kiss1*에 의한 것인지 확인할 수 있다고 생각하였다. 혈청 kisspeptin의 농도는 난소를 제거한 후 감소하였으나, 여성호르몬을 투여했음에도 여전히 감소한 상태였다. 또한 앞배쪽뇌실주위핵과 궁상핵에서의 *Kiss1* 발현은 기존의 연구 결과와 동일하게 여성호르몬에 대하여 각각 양성되먹임 반응과 음성되

먹임 반응을 보였다. 이는 혈청 kisspeptin의 변화가 앞배쪽뇌실주위핵이나 궁상핵에서의 *Kiss1* 발현 양상과 다르며, 혈청 kisspeptin이 난소의 *Kiss1*에서 유래하는 것임을 시사한다.

이러한 결과를 토대로 혈청 kisspeptin은 사춘기 발달에 따라 증가하나, 이는 사춘기 발달에 따른 시상하부의 kisspeptin/KISS1 시스템의 후속 반응이 아닌, 난소의 *Kiss1* 발현의 변화에 따른 것임을 확인하였다. 본 연구 결과는 향후 혈청 kisspeptin의 임상 적용에 있어 중요한 기초가 될 것으로 기대된다.

핵심되는 말 :kisspeptin, 난소, 시상하부, 성발달, 암컷, 쥐