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The inhibitory effects of gonadotropin-releasing hormone(GnRH) agonist on ovarian functions in immature rats pretreated with pregnant mare serum gonadotropin(PMSG)

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Abstract : In the present study, to understand how gonadotropin-releasing hormone (GnRH) affects ovarian functions in superovulated rats, we examined the effects of GnRH agonist on the ovulatory response, the morphological normality and nuclear maturation of ovulated oocytes, the ovarian weight, the ovarian histology, and the circulating steroid hormone (17β-estradiol, progesterone and testosterone) levels in immature rats pretreated with 30IU pregnant mare serum gonadotropin (PMSG) and supplemented with 10IU human chorionic gonadotropin(hCG). GnRH agonist was intravenously injected via jugular vein catheter every 20min for 4hrs in early follicular phase (from 6hr after PMSG) of superovulated rats. In addition, GnRH antagonist, Antide, was intravenously injected in combination with GnRH agonist to verify the effects of GnRH agonist on ovarian functions. All animals were sacrificed at 72hr after PMSG administration.

The administration with GnRH agonist in early follicular phase of superovulated rats caused inhibition of ovulatory response, increased the proportion of abnormal appearing oocytes(especially, in the rats of the group treated with 500ng GnRH agonist), decreased ovarian weight and promote follicular atresia, compared to those from the rats of control regimen that were not treated with GnRH agonist. In addition, the treatment with GnRH agonist in the superovulated rat distinctly decreased serum steroid hormone (17β-estradiol, progesterone and testosterone) levels in preovulatory phase. On the other hand, the inhibitory effects of GnRH agonist treatment in superovulation-pretreated rats on ovarian functions were totally reversed by the combination with GnRH antagonist, Antide. The nuclear maturation of oocytes recovered from the oviducts in immature rats treated with GnRH agonist and/or GnRH antagonist was characterized by prematurity and asynchronization in early follicular phase, which was similar to control group.

The overall results of this study indicate that GnRH agonist disturbs directly ovarian function

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in early follicular phase of superovulated immature rats in terms of ovulatory response and morphological normality of ovulated oocytes. This concept has been further evidenced by the findings of a great decrease in ovarian weight, a marked increase in follicular atresia and a distinct decrease circulating steroid hormone (17β-estradiol, progesterone and testosterone) levels in GnRH agonist treatment regimen in early follicular phase.

Key words : GnRH agonist, ovarian function, PMSG, superovulation, rat.

Introduction

Superovulation protocols employed with exogenous gonadotropins such as pregnant mare serum gonadotropin(PMSG) have been widely used in many laboratories and livestock industries for the retrieval of multiple oocytes and preimplantation embryos. However, it has been reported that protocols result in reduced fertility in large domestic[1,2] and small laboratory animals[3~7]. In immature rats, superovulatory doses of PMSG lead to a progressive failure in early stages of pregnancy[7]. This failure was shown to be due to excessive estrogenic stimulation of genital tract following ovulation and asynchrony in embryonic and uterine development by disturbances in the oviductal and uterine microenvironment[8]. Early defects following superovulation with PMSG could primarily occur in the process of follicular development and oocyte maturation before ovulation by hyperstimulation of ovarian tissues[9,10]. Pre- or peri-ovulatory endocrine aspects including the excessive secretion of follicular steroids relate to the affection of the final maturation, subsequent fertilization, and developmental capability of ovulated oocytes[9].

The use of hypothalamic regulator or pituitary gonadotropin preparations with shorter biological half-life might overcome some of the endocrine problems associated with the use of PMSG. It is reported that the employment of gonadotropin-releasing hormone(GnRH) agonists in combination with exogenous gonadotropin stimulation had gained multiple oocytes and embryos[11~16] in human *in vitro* fertilization and embryo transfer(IVF & ET) program.

This protocol of ovulation induction reduces the risk of a premature luteinizing hormone(LH) surge and when performed for IVF & ET17, increases the average number of oocytes obtained at retrieval[16]. Besides, administration of GnRH agonists in late follicular phase of IVF patients increases serum levels of endogenous LH and follicle stimulating hormone (FSH) similar to a spontaneous gonadotropin surge with subsequent retrieval of mature oocytes which are capable of fertilization, implantation, and normal pregnancy[18,19].

Therefore, the objective of this study is to investigate the GnRH action on the ovarian functions of superovulated immature rats pretreated with PMSG. In detail, this study examined the effects of GnRH agonist on the ovulatory response, the morphological normality and nuclear maturation of ovulated oocytes, the ovarian weight, the ovarian histology, and the circulating steroid hormone(17β-estradiol, progesterone and testosterone) levels in these rats.

Materials and Methods

Immature female Sprague-Dawley rats were kept under temperature- and light-controlled conditions(20~25℃, 12L : 12D) and fed standard rat chow and water *ad libitum* throughout the experimental period. One day before the experiment(on the age of 27 days), the animals were installed with chronically indwelling catheters using the method described by Harms and Ojeda[20]. Briefly, a catheter made of silastic tubing(Dow-Corning Corp., Midland, MI) was slid into the external jugular vein to enter or approach the right atrium under pentobarbital anaesthesia(35mg/kg body weight). To prevent clot formation in the catheter, catheter was filled

with dilute 25IU/ml heparin-saline solution.

On the following day(day 28 of age), the rats received a single subcutaneous dose(30IU/0.2ml saline) of PMSG(Equinex, Ayerst, Montreal, Quebec, Canada) for superovulation induction. Following PMSG treatment, animals were given 100ng or 500ng GnRH agonist(pGLU-HIS-TRP-SER-TYR-$_D$-ALA-LEU-ARG-PRO-NHEt, Sigma Chemical Co., St. Louis, MO) and/or 1μg GnRH antagonist, Antide(Acetyl-β-[2-Naphthyl]-$_D$-Ala-$_D$-p-Chloro-Phe-β-[3-Pyridyl]-$_D$-Ala-Ser-Nε-[Nicotinoyl]-Lys-Nε-[Nicotinoyl]-$_D$-Lys-Leu-Nε-[Isopropyl]-Lys-pro-$_D$-Ala-NH_2, Sigma Chemical Co., St. Louis, MO) starting at 6hr(in early follicular phase). GnRH agonist was intravenously injected via the jugular vein catheter every 20min for 4hrs in 0.1ml of sterile saline, and in a separate experiment, 1μg GnRH antagonist, antide was intravenously administered in 0.1ml of sterile saline in combination with GnRH agonist to certify the real effects of GnRH on ovarian functions. The females were subcutaneously injected with 10IU human chorionic gonadotropin(hCG, Sigma Chemical Co., St. Louis, MO) in 0.2ml of sterile saline at 54hr after PMSG injection to ensure ovulation. In order to analyze circulating steroid hormone(17β-estradiol, progesterone and testosterone) concentration, 0.5ml of whole blood was collected from females via the jugular catheter at 54hr after PMSG administration. The blood was allowed to stand at room temperature for 6~8hrs to clot. The samples were centrifugated at 2,000rpm for 20 minutes to separate serum. The sera were stored at -20℃ for subsequent assay of steroid hormones using radioimmunoassay(RIA).

Animals were sacrificed at 72hr after PMSG injection. To measure ovarian weight, ovaries dissected free from oviducts were cleaned of ovarian bursae, connective tissue and fat. They were then dried, paired and weighed in mg tissue. At the same time, to count oviductal oocytes, oviducts were separated from uterine horns at the uterotubal junction and flushed with a few drops of Dulbecco's phosphate-buffered saline(DPBS) under a stereo dissecting microscope(10×magnification, Nikon, SMZ-U), as described previously by Yun [21]. Subsequently, in order to facilitate the oocyte counting, the extracoronal cumulus cells surrounding the oocytes were dispersed after being exposed to DPBS solution containing 0.1% of hyaluronidase(type I-S : from bovine testes, Sigma Chemical Co., St. Louis, MO) for 10~15 minutes. The recovered oocytes were counted under the stereo dissecting microscope(40×magnification), and were subjected to assess the occurrence of fragmentation and other degenerative changes. Briefly, those eggs showing an irregular cell mass with debris and empty zona pellucida were classified as abnormal.

To assess oocyte nuclear maturation, the recovered normal appearing oocytes were stained as described previously [21]. Briefly, only normal appearing oocytes were placed into a 10×35mm petri dish containing 1.0% hypotonic sodium citrate and swollen at room temperature for 10min. Subsequently, the oocytes were transferred onto a grease-free slide with a thin coat of Mayer's albumen. The oocytes were allowed to dry over a hot plate(45~53℃) to enhance chromosome spreading. Then the dried oocytes were fixed with acetic alcohol(one part of glacial acetic acid and two parts of absolute ethyl alcohol) for 45min, and stained with 2% aceto-orcein for 30min. A stock solution of this stain had been made up in a fumehood by dissolving 2.2g of orcein (BDH Chemical Ltd. Toronto, Ontario) in 45ml of hot glacial acetic acid with continuous stirring for 30min and by adding 55ml of 0.9% NaCl solution. A working staining solution was prepared immediately before use by mixing two parts of the stock solution with three parts of 0.9% NaCl solution and by filtering with 0.8μm syringe filter. Finally, the stained oocytes were allowed to a serial dehydration through 50%, 60%, 80% and 100% ethyl alcohol followed by xylene for 5min in each step, and subjected to a microscopic evaluation of nuclear maturation. Various stages of meiosis were identified according to the criteria described previously[21].

To observe ovarian histology, ovaries obtained at 72hr after PMSG injection were immediately fixed in Bouin's solution(75% saturated picric acid, 20% buffered formalin and 5% glacial acetic acid) for about 6hrs and washed for 12hrs in 70% ethanol to remove excess fixation. The ovarian tissue was subsequently dehydrated in sequential concentrations of ethanol(70%, 80%, 90% and 100%), cleared, and embedded in paraffin wax. Then serial sections of the tissue block were cut 5μm thick and stained with hematoxylin and

eosin. All sections were examined for evidence of precocious ovulation or follicular atresia, and representative sections were taken for photomicroscopy(Olympus, BX50, Japan). Advanced stages of follicular atresia were defined by nuclear pyknosis and a loss of homogeneity of the granulosa cell layers in conjunction with thecal hypertrophy and varying degrees of oocyte degeneration.

To determine serum steroid hormones(17β-estradiol, progesterone and testosterone), 0.5ml aliquots of sera were extracted with 3ml ethyl ether by vigorously vortexing. These extracts were evaporated for 60 minutes and reconstituted in 1ml GPBS(buffer). 100μl aliquots of the extracts were assayed in duplicate for steroid hormones by specific RIA using the antisera. In the assay procedures, approximately 6,000cpm of tracer(^{3}H) was added to each tube. The unbound steroids were removed by the 0.2ml cold dextran coated charcoal, and the bound steroids were counted in a liquid scintillation analyzer(TRI-CARB 2300TR, Packard). The binding efficiency(%B_0) of the steroid antibodies was 40~50% and %NSB was less than 5%. The coefficient of variation(*CVs*) was 9~10% for 17β-estradiol, 8~9% for progesterone and 7~9% for testosterone, respectively. Hormone concentrations were expressed as ng/ml sera.

The significance of differences between the means of treatment groups was evaluated statistically by analysis of variance employing the LSD(Least Significant Difference) of Student's *t*-test.

Results

Ovulatory responses modulated by treatments with GnRH agonist and/or GnRH antagonist in early follicular phase of immature rats given superovulatory dose of PMSG are presented in Fig 1. The oocyte count(1.00$\pm$0.68 oocytes/rat) obtained after treatment with 500ng GnRH agonist in early follicular phase of superovulated rats was significantly($p < 0.01$) decreased, compared to that(48.00$\pm$4.57 oocytes/rat) from the rats of control group that was not treated with GnRH agonist. In contrast, the treatment with 1μg GnRH antagonist in conjunction with 500ng GnRH agonist recovered the ovulatory response decreased following GnRH agonist treatment to the value of controls ; the oocyte count in GnRH antagonist-cotreated group was 33.63$\pm$6.41 oocytes/rat.

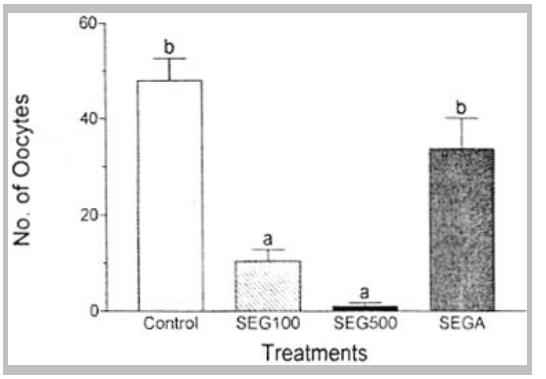

Fig 1. Ovulatory responses after treatments with GnRH agonist and/or GnRH antagonist in early follicular phase of immature rats pretreated with 30IU PMSG and supplemented with 10IU hCG.
100ng GnRH agonist(SEG100), 500ng GnRH agonist(SEG 500), or 1μg GnRH antagonist and 500ng GnRH agonist (SEGA). GnRH agonist and GnRH antagonist were injected intravenously every 20min for 4hrs from 6hr after PMSG-pretreatment. Control rats were administered with saline at the same time of GnRH treatment. The number of oocytes recovered from oviducts at 72hr after PMSG-pretreatment, are expressed as the mean$\pm$SE(n = 8). The means with no superscripts in common are significantly($p < 0.01$) different.

Morphological normality(43.34$\pm$23.34%) of oocytes recovered from the rats treated with 500ng GnRH agonist was significantly($p < 0.05$) decreased, compared to that(85.03$\pm$2.20%) obtained from the rats of control group. However, the percentage of oocyte normality in the rats treated with 1μg GnRH antagonist in combination with 500ng GnRH agonist was 84.85$\pm$3.67%, which was comparable to control value (Fig 2). This value was shown greatly to recover the normality reduced by 500ng GnRH agonist treatment exclusively.

The weight(8.60$\pm$0.49mg) with paired ovaries in the rats treated with 500ng GnRH agonist was significantly($p < 0.05$) decreased, compared to that(20.67$\pm$3.23mg) from the rats of control group. The ovarian weight following the treatment with 1μg Antide and 500ng GnRH agonist was 19.78$\pm$1.71mg per rat, which was comparable to the control value(Fig 3). This value was found greatly to recover the weight loss caused by the treatment with 500ng GnRH agonist exclusively.

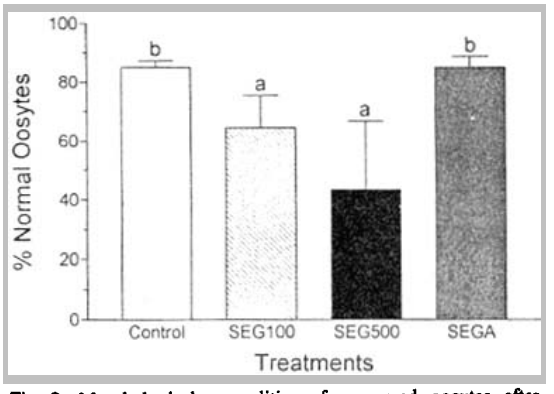

Fig 2. Morphological normalities of recovered oocytes after treatments with GnRH agonist and/or GnRH antagonist in early follicular phase of immature rats pretreated with 30IU PMSG and supplemented with 10IU hCG.
100ng GnRH agonist(SEG100), 500ng GnRH agonist(SEG 500), or 1μg GnRH antagonist and 500ng GnRH agonist (SEGA). GnRH agonist and GnRH antagonist were injected intravenously every 20min for 4hrs from 6hr after PMSG-pretreatment. Control rats were administered with saline at the same time of GnRH treatment. The results of % normal oocytes recovered from oviducts at 72hr after PMSG-pretreatment, are expressed as the mean ± SE(n = 8). The means with no superscripts in common are significantly($p < 0.05$) different.

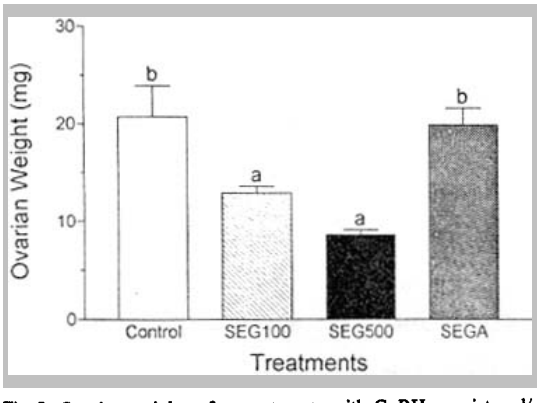

Fig 3. Ovarian weights after treatments with GnRH agonist and/or GnRH antagonist in early follicular phase of immature rats pretreated with 30IU PMSG and supplemented with 10IU hCG.
100ng GnRH agonist(SEG100), 500ng GnRH agonist(SEG500), or 1μg GnRH antagonist and 500ng GnRH agonist(SEGA), GnRH agonist and GnRH antagonist were injected intravenously every 20min for 4hrs from 6hr after PMSG-pretreatment. Control rats were administered with saline at the same time of GnRH-pretreatment. The results of dried and paired ovarian weight measured at 72hr after PMSG treatment, are expressed as the mean ± SE(n = 8). The means with no superscripts in common are significantly($p < 0.05$) different.

The oocytes recovered from oviducts after treatments with GnRH agonist in early follicular phase of superovulated rats were classified for each stage of nuclear maturation. The proportion of normal-appearing oocytes analyzable for the classification of each stage was actually 82~100%, since some oocytes lost or scattered their chromosomes by occasional rupture of cell membrane during the process of preparation and staining. Furthermore, the stained oocytes were actually not possible to distinguish between metaphase I and metaphase II, because of rapid deterioration or inconsistent formation of typical polar body. Percentages of oocyte nuclear maturation after treatments with GnRH agonist in early follicular phase of PMSG-pretreated control rats are presented in Fig 4. The nuclear maturation of superovulated oocytes

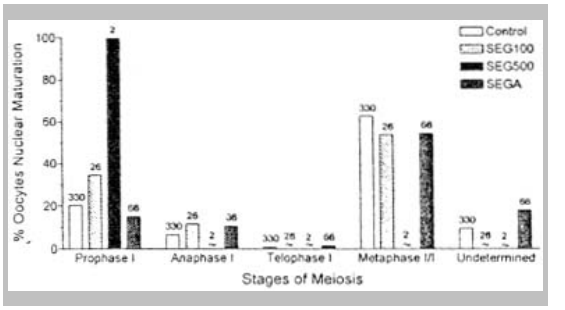

Fig 4. Nuclear maturation of recovered oocytes after treatments with GnRH agonist and/or GnRH antagonist in early follicular phase of immature rats pretreated with 30IU PMSG and supplemented with 10IU hCG.
100ng GnRH agonist(SEG100), 500ng GnRH agonist(SEG 500), or 1μg GnRH antagonist and 500ng GnRH agonist(SEGA), GnRH agonist and GnRH antagonist were injected intravenously every 20min for 4hrs from 6hr after PMSG-pretreatment. Control rats were administered with saline at the same time of GnRH treatment. The number above each bar represents oocyte counts examined; the oocytes recovered with visible signs of degeneration were excluded. ~denotes 0%.

recovered from the PMSG-pretreated control rats was characterized with prematurity and asynchronization : 20.39% in prophase I stage, 6.67% in anaphase I stage, 0.61% in telophase I stage, and 62.73% in metaphase I / II stage. The meiotically aberration phenomena of superovulated control oocytes were not altered by treatments with 100ng GnRH agonist. On the other hand, the oocytes recovered from 500ng GnRH agonist-treated rats consistently exhibited

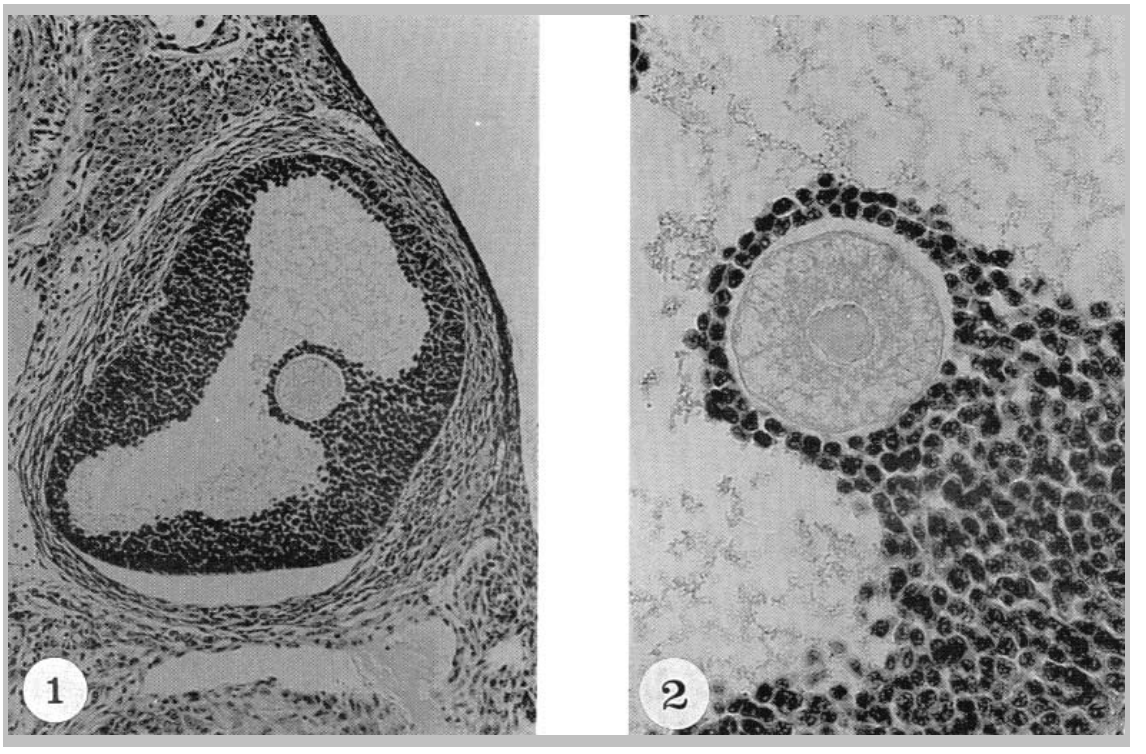

Fig 5. Light microscopic analysis of ovarian morphology obtained at 72hr after 30IU PMSG supplemented with 10IU hCG in the immature rat. Sections(5μm) were stained with hematozylin and eosin. ① Ovary showing a large tertiary follicle. Magnification. ×100. ② Normal oocyte. Magnification, ×200.

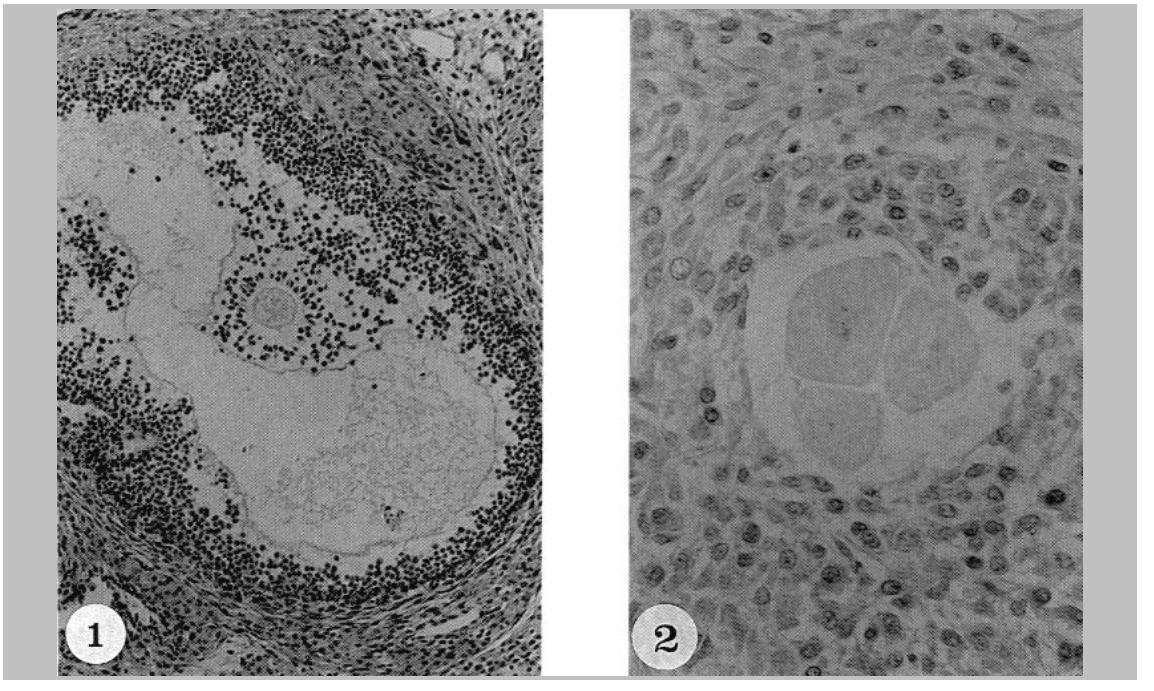

Fig 6. Light microscopic analysis of ovarian morphology obtained after treatment with 500ng GnRH in early follicular phase of the immature rat pretreated with 30IU PMSG supplemented with 10IU hCG at 72hr. Sections(5μm) were stained with hematozylin and eosin. ① Atretic antral follicle with typical atretic signs. Magnification ×100. ② Corpus luteum with a non-ovulated and fragmented oocyte. Magnification, ×400.

the stage of prophase Ⅰ, while a total of only two normal-appearing oocytes were analyzed because of high recovery of abnormal oocytes. The treatment with 1μg GnRH antagonist in combination with 500ng GnRH agonist in early follicular phase of PMSG-pretreated rats also advanced the oocyte nuclear maturation, compared to the group of GnRH agonist treatment exclusively. However, GnRH antagonist treatment did not totally synchronize the oocyte nuclear maturation to the stage of metaphase Ⅱ. Each percentage of oocyte nuclear maturation in this group was 15.15% in prophase Ⅰ stage, 10.61% in Anaphase Ⅰ stage, 1.52% in Telophase Ⅰ stage, and 54.55% in Metaphase Ⅰ/Ⅱ stage.

In ovarian histology, a majority of the follicles in superovulated control rats treated with 30IU PMSG were in tertiary stage of growth(Fig 5). The follicles exhibited not only healthy corpora lutea but a few signs of atresia. In contrast, ovarian morphology after treatment with GnRH agonist in early follicular phase of superovulated rats displayed lots of atretic follicles with granulosa cells containing pronounced pyknotic nuclei(Fig 6). The granulosa layers of the many atretic follicles were characterized to be thinned with the distinct signs of degeneration. There was also a marked loss of structural integrity in the cumulus oophorus granulosa cells. Some large Graafian follicles had become luteinized with non-ovulated and fragmented oocytes. On the other hand, 1μg GnRH antagonist treatment in combination with 500ng GnRH agonist improved the occurrence of follicular atresia which was pronounced by GnRH agonist treatment in early follicular phase(Fig 7).

The concentrations of steroid hormones(17β-estradiol, progesterone and testosterone) in blood of the rats treated with GnRH agonist and/or GnRH antagonist in early follicular phase are presented in Figure 8. The serum levels of steroid hormones after treatment with 500ng GnRH agonist in early follicular phase of PMSG-pretreated rats were measured at 54hr after PMSG treatment. The mean levels of serum steroid hormones in the rats treated with 500ng GnRH agonist were significantly($p < 0.05$) decreased, compared to those from

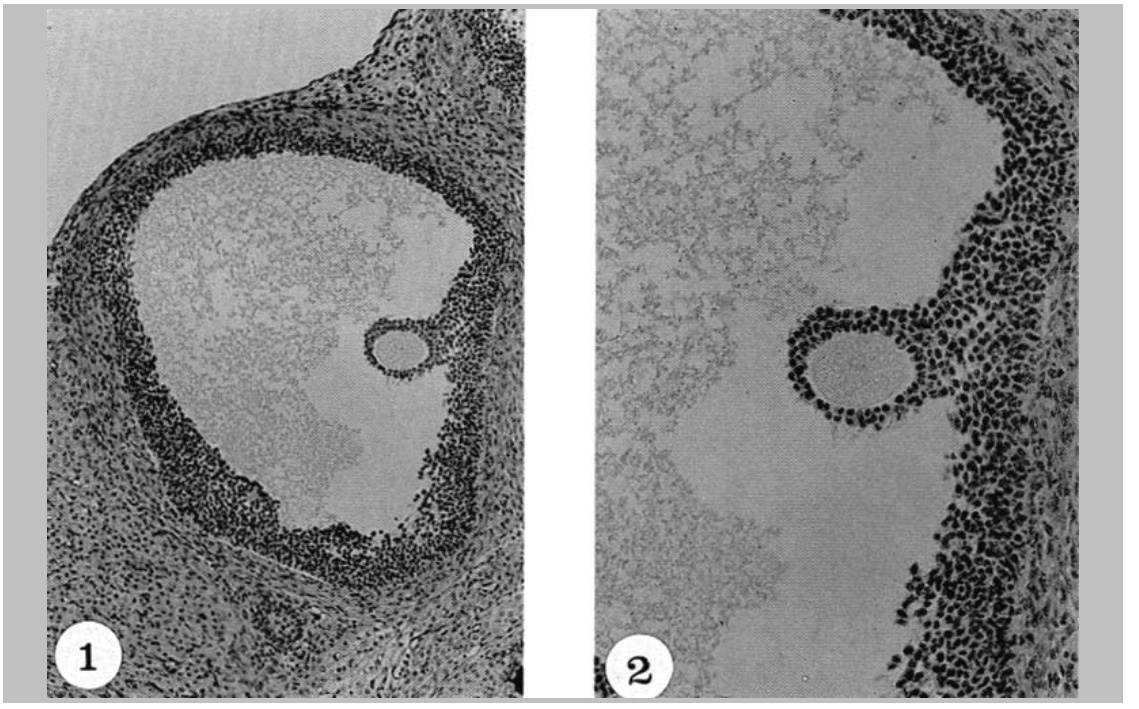

Fig 7. Light microscopic analysis of ovarian morphology obtained after 1μg GnRH antagonist treatment in combination with 500ng GnRH agonist in early follicular phase of the immature rat pretreated with 30IU PMSG supplemented with 10IU hCG at 72hr. Section(5μm) were stained with hematoxylin and eosin. ① Healthy antral follicle. Magnification, ×100. ② Healthy corpus luteum. Magnification, ×100.

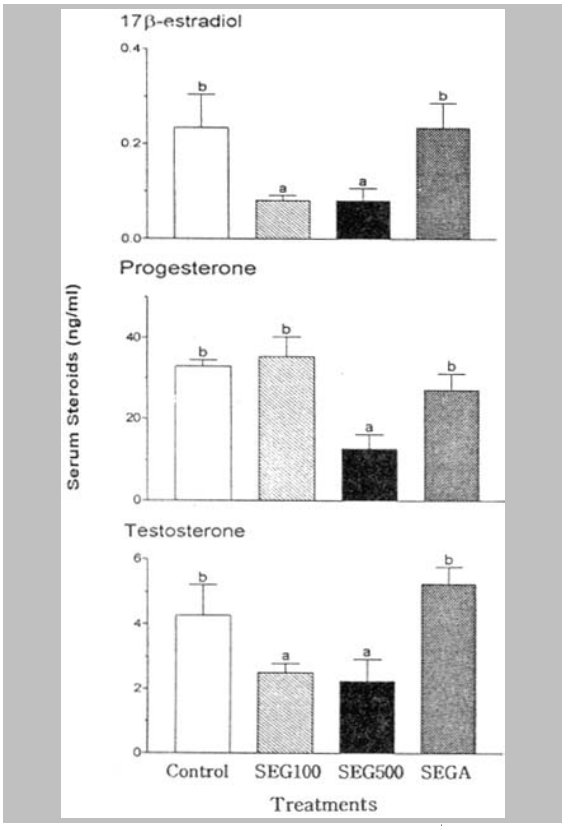

Fig 8. Preovulatory serum levels of 17β-estradiol, progesterone and testosterone after treatments with GnRH agonist and/or GnRH antagonist in early follicular phase of immature rats pretreated with 30IU PMSG and supplemented with 10IU hCG. 100ng GnRH agonist(SEG100), 500ng GnRH agonist(SEG 500), or 1μg GnRH antagonist and 500ng GnRH agonist (SEGA). GnRH agonist and GnRH antagonist were injected intravenously every 20min for 4hrs from 6hr after PMSG-pretreatment. Control rats were administered with saline at the same time of GnRH treatment. The levels of serum steroids measured at 54hr after PMSG-pretreatment, are expressed as the mean$\pm$SE(n = 5). The means with no superscripts in common are significantly($p < 0.05$) different.

control group : the levels of 17β-estradiol, progesterone and testosterone were 0.08$\pm$0.03ng/ml, 12.70$\pm$3.47ng/ml and 2.23$\pm$0.67ng/ml in the rats treated with 500ng GnRH agonist, and 0.23$\pm$0.07ng/ml, 32.86$\pm$1.58ng/ml and 4.26$\pm$0.94 ng/ml in control rats, respectively. On the other hand, 1μg GnRH antagonist treatment in combination with 500ng GnRH agonist recovered serum steroid levels decreased following GnRH agonist treatment, exclusively, to the values of the controls : the levels of 17β-estradiol, progesterone and testosterone in GnRH antagonist-cotreated group were 0.23$\pm$ 0.05ng/ml, 27.10$\pm$4.02ng/ml and 5.24$\pm$0.52ng/ml, respectively.

Discussion

In the present experiment, the ovulatory responses of immature rats treated with 30IU PMSG and supplemented with 10IU hCG were similar to the result reported previously[22]. This hormone treatment certainly induced superovulation at the average of 48 oocytes per rat. In addition, superovulatory treatment with PMSG increased the proportion of abnormal appearing oocytes, and caused premature or asynchronous nuclear maturation of the ovulated oocytes as confirmed previously[10,22].

The results of this study have shown that treatment with GnRH agonist in early follicular phase of the immature rat superovulated with PMSG markedly inhibited ovulatory response. This treatment with GnRH agonist additionally resulted in a great increase in the percentage of abnormal oocytes. The inhibitory activities of GnRH agonist on ovarian function were further evidenced by a significant decrease of ovarian weight and promotion of follicular atresia with pyknosis in granulosa cells. Furthermore, treatment with GnRH agonist in the rat superovulated with PMSG distinctly decreased serum steroid hormone(17β-estradiol, progesterone and testosterone) levels during preovulatory period.

These overall results indicate that *in vivo* GnRH agonist has an adverse effect on the ovary, inhibiting the follicular growth and maturation in early follicular phase following superovulatory dose of PMSG. Vickery[23] reported that continuous administration of GnRH agonist could decrease ovarian estrogens, progesterone and androgens production by interfering with ovarian function. A GnRH-induced decrease in estrogen production offers an explanation for blocking effect of GnRH on various estrogen-dependent ovarian functions inducing folliculogenesis and ovarian weight gain.

Ovarian morphology after treatment with GnRH agonist in early follicular phase of superovulated rats displayed lots of atretic follicles. In this respect, an intriguing finding was that GnRH agonist produced a significant pyknosis in gran-

ulosa cells. Since pyknosis is a typical indicator of physiological atresia, it has been suggested that GnRH may act as an atretogenic factor for follicles. Billig *et al*[27] have demonstrated that GnRH acts as an atretogenic factor, since GnRH agonist increased apoptotic DNA fragmentation of the ovary in a time- and dose-dependent manner. It is noteworthy that Erickson *et al*[24] have shown that GnRH treatment in hypophysectomized estrogen-primed rats caused a rapid and dramatic increase granulosa cell pyknosis in atretic as well as healthy antral follicles. Furthermore, they have suggested that autocrine and paracrine secretion of ovarian GnRH might cause atresia by the mechanism(s) involving increased insulin-like growth factor-binding protein-4(IGFBP-4) synthesis, a physiological marker of atresia; GnRH agonist stimulates the expression of IGFBP-4 protein in rat granulosa cells *in vitro*, and abolishes the ability of FSH to inhibit IGFBP-4 expression and to induce IGFBP-4 protease activity. However, this mechanism involved in GnRH action in the granulosa cells is not yet clear.

The inhibitory action of GnRH was completely reversed by the combination with GnRH antagonist. These results indicate that the inhibitory action of GnRH agonist mediated by specific GnRH receptors in the ovarian granulosa cells[28] could be recovered using GnRH antagonist. These findings strongly suggest that the action of GnRH agonist and GnRH agonist and GnRH antagonist is mediated by homologous receptors[29].

Although the physiological significance of this relationship is not yet known, GnRH and GnRH-like peptides are found in extrapituitary tissues, including the ovary, placenta, and testis[30,31]. Based on the finding that treatment with GnRH antagonist *in vivo* increases follicular development in gonadotropin-treated hypophysectomized rats, the presence of an ovarian GnRH-like substance, gonadocrinin, has also been suggested[32]. Although isolation of GnRH or a GnRH-like peptide in the ovary has not been accomplished, low levels of mRNA for GnRH have been determined by the use of reverse trascription-polymerase chain reaction[33]. Whether an ovarian GnRH or GnRH-like material is involved in ovarian function remains to be considerate. However, the present study indicates that exogenous GnRH administration *iv vivo* inhibits ovarian function in early follicular phase.

In summary, the overall results of this study indicate that GnRH agonist directly disturbs ovarian functions in early follicular phase of superovulation-pretreated immature rats in terms of ovulatory response and morphological normality of ovulated oocytes. This concenpt has been further evidenced by the findings of a great decrease in ovarian weight, a marked increase in follicular atresia of follicle and a distinct decrease in circulating steriod hormone(17β-estradil, progesterone and testosterone) levels in GnRH agonist treatment regimen in early follicular phase.

These data provide a basis for explaining the inhibitory effect of GnRH on reproduction functions, and for future studies on the mechanism involved in GnRH regulation of the ovary.

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