Modulating Effects of Korean Ginseng Saponins on Ovarian Function in Immature Rats

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The modulating effects of Korean ginseng saponins on ovarian functions were investigated in immature rats superovulated with pregnant mare serum gonadotropin (PMSG). A single dose of 1 mg (0.1 ml/head) of Korean ginseng total saponin (GTS), Korean ginseng protopanaxatriol saponin (GPT), Korean ginseng protopanaxadiol saponin (GPD), or ginsenoside-Rb1 (Gin-Rb1) was intravenously injected via jugular vein catheter three times at 1 h (early follicular phase), 25 h (middle follicular phase), and 50 h (late follicular phase) after 30 IU PMSG administration. GPD and Gin-Rb1 significantly suppressed excessive ovulatory response caused by PMSG (p < 0.05). All Korean ginseng saponins significantly improved oocyte quality by decreasing the proportion of abnormal oocytes (p < 0.05). Gin-Rb1 significantly decreased preovulatory serum levels of androgens and 17ß-estradiol, while GPD increased preovulatory serum progesterone level (p < 0.05). GPD significantly increased the increased postovulatory serum progesterone level (p < 0.05). These results provide strong evidence that Korean ginseng saponins have a curative effect on ovarian dysfunction caused by excessive stimulation with PMSG.

Key words ginseng saponin; ovarian function; superovulation; oocyte; androgen; progesterone

Superovulation induction with exogenous gonadotropins such as pregnant mare serum gonadotropin (PMSG) and human menopausal gonadotropin (hMG) is a well-established practical technique. The purpose of this technique is to achieve enhanced commercial production of genetically meritorious individuals in the livestock industry, to retrieve a number of oocytes and early embryos in various research fields including genetic engineering, and to treat medically diverse types of female infertility in human. In spite of these advantages, the results obtained from its application on large domestic and small laboratory animals generally appear very disappointing.1—5 In immature rats, administration of superovulatory doses of PMSG leads to a great reduction in fertilization rates, a substantial loss and degeneration of preimplantation embryos, and a partial or complete failure of implantation.6—9 Major defects following superovulatory treatment with PMSG could occur in the process of follicular development and oocyte maturation by hyperstimulation of PMSG on ovarian tissues because of prolonged biochemical action of PMSG that has long half-life.10,11 Hyperstimulation of PMSG on ovarian tissues also induced atypical ovulation of abnormal follicles that would not ovulate and regress under a regular estrous cycle.11—13 However, the precise mechanisms of these negative aspects exerted by superovulatory treatment are not fully understood.

Ginseng has been regarded as one of the important remedies in oriental medicine for one thousand years. A review of classical oriental medicine literature showed that ginseng improves the functions of almost all the organs in the body, including reproductive organs.14 It has been sound that the most effective components in ginseng are saponins including ginsenosides which are plant glycosides having a triterpenoid dammarane skeleton as an aglycone, with three sites called R1, R2 and R3 for ester bonding with the glycones: glucose, rhamnose, xylose and arabinose.15 The specific structure of ginsenosides, a triterpenoid dammarane skeleton, is quite similar to that of ovarian steroid hormones, namely, cyclopentanoperhydrophenanthrene nucleus, implying that ginsenosides are biochemically active substances and that they act as functional ligands in steroid related reproductive organs.15 Recently, the action mechanisms of various ginsenoside fractions have been elucidated in many laboratories. For example, the fractions ginsenoside-Rg1, -Rh1 and -Rh2 exert, via glucocorticoid receptor, their biological actions which are to have glucocorticoid-response gene expressed, and/or to stimulate specific cell differentiation.15—17 However, there is scarcely any available data on the effects of ginseng components on the male or female reproductive system. It has been reported in clinical medicine that in mildly infertile men Korean ginseng components stimulated spermatogenesis and enhanced spermatzoa motility.18 In addition, in menopausal sterile women, the ingestion of Korean ginseng flour cured menopausal disorders by improving the ovarian function by increasing the blood supply into the ovary and raising serum 17ß-estradiol level.19 In laboratory animals, there is only a single report on the ginseng effects on the male gonad. In adult male rats Korean ginseng saponin stimulated both the transportation of cholesterol to testicular cells and the biosynthesis of androgens from cholesterol in the testis.20 Therefore, the objective of this study was to investigate whether or not Korean ginseng saponins have a curative effect on ovarian dysfunctions caused by the administration of superovulatory doses of PMSG. In detail, this study examined the effects of Korean ginseng saponins on the ovulatory response, the morphological normalcy and nuclear maturation of ovulated oocytes, the ovarian weight and histology, and serum steroid hormone response at pre- and post-ovulatory phase in these rats.

MATERIALS AND METHODS

Materials Saponins (>95% purity) including Korean ginseng total saponin (GTS), Korean ginseng propanaxatriol saponin (GPT), Korean ginseng propanaxadiol saponin (GPD), ginsenoside-Rb1 (Gin-Rb1) were the gener-

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ous gifts of Dr. Chong-Woon Baek at the Korea Ginseng and Tobacco Research Institute (Taejon, South Korea), and stock solutions of saponins were prepared at a final concentration of 10 mg/ml with physiological saline.

**Animals** Immature female Sprague-Dawley rats were purchased from Yu-Han Co. (Anyang, Korea) at 22 d of age. All animals were kept under temperature- and light-controlled conditions (20—25 °C, 12 light : 12 dark) and fed standard rat chow and water *ad libitum* throughout the treatment period. One day prior to an initial treatment (the age of 27 d), the animals were implanted with chronically indwelling catheters as described by Harms and Ojeda.21) Briefly, a catheter made of silastic tubing (Dow-Corning Corp. Midland, MI) was inserted under pentobarbital anesthesia (35 mg/kg body weight) into the jugular vein to approach the outer right atrium and was connected to a flexible piece of heparinized polyethylene tubing (Fisher Co. PE 50).

**Experimental Procedures** At the age of 28 d, the animals were treated with 30 IU PMSG (superovulation dose). PMSG (Equinex, Ayerst) was subcutaneously administered as a single dose in 0.3 ml physiological saline. Fifty-four hours after PMSG injection, all animals were subcutaneously treated with 10 IU hCG (Sigma Chemical Co.) in 0.2 ml of physiological saline. Thereafter they received a single dose of 1 mg (0.1 ml/head) of GTS, GPT, GPD, Gim-Rb, or physiological saline via jugular vein catheter. In the treatment regimen each Korean ginseng saponin was intravenously administered three times via this catheter in 0.1 ml physiological saline at 1 h, 25 h, and 50 h after PMSG treatment, and in the control regimen 0.1 ml physiological saline was injected as a vehicle at the same periods. To examine circulating steroid control regimen 0.1 ml physiological saline was injected as a saline at 1 h, 25 h, and 50 h after PMSG treatment, and in the men each Korean ginseng saponin was intravenously administered physiological saline. Thereafter they received a single dose of 1 mg (0.1 ml/head) of GTS, GPT, GPD, Gim-Rb, or physiological saline via jugular vein catheter. In the treatment regimen each Korean ginseng saponin was intravenously administered three times via this catheter in 0.1 ml physiological saline at 1 h, 25 h, and 50 h after PMSG treatment, and in the control regimen 0.1 ml physiological saline was injected as a vehicle at the same periods. To examine circulating steroid hormone (androgens, 17β-estradiol and progesterone) levels in the pre- and post-ovulatory stage, 0.5 ml whole blood was collected from all animals via indwelling catheter at 54 h and at the time of sacrifice, respectively. To prevent acute shock from blood loss and clot formation in the catheter, each blood sampling was followed by replacement of an equal volume of a dilute heparin–saline solution (25 IU/ml). Serum samples were separated by centrifugation and stored at −20 °C until the subsequent determination of androgens, 17β-estradiol and progesterone using radioimmunoassay (RIA). All animals were sacrificed at 72 h after PMSG injection and examined to assess the ovulatory response and oocyte gross morphology, oocyte nuclear maturation, and ovarian histology.

**Assessment of Ovulatory Response, Oocyte Morphological Abnormality and Ovarian Weight** At the time of sacrifice, the ovaries were dissected free from oviducts, cleaned of bursae, connective tissue and fat, and weighed as dried and paired. The oviducts were separated from the uterine horns and oocytes were collected in a few drops of Dulbecco’s phosphate buffered saline (DPBS) under a dissecting microscope (10×magnification). Each oviduct was flushed with 0.2 ml DPBS by inserting a blunt-ended 30-gauge needle through the infundibulum. Subsequently, in order to facilitate the oocyte counting and examination, the extracoronal cumulus cells surrounding the oocytes were dispersed after being exposed to a few drops of DPBS containing 0.1% hyaluronidase (Sigma Chemical Co., St. Louis, MO, U.S.A.) for 10—15 min. The recovered oocytes were counted under a stereo dissecting microscope (40×magnification), and assessed for the occurrence of fragmentation and other degenerative changes as described previously.8,10,22) Those showing fragmentation, parthenogenesis, irregular shape or amorphous opaque mass of vitelline material and empty zona pellucida were classified as abnormal.

**Assessment of Oocyte Nuclear Maturation** Normal appearing oocytes were placed in a 10×35 mm petri dish containing 1.0% hypotonic sodium citrate (Tedia Company Inc., Fairfield, OH, U.S.A.) and allowed to swell at room temperature for 10 min. They were then the oocytes were transferred onto a grease-free slide with a thin coat of Mayer’s albumen to attain their better adhesion of the oocytes to the slide and to prevent their loss in the next step of fixation. The oocytes were then allowed to dry on a hot plate (45—53 °C) to enhance chromosome spreading. Dried oocytes were fixed with acetic alcohol (one part glacial acetic acid and two parts absolute ethyl alcohol) in a Coplin jar for 45 min and stained with 2% aceto-orcein for 30 min. Finally, the stained oocytes were allowed to serially dehydrate with 50%, 60%, 80% and 100% ethyl alcohol followed by xylene for 5 min in each step, and subjected to a microscopic evaluation of nuclear maturation in accordance with the criteria suggested by Austin23) and Yun et al.10)

**Histological Examination** Ovaries obtained at 72 h after PMSG administration were immediately fixed in Bouin’s solution for about 6 h and washed for 12 h in 70% ethanol to remove excess fixative. The ovarian tissue was subsequently dehydrated in sequential concentrations of ethanol (70, 80, 90, 100%), cleared, and embedded in paraffin wax. Then serial sections of the tissue block were cut 5 μm thick and stained with hematoxylin (Sigma Chemical Co.) and eosin (The Coleman & Bell Co., Norwood, OH, U.S.A.). All sections were examined for evidence of precocious ovulation or follicular atresia, and representative sections were taken for photomicroscopy. Advanced stages of follicular atresia were defined by nuclear pyknosis and a loss of homogeneity of the granulosa cells in conjunction with thecal hypertrophy and varying degrees of oocyte degeneration.

**Determination of Circulating Steroid Hormone Levels** To determine circulating steroid hormone (androgens, 17β-estradiol and progesterone) levels, 0.5 ml aliquots of sera were extracted with 3 ml ethyl ether by vortexing vigorously. These extracts were evaporated for 60 min and reconstituted in 1 ml GPBS (buffer). One hundred μl aliquots of the extracts were assayed in duplicate for each steroid hormone by specific RIA using the antisera. In the assay procedures, approximately 6000 cpm of tracer [3H] was added to each tube. The unbound steroid was removed by 0.2 ml of cold dextran coated charcoal, and the bound steroid was counted in a liquid scintillation analyzer (TRI-CARB 2300 TR, Packard). The binding efficiency (B0) of the steroid antibodies was 40—50% and NSB was less than 5%. The coefficient of variations (CVs) was 9—10% for 17β-estradiol, 8—9% for progesterone and 7—9% for androgens, respectively. Hormone concentrations were expressed as ng/ml sera.

**Statistical Analysis** Group data were analyzed by ANOVA and the significant difference between the mean of each treatment group and that of the control group was determined by Dunnett’s test at *p*<0.05 or *p*<0.01.
RESULTS

Effects of Korean Ginseng Saponins on Ovulatory Response
Ovulatory responses modulated by treatments with GTS, GPT, GPD and Gin-Rb1 three times in early, middle, and late follicular phase of immature rats given a superovulatory dose of PMSG are presented in Fig. 1. The oocyte counts obtained from the groups of GTS (58.4 ± 5.9 oocytes/rat), GPT (60.0 ± 6.1 oocytes/rat), GPD (53.7 ± 3.3 oocytes/rat), and Gin-Rb1 (53.1 ± 4.6 oocytes/rat) showed a decreasing trend, compared to that from the control group (66.3 ± 3.9 oocytes/rat). Especially, the oocyte counts obtained from the rats treated with GPD and Gin-Rb1 were significantly decreased below the control count (p < 0.05).

Effects of Korean Ginseng Saponins on Oocyte Quality
Abnormal oocytes characterized by different types of degeneration, such as fragmentation, parthenogenesis, irregular shape or amorphous opaque mass of vitelline material and empty zona pellucida, were recovered from all the groups. As shown in Fig. 2, morphological abnormality of oocytes recovered from the control group was 15.5 ± 2.1%. In contrast, in all groups treated with Korean ginseng saponins following PMSG administration, the percentages of abnormal appearing oocytes decreased significantly compared to that of the controls (p < 0.05). The percentages of morphological abnormality of oocytes recovered from GTS, GPT, GPD, and Gin-Rb1 groups were 7.6 ± 2.2, 7.5 ± 1.5, 8.0 ± 2.2, and 8.3 ± 1.7%, respectively. The proportion of oocytes analyzable for the classification of each meiotic stage was actually 78—97%, 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 impossible to distinguish between metaphase I and metaphase II, because of rapid deterioration or inconsistent formation of the typical polar body in the rat. The nuclear maturation of oocytes recovered from the PMSG-pretreated control rats was characterized by prematurity and asynchronization; the percentages for 310 superovulated oocytes at prophase I, anaphase I, telophase I, and metaphase I/II stage were 39.7, 11.9, 0.3, and 44.2%, respectively (Fig. 3). In contrast, by treatment with Korean ginseng saponins, the meiotical aberration phenomena of superovulated control oocytes were substantially altered. In the GTS treatment group, the percentage for each nuclear maturation stage for 386 recovered oocytes was 13.0% in prophase I stage, 3.1% in anaphase I stage, 0% in telophase I stage, and 79.8% in metaphase I/II stage. In the GPT treatment group,

Fig. 1. The Effects of Korean Ginseng Saponins (Total Saponin: GTS, PT Saponin: GPT, PD Saponin: GPD, Ginsenoside-Rb1: Gin-Rb1) on Recovery of Oocytes from Oviducts in 30 IU PMSG-Pretreated Rats
Each Korean ginseng saponin (1 mg/0.1 ml) was injected intravenously three times at 1, 25, and 50 h following PMSG administration. Controls were simultaneously treated with physiological saline. Values obtained at 72 h following PMSG represent the mean ± S.E. (n = 10).

Fig. 2. The Effects of Korean Ginseng Saponins (Total Saponin: GTS, PT Saponin: GPT, PD Saponin: GPD, Ginsenoside-Rb1: Gin-Rb1) on Abnormality of Oocytes Recovered from Oviducts in 30 IU PMSG-Pretreated Rats
Each Korean ginseng saponin (1 mg/0.1 ml) was injected intravenously three times at 1, 25, and 50 h following PMSG administration. Controls were simultaneously treated with physiological saline. The results of % abnormal oocytes recovered at 72 h following PMSG are expressed as the mean ± S.E. (n = 10). *p < 0.05, compared to controls.

Fig. 3. The Effects of Korean Ginseng Saponins (Total Saponin: GTS, PT Saponin: GPT, PD Saponin: GPD, Ginsenoside-Rb1: Gin-Rb1) on Nuclear Maturation of Oocytes Recovered from Oviducts in 30 IU PMSG-Injected Rats
Each Korean ginseng saponin (1 mg/0.1 ml) was injected intravenously three times at 1, 25, and 50 h following PMSG administration. Controls were simultaneously treated with physiological saline. Rats were sacrificed at 72 h following PMSG. The number above each bar represents oocyte counts examined; oocytes recovered with visible signs of degeneration were excluded. ~ denotes 0%.
the maturation stage for 473 recovered oocytes was 15.1% in prophase I stage, 11.2% in anaphase I stage, 1.5% in telophase I stage, and 66.6% in metaphase I/II stage. In the GPD treatment group, the maturation stage of 375 recovered oocytes was 14.1% in prophase I stage, 15.5% in anaphase I stage, 2.1% in telophase I stage, and 47.1% in metaphase I/II stage. In the Gin-Rb1 treatment group, the maturation stage of 290 recovered oocytes was 17.2% in prophase I stage, 9.0% in anaphase I stage, 0.7% in telophase I stage, and 66.2% in metaphase I/II stage, respectively. Especially, in all treatment regimens, the percentages of oocytes in prophase I stage, the most premature, were decreased to less than half that of the control group. In addition, oocyte nuclear maturation in all treatment groups was conspicuously synchronized to the advanced maturing stage, metaphase I/II, as compared to that in the control group.

**Effects of Korean Ginseng Saponins on Ovarian Weight**

The weights of the paired ovaries obtained from the control regimen were 22.5 ± 2.1 mg (Fig. 4), while those obtained from the GTS, GPT, GPD, and Gin-Rb1 treatment regimens were 19.4 ± 1.5, 20.2 ± 1.6, 20.0 ± 0.9, and 19.6 ± 1.0 mg, respectively (Fig. 4). The dried ovarian weight obtained from each treatment group was lower than that of the controls, but not significantly different.

**Ovarian Histology**

In ovarian histology, a majority of the follicles in all superovulated rats pretreated with 30 IU PMSG was in the tertiary stage of follicular development (Fig. 5). The histological findings of the ovary from controls were indicated by the events of typically atretic follicles, even though there were a few normal appearing growing follicles. The atretic findings were characterized by the disintegration of granulosa and theca cell layers and by the advent of desquamated cell and/or cell debris. In addition, in the atretic follicles, a number of granulosa cells with pyknotic nuclei were detected, cumulus oophorus was disappearing and aged oocytes with germinal vesicle breakdown (GVBD) were observed. In contrast, ovarian morphology after treatment with Korean ginseng saponins in early, middle, and late follicular phase of superovulated rats showed the normal appearance of growing antral follicles, and decreasing propensities for atresia compared to the control regimen. It was observed that in normal growing antral follicles granulosa and theca cell layers maintained their normal integrities and the oocytes without GVBD appeared normal.

**Effects of Korean Ginseng Saponins on Serum Steroid Hormone Levels**

In the preovulatory phase, the concentrations of steroid hormones (androgens, 17β-estradiol and progesterone) in circulating blood of the superovulated rats treated with GPD or Gin-Rb1 were compared with those in the control regimen treated with physiological saline. The levels of androgens, 17β-estradiol, and progesterone in the serum collected from control rats at 54 h (preovulatory phase) after 30 IU PMSG administration were 2.11 ± 0.44, 0.67 ± 0.17, and 4.66 ± 0.65 ng/ml, respectively (Fig. 6). On the other hand, in the GPD treatment group, the serum steroid level for androgens, 17β-estradiol, and progesterone was 1.55 ± 0.34, 0.38 ± 0.11, and 8.33 ± 1.16 ng/ml, respectively. In the Gin-Rb1 treatment group, the serum steroid level for androgens, 17β-estradiol, and progesterone was 0.76 ± 0.15, 0.2 ± 0.05, and 3.49 ± 0.44 ng/ml, respectively. The serum levels of androgens and 17β-estradiol in GPD and Gin-Rb1 treatment regimens were shown to have a declining inclination, and especially in the Gin-Rb1 treatment group, these steroid hormone levels were significantly reduced compared to those of controls (p<0.05). Serum progesterone

![Fig. 4. The Effects of Korean Ginseng Saponins (Total Saponin: GTS, PT Saponin: GPT, PD Saponin: GPD, Ginsenoside-Rb1: Gin-Rb1) on Ovarian Weight in 30 IU PMSG-Pretreated Immature Rats](image)

Each Korean ginseng saponin (1 mg/0.1 ml) was injected intravenously three times at 1, 25, and 50 h following PMSG administration. Controls were simultaneously treated with physiological saline. The results of dried and paired ovarian tissue weight at 72 h following PMSG are expressed as the mean ± S.E. (n=10).

![Fig. 5. Ovarian Histology of Control and GPD (1 mg/0.1 ml) Treatment Regimen Following 30 IU PMSG Administration](image)

Sections (5 μm) were stained with hematoxylin and eosin. A, View of the control ovary. There are several atretic follicles which are characterized by the following atretic events: the follicular fluid contains desquamated cells and cell debris, and granulosa cells and basal laminae lose their normal integrities. However, some normal oocytes remain at the dictyate stage. Magnification, ×40. B, View of the GPD treatment ovary. There are several healthy growing antral follicles. Their granulosa cell layer and basal laminae display normal integrities. Magnification, ×40.


level in the GPD treatment group was significantly elevated above the control value (p<0.05). In the postovulatory phase, serum steroid hormone (androgens, 17β-estradiol and progesterone) levels in the GPD or Gin-Rb treatment group were compared with those of the control regimen (Fig. 7). The serum androgens, 17β-estradiol and progesterone concentrations in the control regimen were 0.98±0.29, 0.08±0.05, and 3.01±0.29 ng/ml, respectively. On the other hand, in the GPD treatment group, these serum steroid levels were 0.89±0.11, 0.12±0.04, and 8.20±1.84 ng/ml, respectively. In the Gin-Rb treatment group, the serum steroid level for androgens, 17β-estradiol and progesterone was 0.67±0.13, 0.17±0.06, and 7.04±1.43 ng/ml, respectively. In particular, the postovulatory serum progesterone level in the GPD treatment group was significantly elevated compared to the control value (p<0.05).

**DISCUSSION**

The ovulatory responses of immature rats treated with 30 IU PMSG and supplemented with 10 IU hCG in this study are quite similar to the results reported previously. The hormonal treatment protocol certainly induced superovulation of 66 oocytes per rat on average. The increased ovulatory response may be associated with the inherent biological properties of PMSG which possesses not only dual activities of follicular stimulating hormone (FSH) coupled with luteinizing hormone (LH) which play an essential role in folliculogenesis, but a long biological half-life with its high component of sialic acid. However, superovulatory treatment with PMSG markedly increased the proportion of abnormal-appearing oocytes, and caused premature or asynchronous nuclear maturation of superovulated oocytes, as compared with physiologically ovulated oocytes.

In this study, the treatments with Korean ginseng saponins to the immature rat superovulated with PMSG in early, middle and late follicular phase reduced excessive ovulatory response. Especially, GPD and Gin-Rb treatments substantially decreased ovulatory response which was still higher than physiological ovulatory response reported early. In Korean ginseng saponin treatments following PMSG administration greatly decreased the proportion of abnormal oocytes recovered from the superovulated rat. In addition, these treatments decreased the incidence of meiotically aberrant superovulated oocytes caused by a superovulatory dose of PMSG. About 51% of superovulated oocytes recovered from controls was actually arrested at the premature stages of prophase I to telophase I; particularly, the percentage of prophase I, which is the most premature stage, showed a noticeable elevation. The current results of atypical ovulations of superovulated oocytes with a high proportion of gross abnormality and with prematurity and asynchrony of nuclear maturation following a superovulatory dose of PMSG confirm our previous reports. In contrast to the control regimen which typically exhibited the deterioration of oocyte quality, Korean ginseng saponin treatments resulted in not only a conspicuous increase in the proportion of oocyte normalcy, but also the synchronization of oocyte nuclear maturation: a predominant decline in the proportion of superovulated oocytes arrested at prophase I and an increase in the proportion of oocytes that were in metaphase II at ovulation. This observation of the present study could suggest that Korean ginseng saponins repair the detrimental effects of superovulation on oocyte quality following a high dose of PMSG. It is additionally thought that the alleviating activity of the inhibitory effect of Korean ginseng saponins on the superovulatory response induced by PMSG is possibly associated with the suppression of excessive follicular growth and/or atypical ovulation of abnormal follicles, which are frequently
shown by superovulatory treatment. Histological findings of the ovary may, in part, account for this concept; in the ovary obtained from the controls, a majority of follicles, though there were several normally growing antral follicles, underwent atresia, but, in the Korean ginseng treatment regimen, a cohort of healthy antral follicles was observed.

The precise mechanisms involved in the yield of superovulated oocytes with poor quality following the administration of a high dose of PMSG in immature rats have not yet been clearly elucidated. An altered endocrine environment, especially elevated ovarian steroid contents, has been closely associated with the deterioration of recovered oocyte quality commonly occurring in superovulatory treatment with PMSG in various mammalian species. As compared to ovarian steroid hormone levels under a physiological condition during pre- and post-ovulatory phase in the rat, those under a superovulatory regimen display significantly different profiles, which are characterized by greatly elevated levels of ovarian steroid hormones. In the current study, administration of a high dose of PMSG in immature rats disturbed ovarian function during the peri-ovulatory phase, resulting in high androgen and 17β-estriadiol levels, but low progesterone levels. It could be suggested that these hormone profiles are closely associated with the yield of morphologically abnormal and meiotically aberrant superovulated oocytes. For normal growth and nuclear maturation of oocytes, it is essential to maintain appropriate ovarian steroid hormone levels in vivo as well as in vitro. Korean ginseng saponin treatments lower serum androgens and 17β-estradiol, but elevate progesterone levels during the peri-ovulatory phase, suggesting that Korean ginseng saponins modulate steroidogenesis in the ovary treated with a superovulatory dose of PMSG and results in improvement in superovulated oocyte quality. Accordingly, the application of Korean ginseng saponins to the superovulation protocol using an exogenous gonadotropin such as PMSG could modulate ovarian steroidogenesis. It could also improve the oocyte quality possibly by restraining the activities of follicular atresia, excessive follicular growth, and atypical ovulation of abnormal follicles which is induced by hyperstimulation of PMSG on the ovarian follicles. Therefore, the treatments with Korean ginseng saponins to immature rats pretreated with a high dose of PMSG exhibited curative effects on ovarian function disturbed by superovulation treatment.

A clinical report indicates that the intake of Korean ginseng powder to women experiencing menopausal disorders improved ovarian function by increasing blood supply to the ovary and exerting its estrogenic activity. However, this curative activity of the ginseng components on ovarian function has not yet been demonstrated using any laboratory animals. On the other hand, the results of our research employing the immature rat model treated with a superovulatory dose of PMSG show clearly that Korean ginseng saponins have anti-estrogenic and anti-androgenic actions. These contrasting results between the previous clinical report and our data could be attributed to the fact that ginseng saponins have two directional effects in several biological parameters, e.g., increasing action of PD saponin and decreasing action of PT saponin in blood pressure, stimulating effects of ginsenoside-Rb1 and sedative effects of gensenoside-Rb1 in central nervous system. It is probable that ginseng saponins exert their bidirectional actions, which moderately suppress the functional ovarian cells hyperstimulated by a high dose of PMSG in the immature rat, while stimulating the non-functional ovarian cells in menopausal women. Further studies are needed to identify the exact action mechanisms of ginseng saponins in the ovary.

In conclusion, the application of Korean ginseng saponin fractions to superovulated rats in early, middle and late follicular phase ameliorated ovarian dysfunction following superovulatory pretreatments with PMSG. This effect of the saponins resulted in suppressing excessive ovulatory response caused by hyperstimulation of the superovulatory PMSG dose, enhancing oocyte quality, and modulating ovarian steroidogenesis.

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