# PMSG and HCG hormones effect on the development and growth of ovarian follicles

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**Objective** Gonadotropins are generally used in animals and humans to stimulate ovulation and increase the number of available oocytes for techniques such as *in vitro* fertilization. Ovulation-inducing drugs are used to achieve multiple oocytes in one cycle, thereby increasing the chances of pregnancy in patients with infertility. The aim of this study was to evaluate the effect of gonadotropins on stimulation of ovulation and their inductive role on the growth and development of follicles in the ovary.
**Methods** To determine the effect of human chronic gonadotrophin (HCG) and pregnant mare serum gonadotrophin (PMSG) on ovary, this study used 20 rats that were randomly divided to experimental and control groups. Rats were subsequently euthanized 48 h after injection and the ovaries were fixed and stained with H&E. Data were analyzed with Tukey's multiple comparisons with one-way ANOVA test in GraphPad Prism software.
**Result** Our analysis demonstrated that HCG and PMSG hormones will significantly increase the number of stimulated oocyte in the ovary but it does not have any significant role on the ovary weight and volume ($P < 0.05$).
**Conclusion** This study's results confirmed the inductive role of PMSG and HCG hormones on folliculogenesis.
**Keywords** HCG, follicle, ovulation, PMSG

## Introduction

Ovulation-stimulating medications are used to increase the chances of pregnancy in patients with infertility by achieving multiple oocytes in one cycle.[1] In animals and humans, there is an emerging use of gonadotropin to induce ovulation and increase the number of oocytes accessible for research and developmental reproduction techniques such as *in vitro* fertilization (IVF). In individuals with poor ovarian response, it is essential to administer ovulation-stimulating drugs to get an adequate amount of oocytes in one cycle, leading to enhanced fertility. Full competence of oocytes to develop the term is acquired depending on the proper timing of hormonal activation. The decrease in follicle numbers because of failure ensures aberrant hormonal regulation as a result of incomplete feedback mechanisms. This hormonal dysregulation will, in turn, accelerate the loss of follicles at the advanced age.[2] The aim of this study was to increase the number of oocyte in each cycle.[3,4] Ovulation is one of the most fundamental processes for successful reproduction. Induction of ovulation would expand the number of healthy follicles, and extracting oocytes is one of the most important reproductive tools that can be used to produce embryo in laboratory from eggs and sperm.[5,6] Maturation and resultant release of oocytes from the ovary are directly influenced by secreted hormones from the anterior pituitary specifically the effects of LH and FSH on the ovary. Gonadotropins are commonly used to stimulate the ovary and increase ovulation induction in human and animal fertilization programs. In laboratory methods, pregnant mare serum gonadotrophin (PMSG) and human chronic gonadotrophin (HCG) can induce similar effects on ovarian hormones as well as triggering a new era of ovulation and stimulation of reproductive behaviors. In the ovulation stimulation process, ovarian steroid hormones (estrogen and progesterone) are secreted more physiologically and can have varied effects on different cells with hormones receptor in one period.[7,8] Follicle growth and ovulation are under the control of FSH and LH, which are secreted directly from the adenohypophysis and prompted by releasing hormones (GnRH) from the hypothalamus. In the early stages of the natural cycle, the secretion of GnRH results in a greater release of FSH and lower levels of LH from the pituitary, which will incite follicular growth.[9] Disruption in any of the above sections results in infertility. Nowadays, various methods are used to remedy infertility worldwide. The use of gonadotropins is a well-known method for increasing the number of follicles and ovulation in animals and humans.[10] Worthington's experiences showed that the use of PMSG during 6-week intervals will increase the weight of the uterus, ovary, stimulating the conversion of follicles to the corpus luteum and increased the number of follicles.[11] The application of PMSG in immature rats increases ovulation and prevents the atresia of periantral and antral follicles.[12] Wang examined the effects of LH and FSH on the growth of ovarian follicles in the mice that had previous pituitary gland removal. They found that FSH increases the number of antral and periantral follicles, while LH increases the primary and secondary follicles.[13] Another study by Popova et al.[14] on immature rats found that PMSG and FSH had similar effects

on ovarian stimulation.[14] The objective of this method is to stimulate folliculogenesis and increase the number of oocytes in a cycle. Following hormonal stimulation of ovulation, the levels of ovarian hormones, especially estradiol and progesterone, extend beyond physiological limits. Increasing ovarian hormones can have various effects on reproductive organs and fetal development. In line with the importance of ovarian stimulation in infertility treatment, most HMG and r-FSH hormones are used to stimulate ovarian follicles and increase their counts. As well as considering the effect of these hormones on *in vivo* these studies did not occur to figure out the development ovarian follicles due to legal and ethical barriers.[7] In existing study, the influences of these two hormones were evaluated on follicle development of rat's ovary.

## Materials and Methods

### *Animals*

This experimental study was performed on 20 adult female Wistar rats weighing 200–220 g and ranging from 2 to 3 months of age. They were placed in individual cages during the study period, with 12 h of darkness and 12 h of light available. Rats had access to water and food *ad libitum*. The housing temperature range was kept between 25 and 30°C. The mice were kept at the animal facility center for 2 weeks to adapt to the new environment prior to the study.

### *Ovulation Stimulation in Ovaries*

To stimulate the ovulation, rats were randomly divided into two major groups: control, experimental. PMSG and HCG (Sigma) were used to induce ovulation. In experimental rats group 100 IU/kg PMSG and 48 h later 100 IU/kg HCG intraperitoneally were injected then 100 IU PBS were injected to the control group.[14]

### *Histological Study*

After completion of treatment, animals were sacrificed by inhalation of ether. Ovarian tissue samples were collected and excess fat tissues were removed from the ovary. Their size was measured and recorded by a stereomicroscope (Olympus, Tokyo, Japan) and weighed by a digital scale (Sartorius, Germany). Subsequently, the number of primary follicles, secondary follicles, growing follicles, grown mature follicles and primary antral follicles were investigated in intermediate sections. To avoid any errors in counting, Image J software (Digital Image Processing for Medical Applications Image J 1.46 r, Java 1.6.0-20, Cambridge University) was used. All of the above items were also performed for control group samples.

### *Statistical Analysis*

To compare the percentage of produced follicle in stimulated ovary vs. control group data were analyzed by Tukey's multiple comparisons in one-way ANOVA test. Also, each type of follicle compared with control group with student *t*-test. All statistical tests were executed on GraphPad Prism and $P < 0.05$ were considered as significant.

## Results

Histologic study of ovarian follicle in both group demonstrate that the number of follicles in the stimulated group with PMSG and HCG hormones are significantly higher than the control group ($P < 0.05$) (Table 1). There was no significant difference in weight and volume of both treated and control group's ovaries ($P > 0.05$) (Figs. 1–3).

## Discussion

Gonadotropins application is an approach for increasing the number of follicles and ovulation in animals and humans. In the present study, the effect of HCG and PMSG on the growth and development of ovarian follicles in adult female rats was assessed. The aim of this study was to evaluate the effects of gonadotropins on ovulation induction. There was a significant difference in the number of follicles in the experimental and control groups secondary to ovarian stimulation ($P < 0.05$) as shown in Table 1.

Table 1. **Histological comparison of developed follicle in experimental group**

| Tukey's multiple comparisons test | Mean diff. | 95.00% CI of diff. | Significant? | Summary | *P*-Value |
|---|---|---|---|---|---|
| Primary follicle vs. secondary follicle | −3.7 | −5.561 to −1.839 | Yes | **** | <0.0001 |
| Primary follicle vs. vesicular follicle | −5.6 | −7.461 to −3.739 | Yes | **** | <0.0001 |
| Primary follicle vs. mature follicle | −18.8 | −20.66 to −16.94 | Yes | **** | <0.0001 |
| Primary follicle vs. atretic follicle | −21.4 | −23.26 to −19.54 | Yes | **** | <0.0001 |
| Secondary follicle vs. vesicular follicle | −1.9 | −3.761 to −0.03915 | Yes | * | 0.0433 |
| Secondary follicle vs. mature follicle | −15.1 | −16.96 to −13.24 | Yes | **** | <0.0001 |
| Secondary follicle vs. atretic follicle | −17.7 | −19.56 to −15.84 | Yes | **** | <0.0001 |
| Vesicular follicle vs. mature follicle | −13.2 | −15.06 to −11.34 | Yes | **** | <0.0001 |
| Vesicular follicle vs. atretic follicle | −15.8 | −17.66 to −13.94 | Yes | **** | <0.0001 |
| Mature follicle vs. atretic follicle | −2.6 | −4.461 to −0.7391 | Yes | ** | 0.0023 |

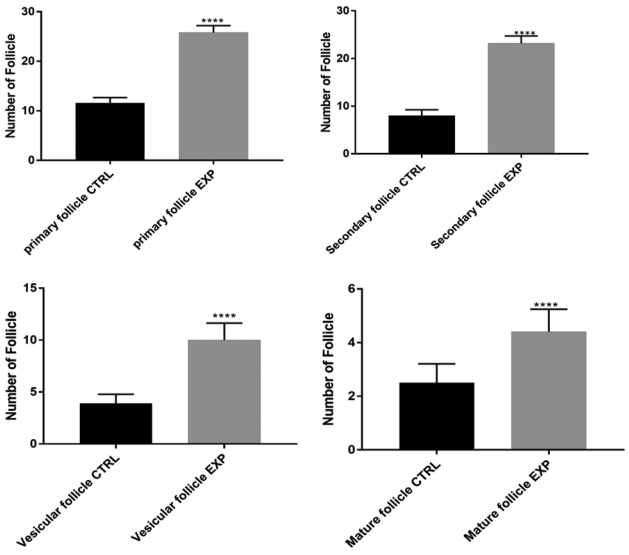


Fig 1. **The average number of follicle in HCG and PMSG stimulated group compared to control group.**

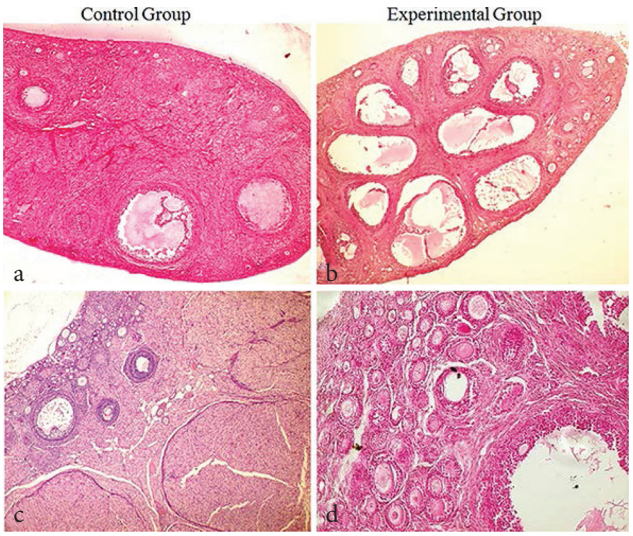


Fig 2. **Histological section of ovary in control and experimental group (HCG, PMSG). Follicle development in the ovary of control group (a) (4×), (b) (10×). Follicle development photomicrograph in the treated group with HCG and PMSG. (c) (4×), (d) (10×).**

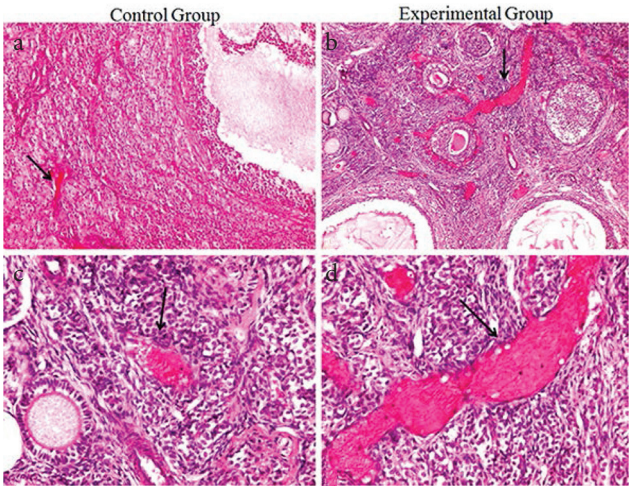


Fig 3. **Histological section of blood vessel distribution in rat ovary in control and experimental group (HCG and PMSG). a and b (10×, 40×) showing natural development of blood vessel in ovaries stroma but with hormonal stimulation of ovary the number of follicle in different stages will increase and these progressive growth demands higher number of blood vessel to provide nutrient in the stroma and close to newly formed follicle. Vasculogenesis is a positive sign for follicle regeneration (c and d) 10×, 40×. Blood vessel is demonstrated by arrow head in each section.**

Treatment with gonadotropin is the common way for improving the number of follicle and ovulation stimulation.[10,16,17] The primary goal of this method is to stimulate folliculogenesis and increase the number of oocytes in a cycle. Following hormonal stimulation of ovulation, the levels of ovarian secretion hormones, especially estradiol and progesterone, go beyond physiological levels. Increasing ovarian hormones can have different effects on reproductive organs and fetal development. Angiogenesis is associated with follicular development and is regulated independently within each follicle potentially making the function of its vasculature critically important in determining its fate. Ovarian follicles require an adequate blood supply of oxygen, nutrients and hormones, in addition to eliminate $CO_2$ and other metabolites. Acquisition of an adequate vascular supply is probably a limiting step in the selection and maturation of the dominant follicle. The degeneration of the capillary network is a relevant phenomenon that causes follicular atresia through the interruption of the metabolic supply for follicular cells. In addition, an increase in vascular density around antral follicles contributes to the inhibition of atresia.[15] The somatic cells of the follicles (granulosa and cumulus cells) recognize ovulation stimulation, leading to the resumption of miosis and the release of oocytes, as well as the change in follicular structure. Ovulation is controlled by several factors including endocrine hormones, metabolic signals, vasculogenesis, angiogenesis and intrafollicular paracrine factors that are secreted by theca, cumulus granulosa cells, and the oocyte itself. Increasing evidence suggests that physiological angiogenesis in ovarian follicles and corpus luteum are fundamental features of mammalian reproduction. Failures in vascular development in these structures may be the reason for several ovarian dysfunctions observed during the estrous cycle and pregnancy. Therefore, it is necessary to evaluate both *in vivo* and *in vitro* influences of the angiogenic factors, alone or in association, on the survival (anti-apoptotic effects) of ovarian cells in different species.[16]

Maturation and oocyte release from the ovary are directly influenced by the secreted hormone from the anterior lobe of pituitary gland. This results from LH and FSH secretion and follicle development in the ovary. One of the signs for ovarian function is dependent on the establishment and continual remodeling of a complex vascular system. The controlled, physiological angiogenesis that accompanies folliculogenesis, ovulation and luteal development requires the coordinated activity of multiple cells and hormone interaction and different angiogenic factors.[16,17] Our studies finding confirms that follicle development is accompanied by blood vessel growth to provide nutrient for growing follicle and move them toward maturation.[17] Gonadotropins are commonly used to stimulate the ovary as well as increase and induce ovulation in animals and humans in order. This brings about the increase in number of oocytes and the chance of getting pregnant. Injection of these hormones in adult mice can trigger a new round of ovulation and stimulate animal reproductive behaviors.[18,19]

In the present study, the effect of HCG and PMSG hormones on the weight, growth and development of ovarian follicles in female rats were investigated. The results of this study showed an increase in the size of the ovary in the HCG group and a small increase in ovarian weight in the PMSG group, which was not significant. Granulosa cells have receptor for GnRH, these hormones can have an immediate effect on the hypothalamic-pituitary gonadal axis, as well as the binding to granulosa cells receptors, which our data are reasonable with this explanation. However, the results of Wang reported ovarian weight gain, also they mentioned that FSH plays an important role in regulating estradiol secretion and antral follicles growth.[20] FSH in combination with LH stimulates the growth of follicles before ovulation. Indeed, the direct response to increased LH during ovulation is related to granulosa cells are due to its more receptors than cumulus cells.

## Conclusion

The results of this study showed that stimulation of ovulation with PMSG and HCG can increase the number of follicles and decrease the size of graph and corpus luteum follicles. As the follicle continues to develop, endothelial cells are recruited to the thecal layer from the blood vessels in the adjacent ovarian stroma. Hormonal stimulation in ovary will increase the number of follicles and follicular growth also associated with the development of an individual capillary network and continued angiogenesis to nourish the rapidly expanding follicle. ■

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