大韓獸醫學會誌(1994) 第34卷 第4號
Korean J Vet Res (1994) 34(4):735-744

Serum luteinizing hormone response and oocyte nuclear maturation in rats superovulated with pregnant mare serum gonadotropin

Young-won Yun

College of Veterinary Medicine, Chungbuk National University

(Received Aug 30, 1994)

임마혈청성 고나도트로핀으로 다배란 처치된 흰쥐에 있어서의 혈청 황체형성 호르몬의 반응 및 난자의 핵성숙

윤 영 원

충북대학교 수의과대학

(1994년 8월 30일 접수)

초록 : 미성숙 래트의 외경정맥에 카테타를 장착하고, 다음날(28일령) 대조군에는 4IU, 다배란처치군에는 20IU의 PMSG를 피하 주사하였다. 각 실험동물은 혈중의 LH농도 변화를 측정하기 위하여 PMSG 투여직전(0시간), 투여후 12시간, 그 이후 6시간 간격으로 혈액을 채취하고 72시간에 희생시켰다.

그 결과 다배란용량의 PMSG 투여는 먼저 배란반응을 대조군에 비하여 4.0배나 현저하게($P<0.05$) 증가시켰다. 또한 난관으로부터 회수된 다배란난자는 상당히 다른 감수분열상의 핵성숙도를 나타내었는데, 즉 prophase Ⅰ이 14.7%, anaphase Ⅰ이 36.2%, telophase Ⅰ이 10.3%, metaphase Ⅰ/Ⅱ가 32.4% 이었다. 그러나 대조군의 래트에서는 대다수(94.0%)의 난자가 한결같이 metaphase Ⅱ상을 보였다. 그리고 혈청 LH농도는 radioimmunoassay(RIA)에 의하여 결정되었는데, 먼저 두군 모두 두개의 분명한 peak을 가진 경시적 변화관계를 보였다. 즉 이들 두군에 있어서 LH농도변화는 0-18시간대에 처음으로 완만한 증가와 54-60시간대에 두번째의 급격한 증가(surge)를 보였다. 그러나 두군간에 LH농도의 크기는 현저하게 달라, 다배란처치군의 동물에 있어서는 두번째의 LH peak에 앞서 전반적인 LH농도가 대조군보다 현저하게($P<0.001$) 높았으나, PMSG 투여후 60시간에 일어나는 peak에 있어서는 LH농도가 대조군보다 현저하게($P<0.001$) 54%나 낮았다. 덧붙여 두 peak간의 증가폭은 대조군에 비하여 다배란처치군에서 훨씬 낮았다. 다배란래트에 있어서 54시간 이전에 최초로 연속적인 증가를 보인 고농도의 혈청 LH는 실제적으로 투여된 PMSG와 측정시의 LH항체와의 교차반응(cross-reaction)에 의한 결과로 판명되었고, 한편 54시간과 60시간에 있어서 두번째로 급격한 증가를 보인 혈청 LH는 주로 뇌하수체로부터 분비되는 내재성 LH surge에 의한 것으로 사료된다.

본 연구 결과는 PMSG투여된 래트에 있어서 혈청 LH의 경시적인 변화상 및 그 특징을 정의한다. 그리고 전체적인 연구결과는 첫째, 다배란용량의 PMSG투여에 따른 배란반응의 증가가 주로 PMSG 자체에 함유된 고나도트로핀 작용과 연관이 있고, 둘째, 미성숙 또는 부동기적 핵성숙을 보이는 다배란난자의 회수는 최초의 혈청 LH의 연속적인 증가 및 이의 연이은 감퇴로 특징지워지는 LH활동도의 비정상적 혈중변화에 기인함을 시사한다.

Key words : superovulation, PMSG, LH, oocyte, nuclear maturation, rat

This work was supported by Grant-in Aid from Korea Science and Engineering Foundation(KOSEF 921-1600-032-1).

Address correspondence and reprint requests to: Dr. Young Won Yun, College of Veterinary Medicine, Chungbuk National University, Cheongju Chungbuk-do, Korea, 360-763, Telephone: (0431) 61-2597, Fax: (0431) 67-3150

Introduction

A single injection of pregnant mare serum gonadotropin(PMSG) is known to initiate induction and synchronization of the ovulatory response in immature rats. Low doses of PMSG elicit a preovulatory gonadotropin surge to generate a "physiological" number of ovulatory follicles wherein a pattern of circulating steroid hormones is similar to that seen in adult rats with spontaneous cycles[1,2]. Time-course studies of the ovulatory response employing the low doses of PMSG in immature rats demonstrated an integration of sequential changes in the endocrine response associated with a synchronized process of oocyte maturation: critical time of the luteinizing hormone(LH) surge at 52-57hr[3-5], meiotic resumption of follicular oocytes 2-3 hr thereafter[6] and ovulation at 60-72 hr after PMSG[7,8]. However, administration of superovulatory doses of PMSG to immature rats produces precocious or multiple waves of ovulations presumably by intrinsic LH activity of high doses of PMSG[8-10]. This atypical ovulation accompanies oviductal recovery of meiotically aberrant oocytes which has been associated with disruption of normal follicular steroidogenesis[11].

In view of above observations, the present study was designed to examine the time-course features of serum LH and nuclear maturity of ovulating oocytes in PMSG-treated rats. To determine more specifically the sequential changes of circulating LH levels after PMSG, a model of chronically catheterized immature rats has been introduced.

Materials and Methods

Immature female Sprague-Dawley rats at 22 days of age were initially housed at a constant temperature of 21℃ with lights on between 0700 and 1900hr and were provided free access of stndard rat chow and water. One day before the experiment, the animals were installed with chronically indwelling catheters as described by Harms and Ojeda[12]. Briefly, a catheter made of silastic tubing(Dow-Corning Corp, Midland, MI) was inserted into the external jugular vein to approach or enter the right atrium under pentobarbital anaesthesia(35mg/kg body wt) and connected with a flexible piece of heparinized polyethylene tubing (Fisher Co, PE50) for withdrawal of blood samples.

On the following day(day 28 of age), to provide the basal level of serum LH, 0.5ml of whole blood was collected from individual rats via the catheter immediately before administration of PMSG(Eqinex, Ayerst). The rats then received a single subcutaneous dose of PMSG for control(4IU/0.4ml saline) or superovulation(20IU/0.4ml saline) treatment between 0830 and 0900hr. All rats in both groups were bled 0.5ml whole blood at 12hr and subsequently at 6 hr intervals until sacrifice at 72hr after PMSG. To alleviate anemia from blood loss and to prevent clot formation in the catheter, each blood sample was followed by replacement of an equal volume of a dilute heparin-saline solution(25IU/ml) as described elsewhere[13]. Serum samples were separated by centrifugation and stored at -20℃ until LH radioimmunoassay (RIA).

Animals were sacrificed by cervical dislocation at 72hr. Ovulation was assayed by counting oocytes flushed out from oviducts as described previously[8]. The recovered oocytes were classified as degenerate or normal appearance as described previously[14]. Only normal-appearing oocytes after exposure to 0.1% hyaluronidase for 5-10 min were placed into hypotonic(1%) sodium citrate at room temperature for 10 min and transferred onto a grease-free slide with a thin coat of Mayer's albumen. Air-dried oocytes were fixed with acetic alcohol(one part of glacial acetic acid, two parts of absolute ethyl alcohol) for 45 min and stained with 2% aceto-orcein for 30 min. After a serial dehydration through 50%, 60%, 80% and 100% ethyl alcohol followed by xylene, the stained oocytes were subjected to a microscopic evaluation of nuclear maturation. Various stages of meiosis were identified according to the criteria of Austin[15](see Fig 1).

Serum LH concentrations were measured by RIA using the procedure outlined by NIADDK, and expressed in terms of ng NIH-rat-LH-RP2/ml. All samples were measured in a single assay, in which a pool of serum from intact cycling adult rats on the day of diestrus had a level of 0.98ng/ml with the intra-assay

coefficient of variation of 6.4%. The minimum detectable value of LH was 40pg per tube. Serum samples were assayed in duplicate in 100μl aliquots. PMSG was found to cross-react with the LH antiserum, although the binding was not parallel to that of NIH-LH-RP2. The addition of 0.125IU PMSG to 100μl serum from intact rats raised the apparent level of "LH" in this assay by about 300pg NIH-LH-RP2 equivalent. This is approximately equal to the increase in serum immunoreactive LH that was measured in the rats of the superovulation group following injection of 20IU PMSG(Fig 1, 12hr vs Ohr).

Experimental data were evaluated statistically by Student's t-test, or when appropriate, by analysis of variance followed by Fisher's PLSD test. Comparisons with $P<0.05$ were considered to be significant.

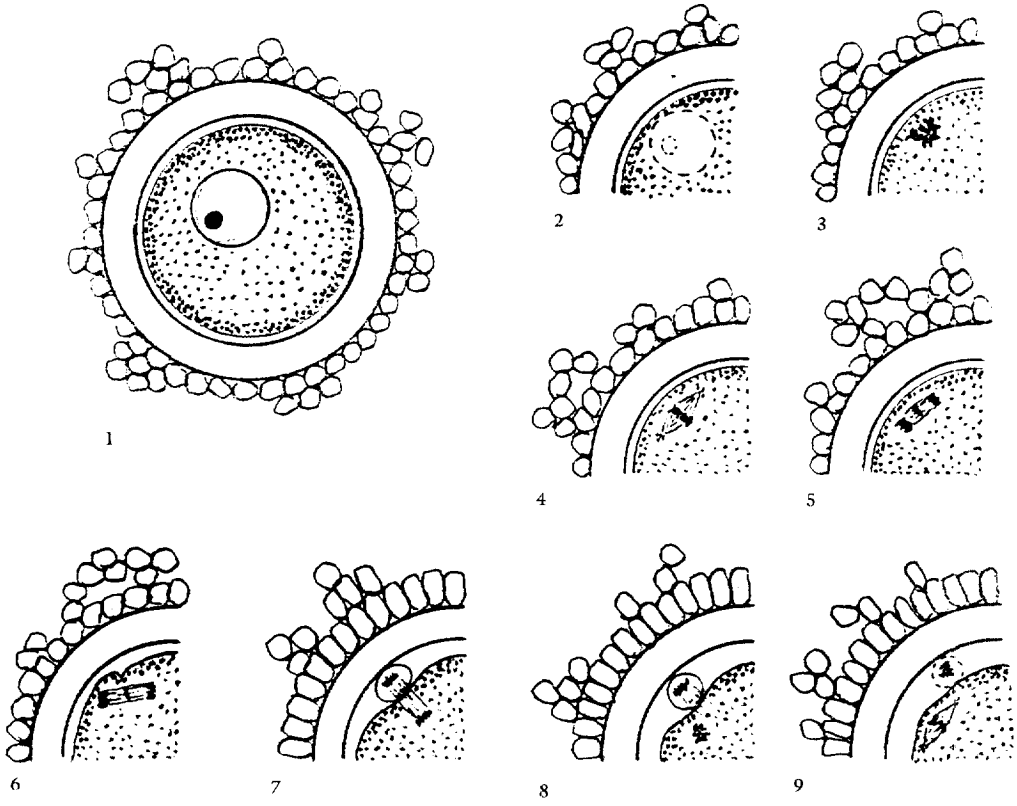


Fig 1. Diagrammatic representation of preovulatory nuclear maturation stages in the rat oocyte(re-drawn from Austin, 1961).

1: germinal vesicle stage.

2: germinal vesicle break-down(GVBD) stage, migration of germinal vesicle to the periphery of ooplasm, and disappearance of nucleolus and nuclear membrane.

3: prophase I , speckled appearance with short segments of chromosomal threads.

4: metaphase I , arrangement of the bivalents at the equator of meiotic spindle.

5: anaphase I , movement of the bivalents to the opposite ends of the spindle.

6&7: telophase I , rotation of the spindle through 90°, and start of polar body separation with elevation of vitellus near to the chromosomes.

8&9: metaphase II , emission of the first polar body with formation of furrow around the outermost set of chromosomes.

Results

The time course and patterns of LH release in blood of control(4IU PMSG) and superovulated (20IU PMSG) rats are illustrated in Fig 2. Both groups showed a similar time relationship of biphasic LH response to PMSG. However, a great difference in the magnitude of serum LH levels between the two groups was noted.

Mean basal levels of serum LH prior to administration of PMSG were 0.37 and 0.50ng/ml. Serum LH levels began to rise significantly($P<0.01$) between Ohr and 12hr, and reached the first peaks of 1.81 ± 0.21ng/ml at 12hr and 3.78 ± 0.18ng/ml at 18hr in control and superovulated rats, respectively. Thereafter, the LH levels in both groups gradually tapered off by 54hr. The first peaks were followed by enormous and significant($P<0.001$) elevations of serum LH with the second peaks synchronized at 60hr in both groups. The peak values of second elevations were 14.98 ± 0.92ng/ml and 6.85 ± 1.29ng/ml in control and superovulated rats, respectively. Subsequently, the LH levels in both groups rapidly fell by 66hr with no further changes apparent at 72hr.

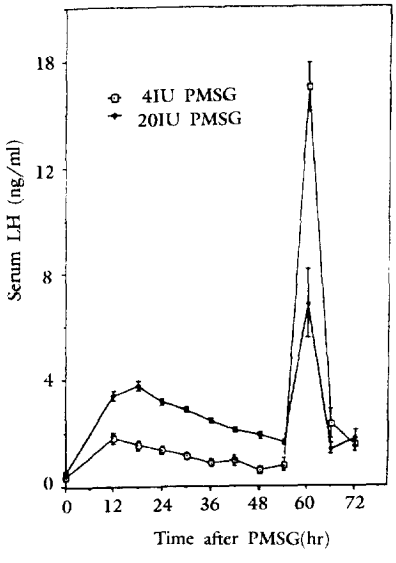


Fig 2. Changes in serum luteinizing hormone(LH) concentrations after administration of 4IU or 20IU PMSG to immature rats. Sequential blood samples were taken from the same individual using chronic catheterization into external jugular vein. Values at each point represent the means±SE(n=7)

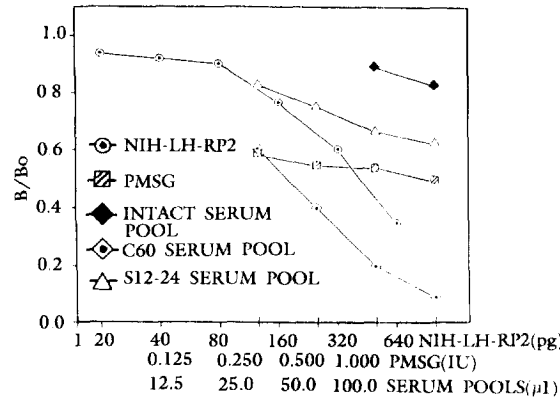


Fig 3. LH assay of PMSG and serum pools, showing the cross-reactivity of the injected PMSG with LH antibody.
NIH-LH-RP2: LH standard preparation.
PMSG: non-injected and diluted preparation.
INTACT POOL: serum pools collected from the diestrous adult rats without injection of PMSG.
POOL C60: serum pools collected from the immature rats at 60hr after injection of a control dose(4IU) of PMSG.
POOL S12-24: serum pools collected from the immature rats at 12hr, 18hr and 24hr after injection of a superovulatory dose(20IU) of PMSG.

As compared to controls, between 12hr and 54hr, cumulative levels of serum LH in superovulated rats were significantly($P<0.001$) elevated. This elevation was consistent at each time point. However, at 60hr, the mean peak value of the second elevation in superovulated rats was markedly reduced by 54% below that of control rats. Similarly, a maximum increase of

mean △LH between the two peaks was much lesser in superovulated than that in control rats. The mean △LH after subtraction of the first peak from the second peak was 3.07ng/ml in superovulated rats and 13.17ng/ml in control rats.

The first increases of serum LH were found to result from the cross-reaction of the injected PMSG with LH antibody in the assay procedure. The initial increases were dose-dependent with a prolonged half-life of 30-36hr which was much longer than that for genuine LH(0.5hr, reported by Bogdanove and Gay[16]). In addition, the result of dilution binding of NIH-LH-RP2, PMSG and serum pools in the LH assay procedure (Fig 3) showed that PMSG actually cross-reacted with LH antibody, and that the serial dilution curve of serum pools obtained from superovulated rats during the initial increase was not parallel to that of the NIH-LH-RP2 standard but was much closer to that of PMSG. On the other hand, the serial dilution curve of serum pools obtained from control rats at 60hr was parallel to the NIH-LH-RP2 standard curve.

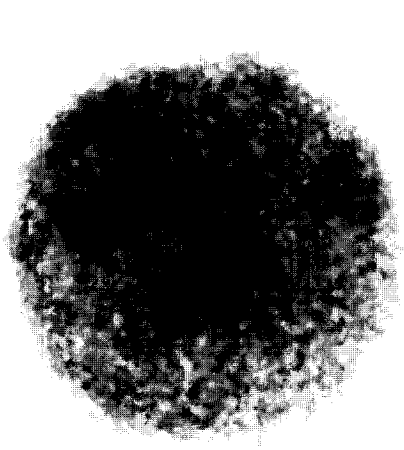
After final collection of blood samples at 72hr, the ovulatory response of the rats treated with two different doses of PMSG were examined(Table 1). In control rats, the total mean number of oocytes recovered was 7.6±0.5 oocytes per rat. On the other hand, a superovulatory dose of PMSG was shown to significantly increase the ovulatory response(36.0±8.5 oocytes per rat, $P<0.05$) above that obtained by a control dose of PMSG.

The various stages of nuclear maturation of oocytes recovered from oviducts after PMSG were recorded (Table 1). Morphologically degenerate oocytes in superovulated rats(3-5 oocytes per rat) were excluded from the meiotic evaluation. The proportion of normal-appearing oocytes actually analyzable for the classification of each stage was 96.6% in control rats and 93.5% in superovulated rats, since some oocytes lost or scattered their chromosomes by occasional rupture of cell membrane during the process of preparing the oocytes. Superovulated oocytes displayed considerably different stages of meiotic maturation: prophase I (14.7%), anaphase I (36.2%), telophase I (10.3%), metaphase I / II (32.4%), while in control rats a majority of the oocytes examined(94.0%) consistently showed a metaphase II configuration(Fig 4-7). In control rats, the observation of oocytes revealed circular indentation of constriction of oocyte membrane and complete aggregation of chromosomes into polar body, typical of metaphase II (Fig 7). In superovulated rats, it was actually not possible to distinguish between metaphase I and metaphase II because of inconsistent formation of typical polar body. Prior to or immediately after the completion of membrane abstriction, polar bodies in some portion of superovulated oocytes became rapidly degenerated.

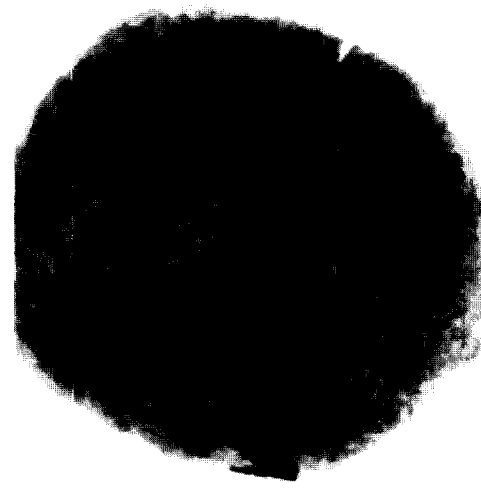
Table 1. Ovulatory response and nuclear maturation of oocytes recovered from oviducts in PMSG-treated rats

Treatment	No Animals[a]	No oocytes recovered (mean±SE)	Stages of meiosis(%)				
			Pro- I	Ana- I	Telo- I	Meta- I / II	Undetermined
4IU PMSG	20	9.4±0.3	-	-	2.6	94.0[c]	3.4
20IU PMSG	24	37.2±7.6[b]	14.7	36.2	10.3	32.4	6.5

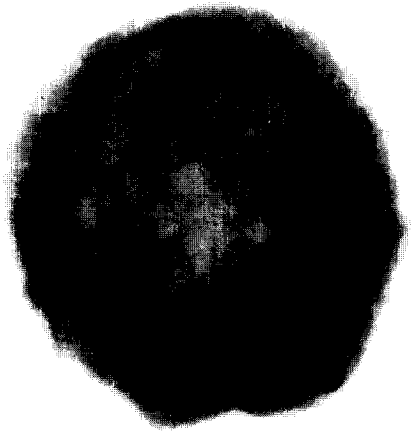
[a]All individuals in each group exhibited ovulations.
[b]$p<0.01$, statistically significant compared to 4IU PMSG-treated group.
[c]Oocytes showed consistently typical metaphase II configuration.



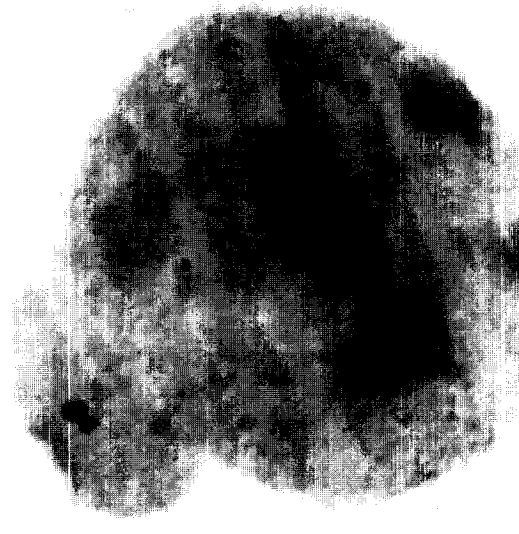
4



5



6



7

Fig 4-7. Typical configuration of nuclear maturation of oocytes recovered from oviducts after PMSG. 4: metaphase I (20IU PMSG). X400. 5: anaphase I (20IU PMSG). X400. 6: telophase I (20IU PMSG). X400. 7:metaphase II (41U PMSG). X400.

Discussion

In the present study, both a control dose(4IU) and a superovulatory dose(20IU) of PMSG elicited two distinct LH peaks in rats: a first slight rise at 0-18hr and a second sharp rise at 54-60hr after treatment. This result, in general, agrees with the patterns produced by a different dose(10IU) of PMSG[6]. Together, these findings indicate a similar and dose-independent time relationship of PMSG to induce LH surge. However, by analyzing the magnitude of the LH response, a superovulatory dose of PMSG was associated with a significant attenuation of the endogenous LH surge accompanied by prolonged elevations of serum LH, as compared to that corresponding to control regimen.

The present observation of two LH increases coupled with previous findings of two distinct ovulations after superovulatory treatment in rats[8,9] suggests that the LH response to PMSG has two different components: a slight and prolonged elevation with the first peak independent of pituitary secretion and a precipitous second elevation of the pituitary-dependent surge. PMSG is known to possess an exceptionally high sialic acid content and a consequent slow clearance rate as well as predominant LH-like activity when measured by bioassay[17]. The 36 hr half-life of circulating PMSG observed in the present study is consistent with the prolonged disappearance rate reported by others[18-20]and considerably longer than the half-lives of native gonadotropins which were reported to be 2.5hr for FSH and 0.5hr for LH in rats[16]. A study of neutralization with anti-PMSG demonstrated the active bilological half-life of PMSG of 54hr to 60hr in mice[19] and its circulating inactivation time of 36hr in rats[21]. On the basis of these chemical properties of PMSG and a cross-reaction between PMSG and rat LH antibody in the current assay system, it is concluded that the elevated serum LH around the time of the first peak is actually an intrinsic component of PMSG. Additionally, the timing of the surge between 54 and 60hr in the present study is well in agreement with the critical period of endogenous LH secretion established in numerous time-inhibition studies using various neuropharmacologic central depressants[3,6] and hypophysectomy[22,23].

It has been previously reported that in immature rats, a low dose of PMSG(3IU) initially stimulates a rapid follicular development during the first 36hr prior to its inactivation in blood, and then by the next 25hr, endogenous gonadotropin secretion is responsible for the maintenance of follicles to ensure final ovulation[21]. In the present study, a superovulatory dose of PMSG resulted in the initial prolonged elevations of serum LH around the first peak and subsequent suppression of the second, endogenous, LH surge as compared to control levels of serum LH. Therefore, in superovulated rats, the current observation of increased ovulatory response as well as the previous findings of precocious ovulation as early as 24hr[8,9], reflect the effectiveness of PMSG-derived intrinsic gonadotropin for ovarian hyperstimulation. However, this interpretation does not rule out the involvement of endogenous gonadotropin secretion in the process of follicular maturation and ovulations even in superovulated rats, since the second elevation of serum LH remains higher than the preceding peak.

There recently have been several lines of evidence that superovulatory regimens using exogenous gonadotropins inhibit the onset of endogenous LH surge and attenuate its magnitude in humans[24,25] and monkeys[26]. The results of the present study are consistent with these findings, and provide further evidence on a nonprimate model. In rats, a superovulatory dose of PMSG significantly suppressed the endogenous LH surge without affecting the timing of its onset. This suppression has previously been attributed to the action(s) of inhibin-like protein and/or nonsteroidal ovarian factor(s) produced from hyperstimulated follicles which mediates directly a negative feedback of LH secretion[24] or interferes with estrogen-mediated positive feedback[26]. The substance responsible for this effect was further shown to be a factor of ovarian origin with a short circulating half-life, since bilateral ovariectomy restored normal pituitary responsiveness within 30min[27]. Therefore, it seems likely that a significant suppression of endogenous LH surge in superovulated rats is a reflection of hyperstimulation of multiple follicles by a superovulatory dose of PMSG.

Superovulated oocytes displayed substantially dif-

ferent stages varying from prophase I to metapase II, while the nuclear maturation of a majority of control oocytes recovered from the oviducts of 4IU PMSG-treated rats. The asynchrony phenomenon of nuclear maturation of superovulated oocytes has been previously ascribed to premature meiotic activation of oocytes from certain follicles[28] which may result from the alteration or imbalance of follicular steroid contents[11]. On the other hand, the microenvironment of ovarian steroids and its related changes in gonadotropins are, in general, essential for the normal follicular development and maturation. Especially, LH among the gonadotropins plays a key role of ovulation process and oocyte maturation. Thus, the recovery of immature or asynchronously mature oocytes at ovulation in the rats treated with a superovulatory dose of PMSG is presumed to be associated with circulatory alteration of LH activity: initial prolonged elevation of serum LH prior to 54hr and subsequent attenuation of endogenous LH surge in superovulated rats.

Summary

Catheters were placed into the external jugular veins of immature female rats. On the following day (day 28 of age), the animals were injected subcutaneously with pregnant mare serm gonadotropin(PMSG): 4IU(control) or 20IU(superovulation). Each animal was sequentially bled at Ohr and 12hr and subsequently at 6hr intervals until sacrifice at 72hr after PMSG.

The superovulatory dose of PMSG significantly($P<0.05$) increased the ovulatory response by 4.0 fold above controls. On the other hand, superovulated oocytes displayed considerably different stages of meiotic maturation: prophase I (14.7%), anaphase I (36.2%), telophase I (10.3%), metaphase I / II (32.4%), while in control rats a majority of the oocytes examined(94.0%) consistently showed a metaphase II configuration. Serum luteinizing hormone(LH) levels were determined by RIA. Both groups exhibited a similar time relationship with two distinct peaks: an initial slight rise at 0-18hr and a second sharp rise at 54-60hr. However, there was a marked change in the magnitude of LH levels between the two groups. In superovulated animals, prior to the second peak, overall LH levels were significantly($P<0.001$) higher than controls. In contrast, at the peak occurring at 60hr, LH concentrations were significantly($P<0.001$) reduced by 54% below that of control. Additionally, a maximum increase of mean $\triangle$LH between two peaks was much less in superovulated as compared to control rats. The initial prolonged elevation of serum LH before 54hr in superovulated rats was found to result from actual cross-reaction of the injected PMSG with LH antibody in the assay, while a precipitous second elevation between 54hr and 60hr resulted primarily from an endogenous LH surge.

This study clearly defines time-course features of serum LH in PMSG-treated rats. The overall results indicate that, following superovulatory treatment with PMSG, the increased ovulatory response is primarily associated with PMSG-derived intrinsic gonadotropin, and that the recovery of immature or asynchronously mature oocytes at ovulation may reult from the circulatory alteration of LH activity characterized by an initial prolonged elevation of serum LH and its subsequent attenuation.

References

1. Wilson CA, Horth CE, Endersby CA, et al. Changes in plasma concentrations of estradiol and luteinizing hormone in immature rats treated with pregant mare serum gonadotrophin. *J Endocrinol* 1974; 60: 293-304.
2. Parker CR, Jr, Costoff A, Muldoon TG, et al. Actions of pregnant mare serum gonadotrophin in the immature female rat: correlative changes in blood steroids, gonadotrophins and cytoplasmic estradiol receptors of the anterior pituitary and hypothalamus. *Endocrinology* 1976; 98: 129-136.
3. Sorrentino S, Jr, Reiter RJ, Lee LA, et al. Timing and neural pathways involved in release of luteinizing hormone after pregnant mare serum administration. *Neuroendocrinology* 1972; 9: 341:348.
4. Costoff A, Eldridge JC, Mahesh VB. Pituitary ultrastructure and serum gonadotropin levels in the PMS-

primed immature rat. *Cell Tiss Res* 1974; 151(1): 79-92.

5. Moon YS, Yun YW, King WA. Detrimental effects of superovulation. *Seminars Reprod Endocrinol* 1990; 8(3): 232-241.
6. Hillensjo T, Barnea A, Nilsson L, et al. Temporal relationship between serum LH levels and oocyte maturation is prepubertal rats injected with pregnant mare's serum gonadotropin. *Endocrinology* 1974; 95: 1762-1766.
7. Walton EA, Evans G, Armstrong DT. Ovulatory response and fertilization failure in immature rats induced to superovulate. *J Reprod Fert* 1983; 67: 91-96.
8. Yun YW, Ho Yuen B, Moon YS. Effects of superovulatory doses of pregnatnt mare serum gonadotropin on oocyte quality and ovulatory and steroid hormone responses in rats. *Gamete Res* 1987; 16: 109-120.
9. De La Lastra M, Forcelledo ML, Serrano C. Influence of the hypophysis on pregnant mare's serum gonadotropin-induced ovulation in immature rats. *J Reprod Fert* 1972; 31: 23-28.
10. Miller BG, Armstrong DT. Effects of superovulatory dose of pregnant mare serum gonadotropin on ovarian function, serum estradiol and progesterone levels and early embryo development in immature rats. *Biol Reprod* 1981; 25: 261-271.
11. Yun YW, Yu FH, Ho Yuen B, et al. Effects of superovulatory dose of pregnant mare serum gonadotropin on follicular steroid contents and oocyte maturation in rats. *Gamete Res* 1989; 23: 289-298.
12. Harms PG, Ojeda SR. A rapid and simple procedure for chronic cannulation of the rat jugular vein. *J Appl Physiol* 1974; 36: 391-392.
13. Waynforth HB. Injection techniques. In: Waynforth HB, ed. *Experimental and Surgical Technique in the Rat*. Academic Press Inc., London, UK, 1980; 50-52.
14. Yun YW, Ho Yuen B, Moon YS. Effects of an antiandrogen, flutamide, on oocyte quality and embryo development in rats superovulated with pregnant mare's serum gonadotropin. *Biol Reprod* 1988; 39: 279-286.
15. Austin CR. The mammalian egg. Oxford: Blackwell Scientific publications. 1961.
16. Bogdanove EM, Gay VL. Studies on the disappearance of LH and FSH in the rat: A quantitative approach to adenohypophysial kinetics. *Endocrinology* 1969; 84: 1118-1131.
17. Schams D, Menzer Ch, Schallenberger E, et al. Some studies on pregnant mare serum gonadotrophin(PMSG) and on endocrine responses after application for superovulation in cattle. In: Sreenan JM, ed. *Control of Reproduction in the Cow*. Martinus Nijhoff, Hague, 1978; 122-143.
18. Parlow AF, Ward DN. Rate of disappearance of LH, PMS and HCG from plasma. In: Albert A, Charles C Thomas, eds. *Human Pituitary Gonadotropins*. Springfield, Illinois, 1961; 204.
19. Sasamoto S, Sato K, Naito H. Biological active life of PMSG in mice with special reference to follicular ability to ovulate. *J Reprod Fert* 1972; 30: 371 379.
20. Aggarwal BB, Papkoff H. Studies on the disappearance of equine chronic gonadotropin from the circulation in the rat: tissue uptake and degradation. *Endocrinology* 1981; 109: 1242-1247.
21. Sasamoto S, Kennan AL. Endogenous gonadotropin requirements for follicular growth and maintenance in immature rats pretreated with PMS. *Endocrinology* 1973; 93: 292-296.
22. Strauss WF, Meyer RK. Neural timing of ovulation in immature rats treated with gonadotrophin. *Science NY* 1962; 137: 860-861.
23. Zarrow MX, Quinn DL. Superovulation in the immature rat following treatment with PMS alone and inhibition of PMS-induced ovulation. *J Endocrinol* 1963; 26: 181-188.
24. Ferraretti AP, Garcia JE, Acosta AA, et al. Serum luteinizing hormone during ovulation induction with human menopausal gonadotrophin for *in vitro* fertilization in normally menstruating women. *Fertil Steril* 1993; 40(6): 742-747.
25. Messinis IE, Templeton A. The effect of pulsatile follicle stimulating hormone on the endogenous luteinizing hormone surge in women. *Clin Endocr* 1986; 25: 633-640.
26. Littman BA, Hodgen GD. Human menopausal gonadotrophin stimulation in monkeys: blockade of the luteinizing hormone surge by a highly tran-

sient ovarian factor. *Fert Steril* 1984; 41(3): 440-447.

27. Schenken RS, Hodgen GD. Follicle stimulating hormone induced ovarian hyperstimulation in monkeys: blockade of the luteinizing hormone surge. *J Clin Invest* 1983; 57: 50-55.
28. Moor RM, Hay MF, McIntosh JEA, et al. Effect of gonadotropins on the production of steroids by sheep ovarian follicles cultured *in vitro*. *J Endocrinol* 1973; 58: 599-611.