大韓獸醫學會誌(1994) 第34卷 第4號
Korean J Vet Res (1994) 34(4):745-757

The effects of superovulatory doses of pregnant mare serum gonadotropin on uterine microenvironment of the rat

Young-won Yun

College of Veterinary Medicine, Chungbuk National University

(Received Aug 30, 1994)

다배란 용량의 임마혈청성 고나도트로핀(PMSG)이 랫트의 자궁내 미세환경에 미치는 영향

윤 영 원

충북대학교 수의과대학

(1994년 8월 30일 접수)

초록 : 고나도트로핀제재에 의한 다배란처치는 난소 스테로이드 호르몬(estrogens, progestins 및 androgens)의 정교한 균형을 깨트림으로써 자궁에 바람직하지 못한 영향을 미친다. 따라서 이러한 다배란처치가 자궁조직에 미치는 영향을 검사하기 위하여, 28일령의 189마리 미성숙래트에 4IU, 20IU 또는 40IU의 임마혈청성 고나도트로핀(PMSG)을 투여하고, 그후 10일까지 매 24시간 간격으로 실험동물을 희생시켰다. 자궁조직의 장기적 효과는 4IU 또는 40IU의 PMSG를 투여한 12마리의 래트를 30일째에 희생시켜 검사하였다. 그리고 일부 성숙래트의 자궁은 PMSG를 투여한 미성숙래트의 자궁과 비교하는데 공시되었다. PMSG투여후 2일부터 5일까지 대조군(4IU) 자궁의 형태학적, 조직학적 변화는 성숙래트의 발정주기 동안의 변화와 거의 동일하였다. 그러나 대배란처치용량(20IU 또는 40IU)의 PMSG는 투여 후 2일째에는 자궁 간질조직의 hypertrophy와 3일째에는 자궁내강 상피세포의 focal papillary hyperplasia를 형성시켰다. 20IU와 40IU의 PMSG를 투여한 후 17β-estradiol의 혈중농도는 투여 1일 후에 대조군(4IU)보다 현저하게($P<0.005$ 및 $P<0.05$) 증가하였고, androgen농도는 투여 1일 후에 baseline으로부터 현저하게($P<0.05$ 및 $P<0.005$) 증가하여 2일과 3일 사이에 최고에 도달하였다. 20IU PMSG 투여군에 있어서, hyperplasia 현상은 투여 3일 후부터 점차 퇴행되어 10일까지는 완전히 소멸되었다. 그러나 40IU PMSG 투여군에서의 hyperplasia는 투여 6일 후까지 뚜렷이 진행되었다. 이러한 결과는 혈중 estrogen 농도의 상승과 밀접한 관계가 있는 것으로 사료된다. 왜냐하면 40IU PMSG 투여군에 있어서의 17β-estradiol 혈중농도가 투여 4일 후에 최고에 도달하였으며, 이는 4IU PMSG를 투여한 대조군과 20IU PMSG 투여군보다 현저하게($P<0.001$) 높았기 때문이다. 그리고 40IU PMSG 투여군에 있어서의 hyperplasia 현상은 투여 6일과 10일 사이에 약간씩 퇴행됨을 보였고, 30일까지는 완전히 소멸되었다.

본 연구결과는, 다배란처치로 인하여 난조조직으로부터 과잉 분비되는 estrogen 및 androgen에 대한 자궁조직의 사전노출이, 위에서 언급한 비정상적 hyperplasia 형성의 가능한 원인적 요소가 됨을 시사한다.

Key words: superovulation, PMSG, endometrium

This paper was supported (in part) by NON DIRECTED RESEARCH FUND, Korea Research Foundation, 1993.

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

Superovulation is a widely accepted technique in synchronizing and inducing ovulation. It is well established that follicular hyperstimulation results in the disruption of the delicate balance of ovarian steroids (estrogen, progesterone, androgens)[1-3]. However the effects of this disruption on steroid-sensitive tissues, such as the uterus, are not well understood.

Recent evidence suggests that superovulatory treatment with PMSG adversely affects uterine structure and function in immature rats. In superovulated(40IU PMSG) mated and non-mated rats, Miller and Armstrong[4] observed a two fold increase in wet uterine weight by D2 of pregnancy or D3 post treatment. This increase was accompanied by the presence of desquamated cellular debris in uterine lavages as early as D2 of pregnancy. Histological examination of debris recovered on D3 suggested the presence of epithelial hyperplasia. In contrast, no such findings were observed in the uterine lavages from controls treated with "physiologic" dose of PMSG(4IU). This result could suggest hyperstimulation of endometrial epithelia. Rennels[5] using 30IU PMSG/20IU hCG observed a marked increase in uterine weight and attributed the increase to hypertrophication of the inner muscularis of the myometrium, beginning on D2.

Studies involving embryo transfer or decidual induction on D5 of pregnancy of PMSG-treated immature rats have provided direct evidence that PMSG impairs implantation. Synchronous embryo transfer between 4IU PMSG-treated donor and recipient rats on D5 of pregnancy has achieved comparable implantation rate to that of 4IU PMSG-treated pregnant rats(80-90%)[4,6]. In contrast, similarly conducted transfer from 4IU PMSG-treated donors to 40IU PMSG-treated recipients results in no implantation[4]. While trauma-induced deciduomata were observed at embryo transfer points in rats treated with physiologic dose, none were observed in 40IU PMSG-treated rats. These findings coincide with the difficulty observed in inducing deciduomata with 50IU PMSG[7].

The functional study results together with the structural findings suggest that high doses of PMSG induce abnormal changes, some manifested structurally, which prevent the uterus from reaching a receptive state. To date, no studies have been perfomed to follow the temporal changes of the uterus under the influence of the superovulatory doses of PMSG. In the present study, the time-course of the uterine response and changes in serum steroidal levels was examined.

Materials and Methods

Experimental animals : Immature female Sprague Dawley rats were obtained at 25 days and 2 months of age. All animals were housed for 3 days under controlled conditions of temperature(20℃) and illumination(12L:12D) prior to PMSG treatment. PMSG(Equinex, Ayerst, Montreal) was administered in a 4, 20 or 40IU dose in 0.2ml of 0.9% NaCl via a subcutaneous dorsal injection between 0930 and 1130h. Standard rat chow and water were available *ad libitum* throughout the treatment period.

Data collection : Three separate studies were conducted to determine the effects of superovulatory doses of PMSG on the uterus. The short term effects of 4, 20 and 40IU PMSG were studied in immature rats from D2 to D10 post injection. In this study, 162 animals were divided into 6 animals per treatment dose and sacrificed at 9 time intervals(D2-D10), 24h apart(54 rats/dose). Groups of 3 animals were sacrificed at a time. The results for all 6 animals were combined later. Simultaneously, in a separate study, the hormonal status of 3 rats per dose was examined at 0, 24 and 60h. The long term effects of 4 and 40IU PMSG were assessed in 12 immature rats 30 days following administration. To provide a reference for induced cyclical changes in immature rats, 12 adult rats were followed by vaginal smears for one full cycle and sacrificed at the appropriate time to achieve samples of proestrus, estrus, metestrus and diestrus phases.

At sacrifice, the animal were weighed, anesthetized using diethyl ether vapour and exsanguinated via the trunk vein. The reproductive tracts were dissected whole and a small crimp was made at the uterotubal junction of the left cornua for identification purposes. The tissue was placed immediately in Bouin's fixative (saturated picric acid-75%, 37% buffered formalin-20%,

glacial acetic acid-5%). During fixation, mesenteric tissue was removed and each uterine horn was dissected from the periovarium and partitioned into the following segments before placement into cassettes denoting left and right sides. The first 2mm of uterus(proximal to the oviduct) was discarded, the next 3mm was used for traverse sections and the rest was discarded. After 4 hours, all tissues were placed in running tap water to remove excess fixative and were stored in 10% buffered neutral formalin until histological processing. From each uterine segment, 4 serial sections(5μm) were takene for routine H&E staining for a total of 24 sections per rat.

Steroid radioimmunoassay : The blood, collected via the trunk vein, was stored 4 hours at room temperature prior to centrifugation. The sera were collected and stored at -20℃. Aliquots(0.5-1.0ml) of sera were extracted twice with sufficient diethyl ether to bring the total volume to 5.0ml. The pooled extacts were evaporated at 35℃ under nitrogen gas before being reconstituted in 1.0ml absolute ethanol. Duplicate 100μl aliquots of the extracts were assayed. Approximately 10,000cpm(H^3-steroid) was added to each tube. The binding efficiency of antibodies of the steroid hormones(17β-estradiol, progesterone, androgens) was 40-60% and non-specific binding was less than 5%. Quality control of the assay was carried out using water blanks and reference sera and recoveries were estimated by addition of tracer to reference sera aliquots. Intra-and inter-assay coefficients of variation were less than 10% and 15%, respectively. Antisera for 17β-estradiol, progesterone, androgens radioimmunoassays were provided by courtesy of Dr. D.T. Armstrong, University of Western Ontario. The respective cross-reactivities of the antisera are as follows: 17β-estradiol antiserum: estrone 2.9%; estriol, 0.5%; other steroids, <0.2%; progesterone antiserum: 5β-pregnane-3,20-dione, 35.5%; 5α-pregnane-3, 20-dione, 15.7%; 3α-hydroxy-5β-pregnan-20-one, 2.0%; 20β-hydroxy-4-pregnen-3-one, 1.3%; 17α-hydroxyprogesterone, 1.2%; other steroids, <0.2%; testosterone antiserum: 5α-dihydrotestosterone, 75.0%; 5α-androstane-3α,17β-diol, 13.5%; 5α-androstane-3β, 17β-diol, 10.9%; 19-hydroxytestosterone, 4.7%; other steroids, <0.1%. Specifically, the cross-reactivity with other androgens was substantial and therefore the steroids measured using testosterone antiserum are referred to as androgens rather than testosterone.

Statistical analysis : The significance of treatment means differences was tested by analysis of varience (completely randomized) and Duncan's multiple range test. Individual interactions were tested by the Student's T-test where significance was defined as $p<0.05$.

Results

Representative photomicrographs of the uteri and endometria from 4, 20, 40IU PMSG-treated and adult rats are illustrated in Photos 1-15.

The uterine morphological and histological patterns observed shortly after 4IU PMSG treatment were strikingly similar to those of adult rats. On D2, the majority(66%) of control uteri resembled those of proestrus adults. The lumen in these uteri were greatly distended. The columnar luminal epithelium displayed basophilic cytoplasm and enlarged nuclei with distinct nucleoli. The glandular epithelia was relatively inactive and did not appear to follow the changes of the luminal epithelium. By D3 the lumen was collapsed resulting in a folded and flaccid appearance similar to that observed in the adult estrus state. The tall columnar epithelium was pseudo-stratified and visibly degenerate. An abundant amount of basophilic debris was observed in numerous inter- and intracellular vacuoles observed in the epithelium. During this episode of degeneration, there was extensive neutrophil infiltration of the endometrial stroma. From D3 to D5, there was an overall trend towards quiescence. The tortuous folds of the endometrium on D3 had mostly disappeared by D4. By D5, further collapse of the lumen and regressive changes of the endometrium had resulted in a slit-like lumen(Photo 1). These morphological changes were analogous to those observed in the adult metestrus and diestrus states, respectively. Epithelial degenerative activity(vacuolation and pyknosis) had greatly subsided by D4 and was absent by D5(Photo 2). From D5 to D8, the epithelium was low columnar and exhibited condensed oval nuclei, con-

sistent with a state of quiescence. The uterine response after D8 varied greatly. While 33% were still quiescent, many uteri(50%) displayed the features of early proestrus. The epithelia in the latter presented opaque nuclei with distinct nucleoli, and crowding of the cells. Further progressive changes of the endometrium toward maximal dilation were evident by D10. The changes in uteri treated with 20IU PMSG followed a course similar to the 4IU PMSG response. However, superimposed upon these changes was an abnormal proliferation and a secretion of a basophilic material. On D2, only 20% of the uteri appeared to have the typical proestrus morphology. The remainder presented with stromal hypertrophy and moderate infolding of the endometrium(Photo 3). Very little glandular epithelia was present, even in the invaginations of these folds. The luminal epithelium was tall columnar and exhibited enlarged nuclei and basophilic cytoplasm(Photo 4). By D3, there was increased infolding and in addition, the appearance of focal papillary hyperplasia(Photo 5). Invariably, this hyperplasia occurred at the antimesometrial end and was attended by stromal rarefication. The glandular epithelia did not appear to be involved in this abnormal proliferation. Epithelial degeneration was prominent in all areas except in hyperplastic foci(Photo 6). Between D3 and D10, the epithelial hyperplasia, stromal hypertrophy and the size of the lumen gradually diminished. The smaller papillary buds had disappeared by D4. By D6, the larger papillary extensions as well as existing folds of the endometrium had markedly recessed (Photo 7). By D8, only the folds remained(Photo 8), which were mostly resorbed by D10. Epithelial activity did not begin to decrease until after D6. Between D4 and D6, the columnar epithelium was basophilic and the nuclei showed diffuse chromatin and distinct nucleoli(Photo 9). By D8, the nuclei had condensed and become tightly packed together, and within 24h the cytoplasmic basophilia had disappeared(Photo 10).

The alterations in the endometrium resulting from 40IU PMSG treatment were similar, but magnified when compared with those in 20IU PMSG-treated rats. On D2, there were deep invaginations into the endometrium. The luminal epithelia was columnar and displayed enlarged nuclei and basophilia of the cytoplasm. By D3, papillary hyperplasia appeared focally at the antimesometrial end(Photo 11). Unlike the 4 and 20IU PMSG responses, there was no evidence of epithelial degeneration or increased neutrophil infiltration. The hyperplasia rapidly progressed and by D5, had spread towards the mesometrial end. By D6, the luminal epithelium had deeply and multicentrically invaded the stroma, forming many flaccid projections with numerous papillary extensions(Photo 12). The epithelium had decreased in height and displayed enlarged, rounded nuclei(Photo 13). The glandular epithelium was not involved in this abnormal proliferation, but appeared secretory, similar to that of luminal epithelium. After D6, while the secretory appearance was absent, quiescence was not attained by D10. Many of the papillary extensions observed between D5 and D6 had regressed by D7. Secretory activity had decreased by D7 and was absent by D8. Further reductions in the lumen size and stromal hypertrophy were apparent by D8(Photo 14). On D10, the epithelium was low columnar and appeared inactive(Photo 15). Full regression of the hyperplasia, as observed after the 20IU PMSG treatment, was not achieved by D 10. Uteri recovered 30 days after treatment, however, showed typical estrous cycle features and no evidence of hyperplasia or any other histological abnormalities.

The temporal patterns of serum levels of 17β-estradiol, progesterone and androgens are shown in Fig 1. Major alterations in serum steroid levels occurred shortly after 4IU PMSG treatment. After D1, 17β-estradiol levels significantly($p<0.001$) increased to a maximum(421 ± 33pg/ml) on D2, before falling to basal levels by D3. Progesterone levels significantly($p<0.01$) increased from D2 to a level of 109 ± 17ng/ml at 60h. This was followed by a return to basal levels by D3. Elevations in 17β-estradiol and androgens were observed between D8 and D10, but these were not significant.

Many striking changes resulted from treatment with 20IU PMSG. By D1, 17β-estradiol levels had significantly($p<0.005$) increased and within 24h had peaked(663 ± 59pg/ml). This peak was comparable to that in 40IU PMSG-treated rats, but significantly($p<0.001$) higher than in controls. Between D2 and D3,

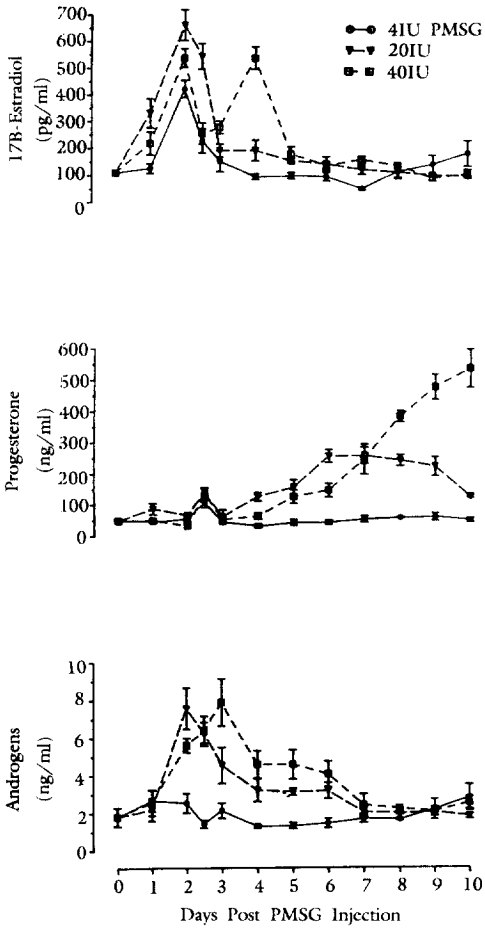


Fig 1. Time-course profiles of serum steroids after administration of 4IU, 20IU or 40IU PMSG to immature rats. Values represent means±SE (n=6).

17β-estradiol levels sharply returned to baseline. Androgen levels rose($p<0.05$) three fold from D1 to D2(7.59±2.1ng/ml) and then decreased stepwise. First, androgen levels significantly($p<0.05$) declined to intermediary levels by D4, but were still significantly($p<0.01$) higher than those of controls. Thereafter, the levels slowly decreased and reached baseline by D7. Progesterone levels followed those of the controls until D3. By D4, however, these levels had increased significantly($p<0.025$) to a maximum level of 261±27ng/ml.

During the study period, steroid levels resulting from the 40IU PMSG regimeng fluctuated greatly. After dramatically($p<0.001$) increasing between D0 and D2(538±36pg/ml), 17β-estradiol levels significantly($p<0.001$) decreased by 68%. The peak on D2 was significantly($p<0.025$) greater than control levels. Unlike the responses to lower doses, 17β-estradiol levels sharply($p<0.001$) rose again after D3 to a maximum on D4(537±43pg/ml), before falling to baseline by D5. A significant($p<0.005$) rise in androgens from 24h to a peak(7.92±1.3ng/ml) on D3 preceded the last increase in 17β-estradiol levels. Analogous to 20IU PMSG response, androgen levels declined in a stepwise manner. They first fell($p<0.05$) between D3 and D4 to plateau levels comparable to that in 20IU PMSG-treated rats. These levels then gradually diminished in a manner similar to the decline of 20IU PMSG levels. Progesterone levels paralleled those of the 20IU PMSG response until D7, but it was not until D5 did the levels became significantly($p<0.01$) greater than those of controls. After D7, progesterone levels continued to rise and reached 539±67ng/ml by D10.

Discussion

The present study confirms that a low dose(4IU) of PMSG can induce immature rats to mimic the physiological events of the adult estrous cycle. The dynamics of 17β-estradiol and progesterone levels between D1 and D5 post PMSG treatment are in fair agreement with those observed by other investigators using low doses(4-8IU) of PMSG[8-10] and the adult cycling rat[11, 12]. In view of the similarity between hormonal patterns of controls and adults, the correspondence of uterine histological changes was not surprising. It has long been recognized that endometrial histological changes during the estrous cycle are controlled by concomitant fluctuations of estrogen and progesterone secretion rates[1]. During proestrus, the uterus becomes maximally dilated by the accumulation and retention of fluid. The accumulation of fluid as well as the increased luminal epithelial proliferation that accomupanies and allows for luminal distension is associated with the elevation of estrogen levels 24h prior to maximum dilation[13-15]. During estrus, declin-

ing estrogen levels and rising progesterone levels synergistically relax the constricted cervix and allow for the drainage of uterine fluid through the vagina[14]. The uterus involutes compressing the endometrium into flaccid folds. Concomitantly, all steroid levels return to baseline inducing stromal hypotrophy and epithelial degeneration, characterized by nuclear debris, cytoplasmic vacuolation and reparation changes[1,16].

Superovulatory treatment with PMSG dose-dependently retarded degenerative activity. Animals treated with 20IU PMSG displayed the classic effects of hormonal withdrawal on D3, albeit subdued in comparison with controls and adults. Although 17β-estradiol and progesterone levels had fallen to baseline by D3, androgen levels remained elevated and may have provided partial hormonal support. Exogenously administered non- and aromatizable androgens may induce uterine epithelial hypertrophy through protein and carbohydrate synthesis associated with growth[12,17]. Despite a sharp fall in 17β-estradiol levels after 60h in 40IU PMSG-treated rats, the levels on D3 were still higher than those of controls. These estrogens in combination with elevated androgens could have provided sufficient epithelial support to account for the absence of degeneration and repair.

While interfering with changes normally associated with ovulation, increased levels of steroids(17β-estradiol, androgens, progesterone) may have concurrently produced endometrial atypia. High levels of 17β-estradiol and androgens together with hypertrophic development of the endometrial stroma followed either 20IU or 40IU PMSG treatment. As there was little difference in the steroid levels and the extent of hypertrophy between the 20IU and 40IU treatment groups, common mechanisms may have been operative. It is well established that endogenous and exogenous estrogens initially hypertrophy the stroma through the development of hyperemia and stromal edema[18-20]. Time course studies of ovariectomized rats given a single dose of 17β-estradiol have revealed that edema rapidly develops to a dose-dependent maximum between 6-10h, but regresses within 20h, even after multiple treatments[1,18]. Fluid is concurrently transferred from the stroma into the lumen resulting in distension[1]. In view of these experiments, the presence of hypertrophy on D2 in relation to elevated 17β-estradiol levels prior to D1, suggests that the affect of estrogens may have been secondary. Exogenous aromatizable and, to a lesser extent, non-aromatizable androgens are capable of inducing sustained hypertrophy up to D3 after treatment[5,17]. Androgens may act directly upon the uterus through their own or in the case of elevated peripheral levels, estrogen receptors[21,22]. As the effects of androgens and estrogens were not delineated, it is possible that these steroids may have synergistically induced and maintained hypertrophy of the stroma for an extended period of time.

The most interesting feature of this study was the production of focal and diffuse luminal papillary hyperplasia with superovulatory doses of PMSG. The development of hyperplasia appeared to follow the onset of stromal hypertrophy in both 20IU and 40IU treatment groups. It is possible that prolonged and elevated levels of 17β-estradiol from D1 to D2 after 20IU or 40IU PMSG treatment initiated this sequential pattern. Similar patterns have been observed in ovariectomized rodents repeatedly treated with estrogens[20]. While prolonged treatment increased uterine weight initially(6-10h) through stromal hypertrophy, subsequent increases(24-48h) apparently depended upon epithelial proliferation and growth. Epithelial hyperplasia, however, was initially focal and invariably appeared at the antimesometrial end. This focal appearance may have been a result of dissimilar hormonal sensitivities of the anti- and mesometrial regions. Autoradiographic studies of ovariectomized mice treated with a single dose of 17β-estradiol showed equal labeling rates up to 16h between both regions, however, after this time while mesometrial rates declined, those of the antimesometrial region remained elevated up to at least 36h[20]. Prolonged and high levels of estrogens associated with superovulatory treatment may have exaggerated the existing elevated proliferation rates in the antimesometrial region resulting in focal hyperplasia. Differential sensitivity was also observed between luminal and glandular epithelia during the development of hyperplasia. It is well recognized that the affects of steroid hormones on the endometrium are dependent upon the sensitivity and proportion of

glandular and luminal epithelial tissue[1,23] in rats and mice, there is proportionately less glandular than luminal tissue. Moreover, the luminal epithelium is far more sensitive to hormonal influence than either glandular or stromal tissue[1,13,15,20]. These features might account for the presence of increased proliferation in the luminal epithelium, and absence in glandular tissue. In addition, the differential sensitivities of the luminal epithelium and the stroma may explain the papillary appearance of the hyperplastic endometrium. The epithelium, growing faster than the stroma, is forced to fold to allow for an increase in surface area within a restricted volume. Had there been synchronized growth between the stroma and epithelium, stromal rarefication would have been absent and the epithelium less convoluted, as observed during the onset of proestrus in controls from D9-D10.

From D4 to D5 post 40IU PMSG treatment, there was increased hypertrophy of the stroma and progressive hyperplasia. While the hormonal event(s) responsible were not identified, the absence of these changes in 20IU PMSG-treated rats provides a basis for comparison. The major difference between the steroidal patterns of the 20IU and 40IU PMSG treatment groups was the marked elevation of 17β-estradiol levels from D3 to D4 post 40IU PMSG. Walton et al[6] simultaneously reduced the 17β-estradiol surge on D3 and abolished the increase in uterine weight usually observed with superovulatory treatment after administering PMSG antisera at 58h post 40IU PMSG injection. As previously mentioned, high doses of estrogens may induce endometrial hypertrophy and hyperplasia. It is possible that earlier elevations of estrogen levels(D1-D2) may have primed or sensitized the endometrium to respond intensely to a second stimulus. The changes induced by this second stimulus would then be superimposed over those elicited by the first stimulus. Martin et al[20], using a single injecion of 17β-estradiol, found that uterine weight and volume, stromal edema and epithelial proliferation rates declined after they reached a maximum between 6-24h. However, if a second injection of the same dose(100ng) was given one day later, the magnitude of all parameters rapidly increased to new and higher maxima within 16-24h. These results of the use of single and double injection of estrogens appear to parallel our observations of 20IU and 40IU PMSG-treated rats, respectively.

The present results suggest that superovulatory doses of PMSG induce pathological structure and metabolic alterations in the uterus. Although reversible, many of these effects may persist after one week following treatment. It is possible that these effects may contribute to the lower fecundity rates associated with superovulation. It is well established that disturbances in the estrogen and progesterone profiles in adult cycling rats with exogenous ovarian steroids, especially estrogens, can reduce or abolish uterine receptivity[24,25]. Perhaps, this interference with uterine receptivity may be mediated through abnormal endometrial growth, whereby preimplantational preparations are incomplete or absent. Loss of these processes may retard or induce abnormal embryo development. Moreover, intraluminal secretions of the endometrium could further augment these deleterious effects.

Summary

Superovulatory treatment with exogenous gonadotropins adversely affects the uterus through the disruption of the delicate balance of ovarian steroids (estrogens, progestins, androgens). To examine the uterine effects of this treatment, 189 rats were given 4IU, 20IU or 40IU pregnant mare's serum gonadotropin(PMSG) at 28 days of age and sacrificed every 24h until day 10(D10) post injection. Long term uterine effects were examined in 12 rats treated with 4IU or 40IU PMSG and killed on D30. Adult rat uteri were examined to provide a reference for comparisons.

Morphological and histological changes of control (4IU) uteri mimicked those of the adult on a comparable time-course form D2 to D5. Administration of superovulatory doses(20IU, 40IU) of PMSG produced stromal hypertrophy by D2 and focal papillary hyperplasia of the luminal epithelia by D3. Levels of 17β-estradiol following 20IU and 40IU PMSG treatment were significantly($p<0.05$, $p<0.005$) elevated above those of controls after D1. Androgen levels of both

groups(20IU, 40IU) significantly $p<0.05$, $p<005$ increased from baseline on D1 and were maximum between D2 and D3. In the 20IU PMSG group, the hyperplasia gradually regressed after D3 and was absent by D10. The hyperplasia in the 40IU PMSG group, however, had become extensive by D6. It is suspected that preceding elevated levels of estrogen may be responsible for this progressive change. On D 4, the levels of 17β-estradiol reached a maximum, which was significantly($p<0.001$) greater than both the controls and 20IU PMSG-treated rats. Between D6 and D10, the hyperplasia in 40IU PMSG-treated rats partially regressed. Examination of uteri from D30 revealed no evidence of the hyperplasia.

It is suggested that previous exposure to high levels of estrogen and androgens, secondary to superovulation, is possible cause for the observed hyperplasia.

Legends for photographs

Photo 1. Uterus, 4IU PMSG, D5(40X, H&E). The lumen appears slit-like. Note the increased stromal cellular density next to the lumen.

Photo 2. Endometrium, 4IU PMSG, D5(400X, H&E). Repair of the epithelium is complete. The epithelium is low columnar and appears to be inactive, Note the condensed nuclei, indistinct nucleoli and eosinophilia of the cytoplasm.

Photo 3. Uterus, 20IU PMSG, D2(40X, H&E). The stroma is edematous and hypertrophied, and as a result appears to be folded.

Photo 4. Endometrium, 20IU PMSG, D2(400X, H&E). The luminal epithelium is tall columnar and appears to be active. Note the diffuse chromatin and distinct nucleoli.

Photo 5. Uterus, 20IU PMSG, D3(40X, H&E). Papillary hyperplasia has appeared focally at the anti-mesometrial end. Note that the epithelia has invaded the stroma almost as far as the myometrium.

Photo 6. Endometrium, 20IU PMSG, D3(400X, H&E). Vacuolation and other degenerative changes are only slight. The nuclei and nucleoli are still enlarged.

Photo 7. Uterus, 20IU PMSG, D6(40X, H&E). Previous endometrial growths have decreased to small buds. The cellular density of the stroma has increased in certain areas.

Photo 8. Uterus, 20IU PMSG, D8(40X, H&E). The papillary hyperplasia as well as the folds of endometria have disappeared. Morphological changes towards the formation of a slit-like lumen are apparent.

Photo 9. Endometrium, 20IU PMSG, D6(400X, H&E). The epithelium has become depolarized and appears to be secretory.

Photo 10. Endometrium, 20IU PMSG, D8(40X, H&E). The luminal epithelium displays compact and organized nuclei. Note the quiescent, glandular tissue.

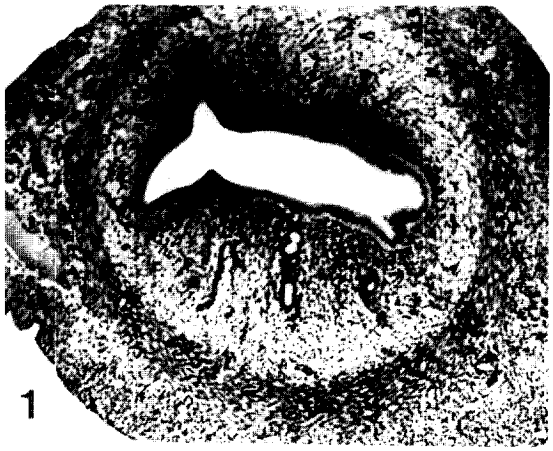
Photo 11. Uterus, 40IU PMSG, D3(40X, H&E). Secretory papillary hyperplasia appears focally at the anti-mesometrial end, similar to that observed on D3 following 20IU PMSG treatment(Photo 6).

Photo 12. Uterus, 40IU PMSG, D6 (40X, H&E). The luminal epithelia has deeply and multicentrically invaded the stroma, forming flaccid projections with papillary extensions.

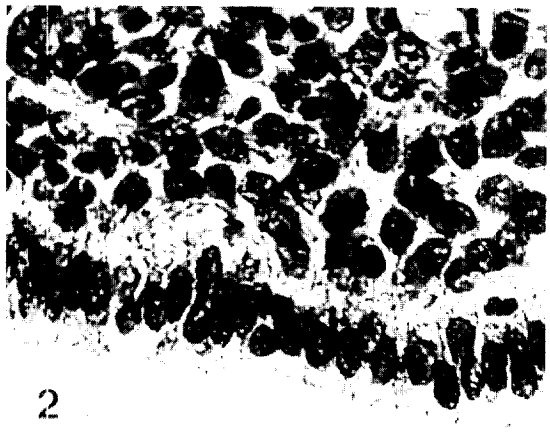
Photo 13. Endometrium, 40IU PMSG, D6(400X, H&E). Secretory activity was more intense than observed after 20IU PMSG treatment(Photo 8). Note the stromal rarefication.

Photo 14. Uterus, 40IU PMSG, D8(40X, H&E). Stromal hypertrophy and lumen size have been reduced. Further regression of the hyperplasia by this time is apparent and the lumen is clear of the luminal material observed on D6(Photo 13).

Photo 15. Endometrium, 40IU PMSG, D10(400X, H&E). The epithelium appears to be less active. The nuclei are smaller and the cytoplasm appears to be eosinophilic.



1

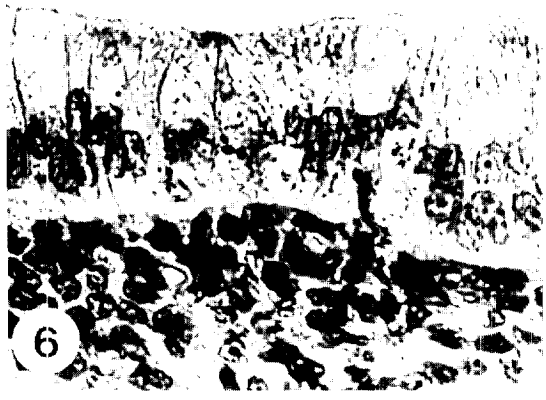


2

3

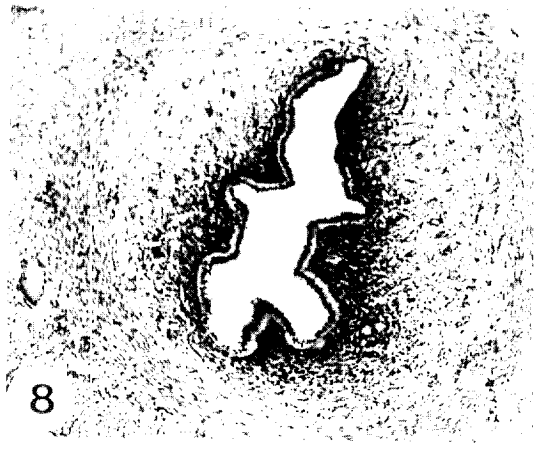
4

5

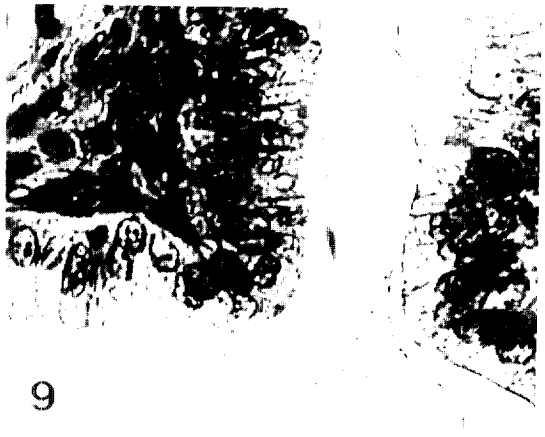


6

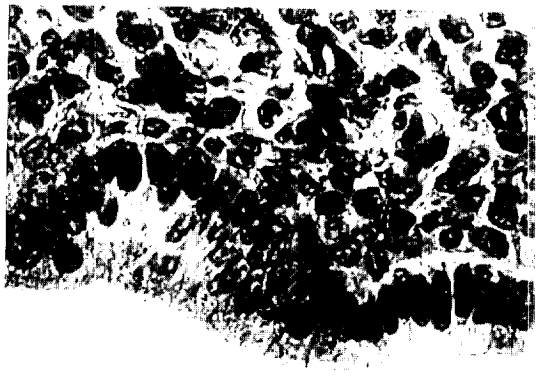
7



8

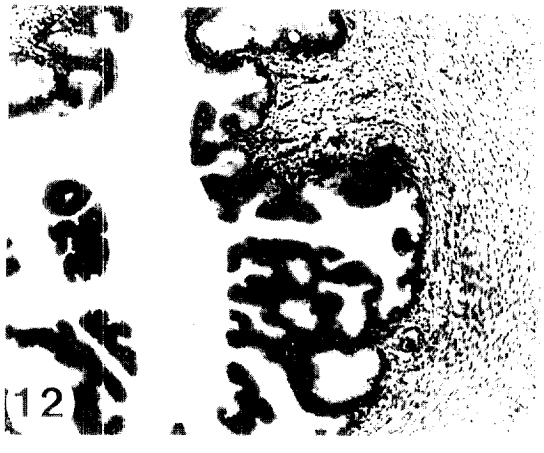


9

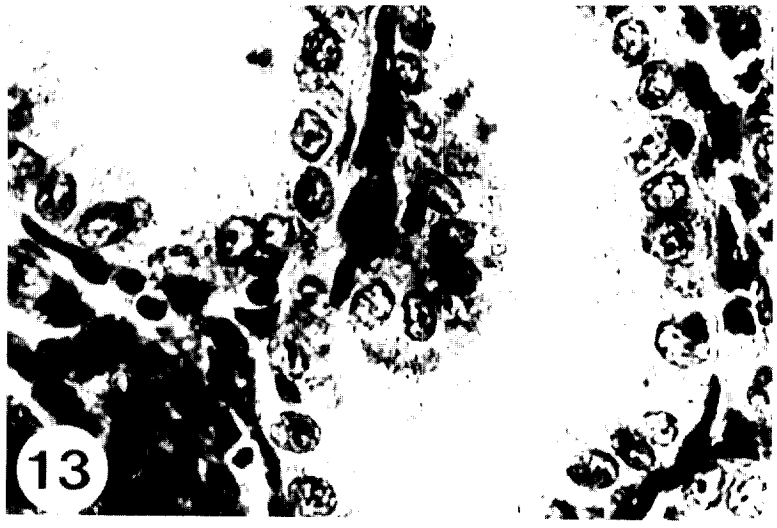


10

11



12


13


14


15

References

1. Finn CA, Porter DG. The endometrium. In: *The uterus*. England: Gresham Press, 1975; 17-132.
2. Yun YW, Ho Yuen B, Moon YS. Effects of superovulatory doses of pregnant mare serum gonadotropin on oocyte quality and ovulatory and steroid hormone responses in rats. *Gamete Res* 1987; 16: 109-120.
3. 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.
4. Miller BG, Armstrong DT. Effects of a superovulatory dose of PMSG on ovarian function, serum estradiol and progesterone levels and early embryo development in immature rats. *Biol Reprod* 1981_b; 25: 261-271.
5. Rennels EG. Influence of hormones on the histochemistry of ovarian interstitial tissue in the immature rat. *Am J Anat* 1951; 88: 63-107.
6. Walton EA, Huntley S, Kennedy TG, et al. Possible causes of implantation failure in superovulated immature rats. *Biol Reprod* 1982; 27: 847-852.
7. Guillet GG, Rennels EG. The experimental production of deciduomata in immature rats. *Texas Rep Biol Med* 1964; 22: 78-93.
8. Wilson CA, Horth CE, Endersby CA, et al. Changes in plasma levels of estradiol, progesterone and LH in immature rats treated with pregnant mare's serum gonadotrophin. *J Endocrinol* 1974; 66: 293-298.
9. Nuti KM, Sridharan BN, Meyer RK. Reproductive biology of PMSG primed immature female rats. *Biol Reprod* 1975; 13: 38-44.
10. Parker Jr CR, Costoff A, Muldoon TG, et al. Actions of pregnant mare's serum gonadotrophin in the immature female rat: Correlative changes in blood steroids, gonadotrophins, and cytoplasmic receptors of the anterior pituitary and hypothalamus. *Endocrinol* 1976; 98: 129-138.
11. Yoshinaga K, Hawkins RA, Stocher JF. Estrogen secretion by the rat ovary *in vivo* during the estrous cycle and pregnancy. *J Endocrinol* 1969; 85: 103-125.
12. Pasqualini JR, Kincl FA. Hormonal mechanisms in reproduction. In: *Hormones and the fetus*. NY: Pergamon Press, 1985; 1-71.
13. Marcus GJ. Mitosis in the rat uterus during the estrous cycle, early pregnancy and early pseudopregnancy. *Biol Reprod* 1974; 10: 447-454.
14. Kennedy TG, Armstrong DT. Loss of uterine luminal fluid in the rat: Relative importance of changing peripheral levels of estrogen and progesterone. *Endocrinol* 1975; 97(6): 1379-1383.
15. Finn CA. The endometrium during implantation. In: Kimball FA, ed. *The endometrium*. NY: Spectrum, 1980: 43-56.
16. Long SA, Evans HM. The oestrus cycle in the rat and its associated phenomena. In: Leuschner AO, ed. *Memoirs of the University of California*. Berkley: Univ Calif Press, 1922; 6: 1-148.
17. Armstrong DT, Moon YS, Leung PCK. Uterotrophic effects of testosterone and 5α-dihydrotestosterone in intact and ovariectomized immature female rats. *Biol Reprod* 1976; 15: 107-114.
18. Schwartz NB. Acute effects on ovariectomy on pituitary LH, uterine weight, and vaginal cornification. *Am J Physiol* 1964; 207: 1251-1259.
19. Galand P, Leroy F, Chretien J. Effect of oestradiol on cell proliferation and histological changes in the uterus and vagina of mice. *J Endocrinol* 1971; 49: 243-252.
20. Martin L, Finn CA, Trinder G. Hypertrophy and hyperplasia in the mouse uterus after oestrogen treatment: An autoradiographic study. *J Endocrinol* 1973; 56: 133-144.
21. Rochefort H, Lignon F. Differences between estradiol and testosterone receptors in the rat uterus. *Eur J Biochem* 1974; 48: 503-512.
22. Lobl L, Maenza RM. The ontogeny of uterine pathology and pathophysiology following neonatal androgen administration. *Biol Reprod* 1977; 16: 182-189.
23. Gondos B. Histological changes associated with oral contraceptive usage. *Anal Clin Lab Sic* 1976; 6(4): 291-299.
24. Yochim JM, Defeo VJ. Hormonal control of the onset, magnitude and duration of uterine sensitivity in the rat by steroid hormones of the ovary. *Endocrinol*

1963; 72: 317-326.

25. Psychoyos A. Endocrine control of egg implantation. In Greep RO, Astwood EG, Gieger SR, eds. *Handbook of Physiology* Sec 7, Vol 2, Part 2. Bethesda, MD: American Physiological Society, 1973; 187-215.