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Physiological Peripubertal Activation of the Ovary Is Not Reproduced by Pregnant Mare Serum Gonadotropin (PMSG) Administration[1] (41992)

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Abstract. During the days preceding the first ovulation the ovary of the rat exhibits a remarkable increase in estradiol (E_2) and progesterone (P) release in response to gonadotropins. No such increase is observed in the case of androgens (A, testosterone + dihydrotestosterone). The present experiments were undertaken to examine the possibility of reproducing these developmental events by stimulating the ovary with a gonadotropin that has substantial FSH-like activity. *In vivo* administration of pregnant mare serum gonadotropin (PMSG) to juvenile 29-day-old rats greatly increased the *in vitro* E_2 and A response to human chorionic gonadotropin (hCG) measured 2 days later in the morning. The magnitude of the A response was significantly larger than that of ovaries from juvenile animals or rats in first proestrus. The E_2 response was much greater than that of juvenile ovaries but similar to that of ovaries from late proestrous rats. In contrast, the P response to hCG was not enhanced by PMSG. In fact the response was similar to that of juvenile ovaries and markedly less than that of first proestrous rats. This decreased P response was not due to a greater conversion of P to its less active metabolite 20α-hydroxy-4-pregnen-3-one (20α-OH-P). The results suggest that PMSG enhances the E_2 and A response of immature ovaries to hCG at the expense of that of P. Treatment of immature rats with PMSG may represent a useful model to study E_2 release from preovulatory ovaries, but it cannot be used to reproduce in its entirety the developmental changes in steroidal response to gonadotropins associated with normal puberty.

At puberty the rat ovary undergoes remarkable changes in steroidogenic capacity, one of them being an increase in estradiol (E_2) and progesterone (P) release in response to gonadotropins (1, 2). The study of ovarian function on the day of first proestrus is hampered by the difficulty of obtaining sufficient animals in this phase of puberty. It would be desirable, therefore, to have an experimental animal model in which ovarian functions were similar to that of normal first proestrous rats. Pregnant mare serum gonadotropin (PMSG)-treated juvenile rats may provide such a model because administration of PMSG has been widely used to stimulate ovarian steroidogenesis (3–5) and to induce ovulation in immature rats (6, 7). Moreover, doses of PMSG of about 4–8 IU have been generally found to produce an LH surge which generates a normal number of ovulatory follicles (8, 9) and a pattern of circulating steroids similar to that seen during the adult proestrus (4).

The present study demonstrates that the ovaries from PMSG-treated rats may be useful to study preovulatory changes in E_2 secretion but they cannot be used as a model to assess the overall pattern of developmental events which occur in the ovary at puberty.

Materials and Methods. Immature female rats were purchased from Holtzman Company (Madison, Wisc.). They were maintained in group cages (5–6/cage) under controlled conditions of temperature (23°C) and light (10 hr dark, 14 hr light; lights on from 0500 hr to 1900 hr). They had free access to pelleted rat chow and tap water.

Experimental procedures. Twenty-nine-day-old rats were injected sc with PMSG (Equinex, Ayerst Laboratories, New York) at 1000 hr at the dose of 7.5 IU/rat. This dose was selected because a similar dose (8 IU) has been previously shown to produce in immature rats a pattern of circulating steroid levels similar to that of the adult estrous

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cycle (4). Forty-eight hours later the animals were decapitated and their ovaries removed for *in vitro* assessment of steroidal response to hCG (vide infra). Age-matched, untreated animals were used as controls.

Other animals were sacrificed between Days 32 and 38 and their pubertal stage assessed according to a criterion previously reported (1, 10). Those rats that exhibited accumulation of uterine fluid and had uterine weights between 120 and 190 mg were considered to be in the early proestrous (EP) phase of puberty, i.e., 1 or 2 days before the day of the first preovulatory surge of gonadotropins (1, 10). When the accumulation of fluid was substantial ("ballooned" uterus) and the uterine weight was greater than 200 mg the animals were classified as late proestrous (LP) rats. This phase corresponds to the day of the first proestrus as the preovulatory surge of gonadotropins occurs in the afternoon (10). The vagina is usually closed and large ovarian follicles are readily seen.

Incubation of ovaries. The ovaries from control and PMSG-treated rats were halved and incubated in Krebs–Ringer bicarbonate buffer, pH 7.4, containing bovine serum albumin (0.1 mg/ml), exactly as described (1, 11). Briefly, the incubation was carried out at 37°C under an atmosphere of 95% O_2–5% CO_2 with constant shaking (60 cycles/min). The ovaries were preincubated for 30 min, at the end of which the medium was discarded and replaced with fresh medium (2 ml) containing different concentrations of a highly purified hCG (13,000 IU/mg, kindly provided by Dr. R. E. Canfield, Columbia University, College of Physicians and Surgeons, New York). The incubation was then continued for an additional 3 hr. At this time the medium was collected, centrifuged at 3000 rpm for 10 min and the supernatant fluid was stored at −20°C for steroid measurement. The ovaries were weighed to the nearest 0.1 mg.

Steroid RIA. E_2, testosterone + dihydrotestosterone (A), and P were measured as previously described (1, 11) utilizing antisera generously supplied by Dr. G. D. Niswender (Colorado State University). Testosterone values are expressed as androgens because the antiserum used (GDN No. 250) has a 69% cross-reactivity with dihydrotestosterone and a 14% cross-reactivity with 3α-androstanediol (12). 20α-Hydroxy-4-pregnen-3-one (20α-OH-P) was kindly measured by Dr. A. Hsueh (University of California at San Diego) as described (13).

Statistics. The results were analyzed with a one-way analysis of variance and the Student–Newman–Keuls multiple comparison test. Differences between two groups exposed to the same dose of hCG were analyzed with the Student *t* test.

Results. *Effect of PMSG treatment on P response to hCG in vitro.* Different doses of hCG induced a dose-related increase in P secretion from both control, juvenile ovaries and ovaries of PMSG-treated rats (Fig. 1). No significant differences between the two groups were observed at any dose tested. In contrast, the ovaries of EP or LP rats exhibited a marked increase in P response to hCG (Fig. 1). This response was significantly ($P < 0.001$) higher than that of both untreated and PMSG-treated rats.

Effect of PMSG treatment on E_2 response to hCG in vitro. The ovaries of untreated, 31-day-old juvenile rats released little E_2 in response to hCG (Fig. 2). Only the high dose tested (50 ng/ml) elicited a significant increase in E_2 secretion. In contrast, and as previously

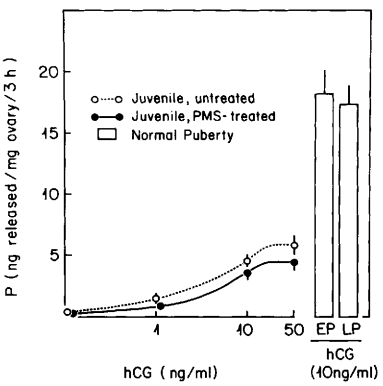

FIG. 1. Effect of PMSG *in vivo* on progesterone (P) release in response to hCG *in vitro* from ovaries of juvenile 31-day-old rats. Progesterone response to hCG of ovaries from animals in the early proestrous (EP) and late proestrous (LP) phases of puberty is also depicted (bars). Basal P release from these ovaries was less than 0.2 ng/ml. PMSG (7.5 IU/rat) was injected sc 48 hr before incubation of the ovaries with hCG. In this and subsequent figures vertical lines are SEM and each point represents the mean of 5–10 determinations.

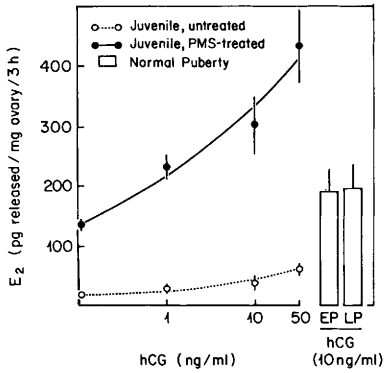

FIG. 2. Effect of PMSG *in vivo* on estradiol (E_2) release in response to hCG *in vitro* from ovaries of juvenile 31-day-old rats. Estradiol response to hCG of ovaries from animals in the early proestrous (EP) and late proestrous (LP) phases of puberty is also depicted (bars). Basal E_2 release from these ovaries was 20 ± 4 and 30.1 ± 3.5 pg/mg ovary, respectively.

reported (1), the ovaries of EP and LP rats exhibited a marked ($P < 0.01$) increase in E_2 response to hCG. The ovaries of PMSG-treated rats had not only a much greater ($P < 0.01$) basal release of E_2 than that of untreated juvenile controls, but also responded to hCG with a much greater increase in E_2 production. Their E_2 response to 10 ng hCG was, however, not significantly greater than that of EP or LP ovaries.

Effect of PMSG treatment on A response to hCG in vitro. As little as 1 ng hCG was capable of eliciting a significant ($P < 0.05$) increase in A release from ovaries of untreated, juvenile rats (Fig. 3). As previously shown (1) the A response to hCG of ovaries from EP and LP rats was not greater than that of juvenile animals. In marked contrast, the ovaries from PMSG-treated rats released significantly ($P < 0.01$) more A under basal conditions and responded much more pronouncedly to hCG than both juvenile and peripubertal ovaries.

Effect of PMSG treatment on 20α-OHP response to hCG in vitro. The failure of PMSG to increase the P response to hCG may have been due to a PMSG-induced increased conversion of P to 20α-OHP (14). This possibility was, however, not supported by direct measurement of the 20α-OHP released from ovaries of peripubertal (EP and LP) or PMSG-treated animals in response to hCG. Levels observed were similar in the three groups (data not shown).

Discussion. The results of the present experiments demonstrate that administration of a low dose of PMSG strikingly increases the E_2 and A response of the prepubertal ovary to hCG *in vitro*. In contrast the P response to hCG was not enhanced. These changes only partially reproduce the developmental alterations in steroidal responsiveness to hCG observed during normal puberty (1, 2). At this time, and particularly during the early proestrus-2 and first proestrous phases of puberty, there is a marked increase in E_2 and P release in response to hCG (1) and LH (2). The ovaries of PMSG-treated rats show a similar, or even more conspicuous change in E_2 response, but failed to develop a comparable change in P release. It would appear that this latter phenomenon is due to an enhanced utilization of P for the production of steroids originated downstream of the steroid biosynthetic pathway and not to an increased metabolism of P to its less active metabolite, 20α-OHP. This notion stems from the findings that both androgens and E_2 release in response to hCG were enhanced in PMSG-treated rats. In contrast, no differences in 20α-OHP released to the incubation medium were discerned between ovaries from first proestrous and PMSG-treated rats.

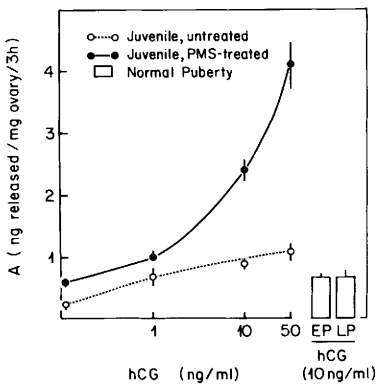

FIG. 3. Effect of PMSG *in vivo* on androgen (A) release in response to hCG *in vitro* from ovaries of juvenile 31-day-old rats. Androgen response to hCG of ovaries from animals in the early proestrous (EP) and late proestrous (LP) phases of puberty is also depicted (bars). Basal A release from these ovaries was 136 ± 9 and 111 ± 8.6 pg/mg ovary, respectively.

Several investigators have previously examined the ovarian response to PMSG and have concluded that the gonadotropin induces increases in the secretion of P, E_2, testosterone, androstenedione, dehydroepiandrosterone, and 17-hydroxyprogesterone (3–5, 15). PMSG has also been found to accelerate the conversion of radiolabeled P to 5α-pregnane-3α,20α-diol and 5α-androstane-3α,17β-diol (5) and to enhance the activity of several enzymes including 20α-hydroxy-steroid dehydrogenase (16) which converts P to 20α-OHP, 17α-hydroxylase, and C_{17}-C_{20} lyase (17) which convert P to the aromatizable androgens testosterone and androstenedione, and aromatase (17) which originates estrogens from aromatizable androgens. Additionally, PMSG greatly decreases Δ^4-5α-reductase activity (17).

By far, however, the greatest effect exerted by PMSG on the ovary is on the enzyme activities associated with estrogen production (17) and this is translated into a preferential secretion of estradiol and aromatizable androgens both *in vivo* (4) and *in vitro* (18) in response to PMSG treatment. The present results are in agreement with these earlier findings. Nevertheless, they also indicate that PMSG cannot be used to reproduce the normal pattern of ovarian steroidogenic response to gonadotropin which characterizes the onset of puberty in the rat. Particularly striking in this regard is the lack of effect of PMSG in enhancing P response to hCG. While PMSG on its own can increase P secretion *in vivo* (4) its lack of effect in facilitating the *in vitro* P response to hCG precludes the use of PMSG-treated rats as a model for the study of peripubertal ovarian P secretion. Acceleration of ovarian development by PMSG appears to alter the delicate control exerted on prepubertal ovarian function by changing pulsatile LH levels and rising PRL and GH titers (19). While PRL and perhaps GH enhance the P responsiveness to gonadotropins (11, 20) subtle changes in circulating LH levels (21, 22) appear to gradually increase the activity of the 17-20 lyase enzyme (23). This results in an increase in available aromatizable androgens which are then utilized in estrogen synthesis. The abrupt enhancement of both 17-20 lyase and aromatase activities induced by PMSG greatly augments the flow of precursors toward estrogen synthesis and this may result in a lower rate of P release in response to hCG. The marked increase in A response to hCG induced by PMSG may indicate a predominant release of T over that of 5α-reduced androgens (DHT and 3α-androstanediol), which are also detected by the antiserum to T used. This is likely because A response to hCG does not increase on the morning of first proestrus (1), but if the steroids are chromatographically separated both a drop in 3α-androstanediol and an increase in T response to hCG/LH are observed (2, 24). Moreover, PMSG is known to suppress Δ^4-5α-reductase activity in immature ovaries (17).

Although the overall changes in ovarian steroidogenesis that occur at puberty may not be reproduced by PMSG treatment the present findings indicate that, in agreement with earlier observations, the PMSG-treated rats may represent a particularly suitable model to study the control of peripubertal changes in estradiol secretion.

1. Advis JP, Andrews WW, Ojeda SR. Changes in ovarian steroidal and prostaglandin E responsiveness to gonadotropins during the onset of puberty in the female rat. Endocrinology **104**:653–658, 1979.
2. Uilenbroek J TH J, Woutersen PJA, van der Linden R. Steroid production in vitro by rat ovaries during sexual maturation. J Endocrinol **99**:469–475, 1983.
3. Sashida T, Johnson DC. The response of the immature rat ovary to gonadotrophins: Acute changes in cyclic AMP, progesterone, testosterone, androstenedione, and oestradiol after treatment with PMS or FSH + LH. Acta Endocrinol (Copenhagen) **82**:413–425.
4. Parker CR Jr, Costoff A, Muldoon TG, Mahesh VB. Actions of pregnant mare serum gonadotropin in the immature female rat: Correlative changes in blood steroids, gonadotropins, and cytoplasmic estradiol receptors of the anterior pituitary and hypothalamus. Endocrinology **98**:129–138, 1976.
5. Lerner N, Eckstein B. Changes in steroidogenesis in preovulatory rat ovaries induced to ovulate with pregnant mare serum gonadotropin. Endocrinology **103**:1039–1047, 1978.
6. Cole HH. Superfecundity in rats treated with mare gonadotropic hormone. Amer J Physiol **119**:704–712, 1937.
7. Zarrow MX, Quinn DL. Superovulation in the immature rat following treatment with PMS alone and inhibition of PMS-induced ovulation. J Endocrinol **26**:181–188, 1963.

8. Herlitz H, Koch Y, Khan MI, Ahren K. Effect of follicle stimulating hormone on cyclic AMP levels in young corpora lutea of the rat. Eur J Obstet Gynecol Reprod Biol **6**:175–179, 1976.
9. Hillensjo T, Barnea A, Nilsson L, Herlitz H, Ahren K. Temporal relationship between serum LH levels and oocyte maturation in prepubertal rats injected with pregnant mare's serum gonadotropin. Endocrinology **95**:1762–1766, 1974.
10. Ojeda SR, Wheaton JE, Jameson HE, McCann SM. The onset of puberty in the female rat. I: Changes in plasma prolactin, gonadotropin and LHRH levels, and hypothalamic LHRH content. Endocrinology **98**:630–638, 1976.
11. Advis JP, Ojeda SR. Hyperprolactinemia-induced precocious puberty in the female rat: Ovarian site of action. Endocrinology **103**:924–935, 1978.
12. Gay VL, Kerlan JT. Serum LH and FSH following passive immunization against circulating testosterone in the intact male rat and in orchidectomized rats bearing subcutaneous Silastic implants of testosterone. Arch Androl **1**:257–266.
13. Wang C, Hsueh AJW, Erickson GF. Induction of functional prolactin receptors by follicle-stimulating hormone in rat granulosa cells in vivo and in vitro. J Biol Chem **254**:11330–11336, 1979.
14. Eckstein B, Raaman M, Lerner N, Cohen S, Nimrod A. The appearance of 20α-hydroxysteroid dehydrogenase activity in preovulatory follicles of immature rats treated with pregnant mare serum gonadotropin. J Steroid Biochem **8**:213–216, 1977.
15. Goff AK, Henderson KM. Changes in follicular fluid and serum concentrations of steroids in PMS treated immature rats following LH administration. Biol Reprod **20**:1153–1157, 1979.
16. Eckstein B, Nimrod A. Effect of human chorionic gonadotropin and prolactin on 20α-hydroxysteroid dehydrogenase activity in granulosa cells of immature rat ovary. Endocrinology **104**:711–714, 1979.
17. Suzuki K, Kawakura K, Tamaoki B. Effect of pregnant mare's serum gonadotrophin on the activities of Δ^4-5α-reductase, aromatase, and other enzymes in the ovaries of immature rats. Endocrinology **102**:1595–1605, 1978.
18. Johnson DC, Hoverland RC. Oestradiol synthesis by granulosa cells from immature rats treated with pregnant mare's serum gonadotropin. Acta Endocrinol **104**:372–380, 1983.
19. Ojeda SR, Smith White S, Urbanski HF, Aguado LI. The onset of female puberty: Underlying neuroendocrine mechanisms. In: Muller EE, MacLeod RM, eds. Neuroendocrine Perspectives. Amsterdam, Elsevier, p225, 1984.
20. Advis JP, Smith White S, Ojeda SR. Activation of GH short loop negative feedback delays puberty in the female rat. Endocrinology **108**:1343–1352, 1981.
21. Andrews WW, Ojeda SR. A detailed analysis of the serum LH secretory profiles of conscious, free-moving female rats during the time of puberty. Endocrinology **109**:2032–2039, 1981.
22. Meijs-Roelofs HMA, Kramer P, Sander HJ. Changes in serum concentration of luteinizing hormone in the female rat approaching puberty. J Endocrinol **98**:241–249, 1983.
23. Bogovich K, Richards JS. Androgen biosynthesis in developing ovarian follicles: Evidence that luteinizing hormone regulates thecal 17α-hydroxylase and $C_{17\text{-}20}$-lyase activities. Endocrinology **111**:1201–1208, 1982.
24. Advis JP, Wiener SL, Ojeda SR. Changes in ovarian 3α-androstanediol response to human chorionic gonadotropin during puberty in the rat: Modulatory role of prolactin. Endocrinology **109**:223–228, 1981.

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