Corticosteroid Regulation of Gonadotropin Secretion and Induction of Ovulation in the Rat (43021)

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Abstract. **In the human polycystic ovarian syndrome, glucocorticoids have been demonstrated to have beneficial effects in inducing ovulation in a number of cases. These beneficial effects were assumed to be due to suppression of adrenal overproduction of androgens. However, the possibility exists that glucocorticoids may directly regulate gonadotropin secretion and thereby improve menstrual rhythm and ovulatory activity. Herein, we report that the corticoid, deoxycorticosterone, and the synthetic glucocorticoid, triamcinolone acetonide, like progesterone (P4), are able to induce luteinizing hormone and follicle-stimulating hormone surges and facilitate ovulation in the pregnant mare serum gonadotropin-primed rat. This effect is not shared by cortisol. Prolactin release was also stimulated by deoxycorticosterone, cortisol, and progesterone, but not by triamcinolone acetonide.**

Similar to progesterone, triamcinolone acetonide and deoxycorticosterone administration caused a loss of fluid retention in the uterus. This effect of triamcinolone acetonide and deoxycorticosterone may be related to progesterone action as opposed to anti-inflammatory action since cortisol had no effect on uterine fluid retention. These findings raise the possibility that the beneficial effects seen with glucocorticoids in inducing ovulation in polycystic ovarian syndrome may be due in part to their direct effects upon the release of gonadotropins. [P.S.E.B.M. 1990, Vol 193]

In human polycystic ovarian syndrome and other syndromes of androgen excess, there have been a number of reports of improvement in menstrual rhythm and ovulatory activity following glucocorticoid therapy (1–3). Since androgens have been shown to inhibit gonadotropin secretion and exert direct effects on the ovary causing follicular atresia and ovulatory failure (4, 5), it has been presumed that the beneficial effects of glucocorticoids were due to their ability to suppress adrenal overproduction of androgens.

However, the possibility exists that glucocorticoids may directly regulate gonadotropin secretion and thereby improve menstrual rhythm and ovulatory activity. Direct stimulatory effects on gonadotropin secretion by both cortisol and corticosterone have been demonstrated in pituitary cell culture studies (6–8). *In vivo* studies have reported variable and contradictory results indicating that the glucocorticoid effect is largely inhibitory to gonadotropin secretion (9–11). Recent studies in our laboratory have shown that in the estrogen-primed ovariectomized rat, the effect of synthetic and natural corticosteroids on gonadotropin secretion *in vivo* depends upon the corticosteroid used (12).

The objective of this study was to determine whether corticosteroid administration results in small changes in gonadotropin secretion or changes that are large enough to be biologically relevant such as induction of ovulation. For this purpose, the immature female rat administered 8 IU of pregnant mare serum gonadotropin (PMSG) was used. When PMSG was given to 28-day-old rats, it caused a preovulatory gonadotropin surge 52 to 56 hr after administration, resulting in ovulation (13). Progesterone administration 24 hr after PMSG advances the preovulatory gonadotropin surge and ovulation by 24 hr (14). The use of the PMSG-primed immature rat thus provided us the opportunity to compare the effects of several corticosteroids on the preovulatory gonadotropin surge and resultant ovulation with that induced by progesterone.

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Materials and Methods

Animals. Immature virus-free Holtzman (Madison, WI) rats were obtained at 26 days of age. They were maintained in air-conditioned rooms with a 14-

hr light:10-hr dark cycle (lights on 0500 hr; off at 1900 hr) and were given water and rat chow *ad libitum*. At 28 days of age the rats were injected subcutaneously with 8 IU of PMSG at 0800 hr. Twenty-four hours later, the animals received either progesterone, cortisol, triamcinolone acetonide, or deoxycorticosterone (all steroids from Steraloids, Wilton, NH) at a dose of 2 mg/rat (sc). The 2 mg/rat dose was chosen based on previous studies with progesterone demonstrating this dose to be optimal in facilitating gonadotropin secretion and ovulation in PMSG-primed immature rats (15). Control animals received vehicle (50% ethanol-saline). In the experiments measuring serum gonadotropin and prolactin levels, the animals were sacrificed 10 hr after steroid treatment (1800 hr). This time of sacrifice was chosen based on previous studies demonstrating that progesterone advances the gonadotropin surge in PMSG-primed immature rats by 24 hr (16). Trunk blood was collected and allowed to clot for 12 hr at 4°C, after which the blood was centrifuged at 2500 rpm for 30 min at 4°C and serum separated and stored at −20°C for subsequent radioimmunoassay of luteinizing hormone (LH), follicle-stimulating hormone (FSH), and prolactin (PRL). In the experiments to determine facilitation of ovulation, the animals were sacrificed via decapitation 24 hr after steroid treatment (0800 hr) and the oviducts were removed, cleaned of fat, mounted and pressed on a slide, and viewed under a microscope for the presence of ova (17). Additionally, the uteri were examined in these animals for the presence or absence of fluid accumulation.

Radioimmunoassay of LH, FSH, and PRL. The concentrations of LH, FSH, and PRL in serum samples were analyzed by a double-antibody radioimmunoassay method as described by Rao and Mahesh (18). The purified hormones and standards and the first antibody for LH (NIAMDD-rLH-S-10 [rabbit]), FSH (NIAMDD-rFSH-S-11 [rabbit]), and PRL (NIAMDD-rPRL-S-(9 [rabbit]) were obtained from NIDDK (National Hormone Pituitary Program). The purified hormone was iodinated with ^{125}I (Amersham, Arlington Heights, IL) by the chloramine-T method (19). The second antibody was purchased from Arnell Inc., Brooklyn, NY, and used at a 1/250 dilution. A 25% binding was obtained at 1/46,875 and 1/25,000 dilutions for LH and FSH antisera, respectively, and at 1/2,500 dilution of the PRL antisera. The assay was linear at 4–128 ng/tube for LH, 32–512 ng/tube for FSH, and 0.05–12.8 ng/tube for PRL. The intra- and interassay variabilities as determined by analysis of replicate serum pool samples were 9.6 and 12.4% for LH, 4.1 and 9% for FSH, and 7 and 11% for PRL. Hormone levels are expressed in terms of NIAMDD-RP-1 standard for LH and FSH and NIAMDD-RP-3 standard for PRL.

Statistical Analysis. The results given in the text are expressed as mean ± SE. Significance of difference was assessed by one-way analysis of variance and comparisons between treatment means were made by the Student-Newman-Keuls multirange test. $P < 0.05$ was considered significant.

Results

As shown in Figure 1, the PMSG-treated control group had basal LH levels. Progesterone, triamcinolone acetonide, and deoxycorticosterone treatment resulted in a significant increase in serum LH levels ($P < 0.05$, $P < 0.01$, and $P < 0.05$, respectively). The magnitude of the LH surges induced by progesterone, triamcinolone acetonide, and deoxycorticosterone were the same as shown by statistical analysis. Cortisol did not have any effect on serum LH levels. As shown in Figure 2, progesterone, triamcinolone acetonide, and deoxycorticosterone treatment also significantly elevated serum FSH levels ($P < 0.01$, $P < 0.01$ and $P < 0.05$, respectively), whereas cortisol treatment was without significant effect. The magnitude of the FSH surge induced by triamcinolone acetonide was statistically greater than that induced by progesterone and deoxycorticosterone

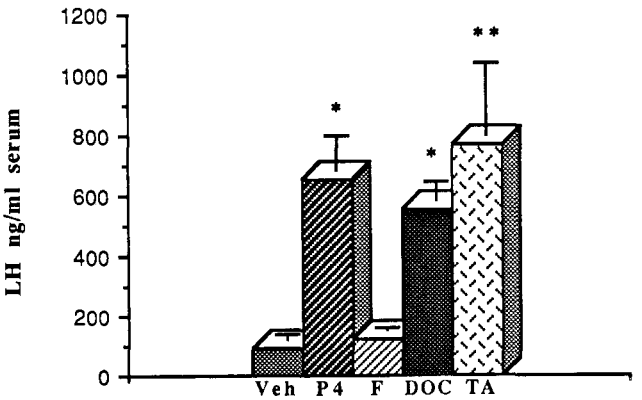

Figure 1. The effect of corticosteroids and progesterone on LH secretion in PMSG-primed immature rats. Twenty-eight-day-old female rats were administered PMSG (8 IU) at 0800 hr. Twenty-four hours later either vehicle (Veh), progesterone (P4), cortisol (F), deoxycorticosterone (DOC), or triamcinolone acetonide (TA) was administered at a dose of 2 mg/rat. Ten hours after steroid treatment (1800 hr), the animals were sacrificed for serum gonadotropin measurements ($n = 8$). $^{*}P < 0.05$, $^{**}P < 0.01$.

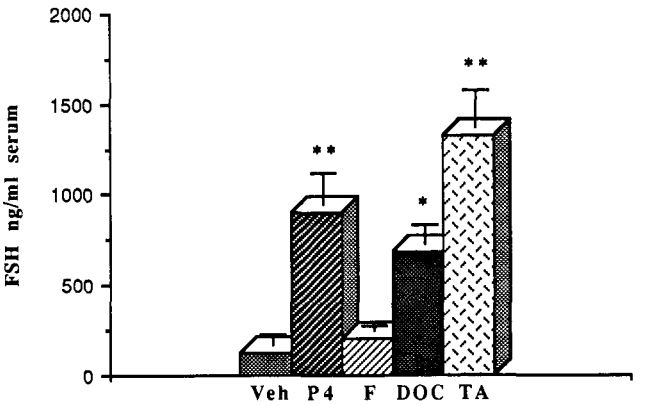

Figure 2. The effect of glucocorticoids and progesterone on FSH secretion in PMSG-primed immature rats. The model is the same as described in Figure 1 ($n = 8$). $^{*}P < 0.05$, $^{**}P < 0.01$.

($P < 0.05$ and $P < 0.01$, respectively). Deoxycorticosterone- and progesterone-induced FSH surges were not statistically different from each other. Progesterone, cortisol, and deoxycorticosterone treatment caused a significant increase in serum PRL levels ($P < 0.05$), whereas triamcinolone acetonide had no significant effect on serum PRL levels (Fig. 3). The magnitude of the PRL surges induced by progesterone, cortisol, and deoxycorticosterone were not statistically different.

To determine whether the immunoreactive gonadotropins released by corticosteroids were biologically active, the bioactivity of the corticosteroid-induced gonadotropin release was assessed using the induction of ovulation as a biologic end point.

As shown in Figure 4, only 25% of the control rats ovulated. Treatment with either progesterone or the synthetic glucocorticoid triamcinolone acetonide resulted in 100% ovulation ($P < 0.01$). Deoxycorticosterone treatment resulted in 88% ovulation ($P < 0.01$), whereas cortisol treatment had no significant effect on ovulation. In the control animals the average number of ova per rat was 2 ± 1.4, whereas in the progesterone-, triamcinolone acetonide-, and deoxycorticosterone-treated groups the average number of ova per rat was significantly increased to 9.5 ± 1.7 ($P < 0.01$), 10 ± 1.4 ($P < 0.01$), and 8 ± 1.6 ($P < 0$ .05), respectively. The number of ova per rat in the cortisol-treated group was not significantly different from the control group.

All of the control animals exhibited distended uteri on the morning of the ova count (Table I). Cortisol-treated animals also had distended uteri. However, progesterone-, deoxycorticosterone-, and triamcinolone acetonide-treated animals had nondistended uteri ($P < 0.01$).

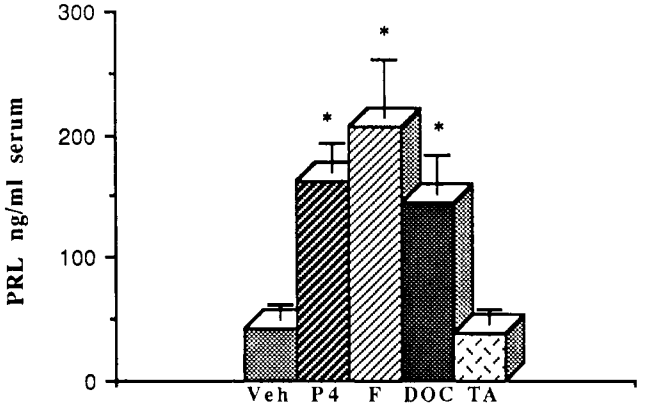

Figure 3. The effect of glucocorticoids and progesterone on PRL secretion in PMSG-primed immature rats. The model is the same as described in Figure 1 ($n = 8$). *$P < 0.05$.

Discussion

The findings in this study demonstrate that the natural corticoid, deoxycorticosterone, and the synthetic glucocorticoid, triamcinolone acetonide, are both able to facilitate gonadotropin secretion in PMSG-primed immature rats. These effects are comparable to those achieved with progesterone. In contrast, cortisol had no significant effect on gonadotropin secretion in the PMSG-primed immature rat. Cortisol did not have any effect on basal gonadotropin levels in intact male rats but was inhibitory in orchiectomized male rats (20). It would appear that elevated serum gonadotropin levels are necessary for the inhibitory effect of cortisol to be apparent. In support of this we have previously shown that cortisol inhibits gonadotropin secretion in the estrogen-primed ovariectomized female rat which has elevated gonadotropin levels as compared with the intact PMSG-primed rat used in this study (12). The divergent effects of deoxycorticosterone and triamcinolone acetonide, as compared with cortisol, may be explained in part on the basis of their ability to interact with the progesterone receptor. Competitive binding studies using the synthetic progestin, R5020, have demonstrated that triamcinolone acetonide and deoxycorticosterone can both bind the progesterone receptor (21, 22). Cortisol was ineffective in competing for the

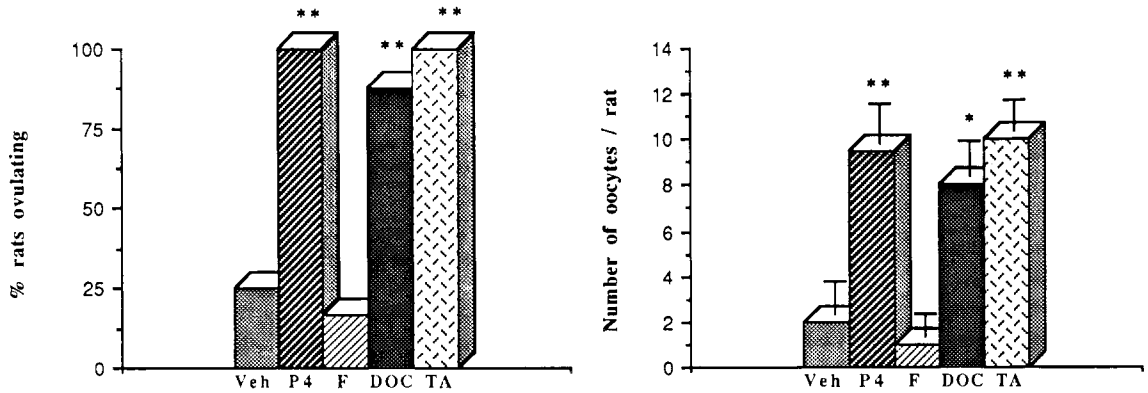

Figure 4. The effect of glucocorticoids and progesterone on facilitation of ovulation in PMSG-primed immature rats. The model is similar to that described in Figure 1, except that all animals were sacrificed 24 hr after steroid treatment (0800 hr) and the oviducts were examined for the presence of ova. The graph on the left demonstrates the effect of the glucocorticoids and progesterone on the percentage of rats ovulating, while the graph on the right demonstrates the effect of glucocorticoids and progesterone on the average number of ova per rat ($n = 8$). *$P < 0.05$, **$P < 0.01$.

Table I. Proportion of Rats with Distended Uteri on the Morning following Steroid Treatment

Treatment	No. of rats with distended uteri
Vehicle	12/12
Progesterone	0/12[a]
Deoxycorticosterone	0/12[a]
Triamcinolone acetonide	0/12[a]
Cortisol	10/10

[a] $P < 0.01$ versus vehicle controls.

progesterone receptor in these studies (21). It is well known that progesterone action requires estrogen priming, presumably for the induction of progesterone receptors (23). Preliminary work in our laboratory has demonstrated that triamcinolone acetonide and deoxycorticosterone can stimulate gonadotropin secretion in estrogen-primed ovariectomized immature rats (12). However, when estrogen priming is omitted these compounds lose their ability to stimulate gonadotropin secretion (12). Since, to our knowledge, glucocorticoid action has not been reported to be estrogen dependent, these findings reaffirm the suggestion that the effects of triamcinolone acetonide and deoxycorticosterone on gonadotropin secretion are progestin receptor mediated.

At the level of the uterus, triamcinolone acetonide and deoxycorticosterone were found to cause a loss of fluid accumulation. Accumulation of fluid in the uterus is known to be estrogen dependent (24) and progesterone can antagonize this estrogen action (25). Therefore, the effect of triamcinolone acetonide and deoxycorticosterone on decreasing uterine distention may be related to progesterone action. In arriving at this hypothesis, one cannot rule out the possibility that these effects are glucocorticoid receptor mediated. There have been several reports in the literature of glucocorticoid antagonism of estrogen action at the level of the uterus (26, 27). Additionally, the anti-inflammatory effects of glucocorticoids such as inhibition of capillary dilation and edema formation are well known (28, 29) and could also explain triamcinolone acetonide and deoxycorticosterone effects on uterine distention. However, the effects of triamcinolone acetonide and deoxycorticosterone may be more related to progesterone action as opposed to anti-inflammatory action of corticosteroids since cortisol had no effect on uterine fluid accumulation.

With respect to prolactin secretion, deoxycorticosterone and cortisol stimulated PRL secretion, as did progesterone. Interestingly, triamcinolone acetonide did not share this effect. In the estrogen-primed ovariectomized rat model, we have found that cortisol enhances the estrogen-induced PRl release, whereas triamcinolone acetonide inhibited the release at every dose tested (12). At present, the precise reason for these divergent effects is unclear.

Recent work has indicated that progesterone plays a significant role in the normal ovulatory process by enhancing the estrogen-triggered gonadotropin surge (18). In the appropriately estrogen-primed rat, progesterone by itself can initiate the gonadotropin surge (30). Therefore, progesterone-like activity of other steroids may be important for their effects on ovulation. The PMSG-primed animals treated with progesterone gave an equivalent complement of ova to those found in ovulating rats (5). Triamcinolone acetonide and deoxycorticosterone at equipotent doses to progesterone induced normal levels of ova found in rats. In view of the need for estrogen priming and the loss of uterine fluid similar to progesterone, triamcinolone acetonide and deoxycorticosterone exhibit progesterone-like activity in inducing ovulation. Finally, the absence of cortisol action on gonadotropin secretion was confirmed by the ovulation studies.

This article demonstrates that the naturally occurring mineralocorticoid, deoxycorticosterone, and the glucocorticoid, triamcinolone acetonide, are able to induce gonadotropin surges and facilitate ovulation in the PMSG-primed rat. This effect is not shared by cortisol. These findings raise the possibility that the beneficial effects seen with glucocorticoids in inducing ovulation in polycystic ovarian syndrome may be due in part to their direct effects upon the release of gonadotropins. In the past, cortisone, and more recently dexamethasone, has been used in polycystic ovarian syndrome to induce ovulation. Triamcinolone acetonide might prove to be particularly intriguing as a candidate for management of polycystic ovarian syndrome by virtue of its dual ability as a glucocorticoid to suppress ACTH secretion and thus reduce adrenal androgen production and as a progestin to facilitate gonadotropin secretion and ovulation.

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1. Greenblatt RB, Barfield WE, Lampros CO. Cortisone in the treatment of infertility. Fertil Steril **7**:203–211, 1956.
2. Smith KD, Steinberger E, Perloff WH. Polycystic ovarian disease. Am J Obstet Gynecol **93**:994–1001, 1965.
3. Jefferies W, Weir W, Weir D, Prouty R. The use of cortisone and related steroids in infertility. Fertil Steril **9**:145–158, 1958.
4. Martini L. The 5α-reduction of testosterone in the neuroendocrine structures: Biochemical and physiological implications. Endocr Rev **3**:1–25, 1982.
5. Bagnell CA, Mills TM, Costoff A, Mahesh VB. A model for the study of androgen effects on follicular atresia and ovulation. Biol Reprod **27**:903–914, 1982.
6. Kamel F, Kubajak C. Modulation of gonadotropin secretion by corticosterone: Interaction with gonadal steroids and mechanism of action. Endocrinology **121**:561–568, 1987.
7. Suter D, Schwartz N. Effects of glucocorticoids on secretion of luteinizing hormone and follicle-stimulating hormone by female rat pituitary cells *in vitro*. Endocrinology **117**:849–854, 1985.
8. Cohen IR, Mann DR. Influence of corticosterone on the response to gonadotropin-releasing hormone in rats. Neuroendocrinology **32**:1–6, 1981.

9. Smith ER, Johnson J, Weick RF, Levine S, Davidson JM. Inhibition of the reproductive system in immature rats by intracerebral implantation of cortisol. Neuroendocrinology **8**:94–106, 1971.
10. Ringstrom SJ, Schwartz NB. Cortisol suppresses the LH, but not the FSH, response to gonadotropin-releasing hormone after orchidectomy. Endocrinology **116**:472–474, 1985.
11. Li PS, Wagner WC. In vivo and in vitro studies on the effect of adrenocorticotropic hormone or cortisol on the pituitary response to gonadotropin releasing hormone. Biol Reprod **29**:25–31, 1983.
12. Brann DW, Putnam CD, Mahesh VB. Corticosteroid regulation of gonadotropin and prolactin secretion in the rat. Endocrinology **126**:159–166, 1990.
13. Parker C, 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.
14. Gallo RV, Zarrow MX. Effect of progesterone and other steroids on PMS-induced ovulation in the immature rat. Endocrinology **86**:296–300, 1970.
15. Ying SY, Meyer RD. Effect of progesterone on induced ovulation in immature rats. J Reprod Fertil **20**:279–285, 1969.
16. McCormack C, Meyer RK. Ovulation hormone release in gonadotropin-treated immature rats. Proc Soc Exp Biol Med **110**:343–346, 1962.
17. Burdick H, Whitney R. Ovulation induced in mice by single injection of follutein or untreated human pregnancy urine. Am J physiol **132**:405–410, 1941.
18. Rao IM, Mahesh VB. Role of progesterone in the modulation of the preovulatory surge of gonadotropins and ovulation in the PMSG primed immature rat and the adult rat. Biol Reprod **35**:1154–1161, 1986.
19. Bolton AE. Experimental protocols for the radioiodination of proteins and other compounds. In: Bolton AE, Ed. Radioiodination Techniques, Review 18. Arlington Heights, IL: Amersham/Searle Corp., p45, 1977.
20. Suter D, Schwartz N, Ringstrom S. Dual role of glucocorticoids in regulation of pituitary content and secretion of gonadotropins. Am J Physiol **254**:E595–E600, 1988.
21. McGuire WL, Horwitz KB, Pearson OH, Segaloff A. Current status of estrogen and progesterone receptors in breast cancer. Cancer **39**:2934–2947, 1977.
22. Booth BA, Colás AE. Binding interactions of progesterone and other C_{21} steroids with rat uterine cytosol. Gynecol Obstet Invest **6**:265–284, 1975.
23. Calderon J, Muldoon TM, Mahesh VB. Receptor-mediated interrelationships between progesterone and estradiol action on the anterior pituitary-hypothalamic axis of the ovariectomized rat. Endocrinology **120**:2428–2435, 1987.
24. Armstrong DT. Hormonal control of uterine lumen fluid retention in the rat. Am J Physiol **214**:754–771, 1968.
25. Kennedy TG, Armstrong DT. Loss of uterine luminal fluid in the rat: Relative importance of changing peripheral levels of estrogen and progesterone. Endocrinology **97**:1379–1385, 1975.
26. Campbell PS. The mechanism of the inhibition of uterotrophic responses by acute dexamethasone pretreatment. Endocrinology **103**:716–723, 1978.
27. Szego C, Davis J. Inhibitions of estrogen-induced cyclic AMP elevation in rat uterus. II. By glucocorticoids. Life Sci **8**:1109–1116, 1969.
28. Cline M, Mulmon K. Plasma kinins and cortisol: A possible explanation of the anti-inflammatory action of cortisol. Science **4**:1135–1137, 1966.
29. Pickup ME. Clinical pharmakinetics of prednisone and prednisolone. Clin Pharmacokinet **4**:111–116, 1979.
30. McPherson JC, Mahesh VB. Dose-related effect of a single injection of progesterone on gonadotropin secretion and pituitary sensitivity to LHRH in estrogen-primed castrated female rats. Biol Reprod **20**:763–772, 1979.