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# THE EFFECTS OF RU486 ON THE LUTEAL PHASE OF THE RHESUS MONKEY

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**Summary**—RU486 is a steroid which possesses great affinity for the progesterone (P) receptor, but which has no P activity. It has been shown to be, as a result, a potent P antagonist. In the present study, we investigated the effect of this compound on the luteal phase of the rhesus monkey.

The day of ovulation was diagnosed with a $\pm 12$ h accuracy, using serial laparoscopies and serum estradiol ($E_2$) determinations, in regularly cycling rhesus monkeys. RU486 was administered by gavage (10 mg daily) in different regimens during the luteal phase: Group 1, days 1–5; Group 2, days 5–9; Group 3, days 9–13; and Groups 4, days 9–13, plus hCG (30, 60, 90, 180 and 360 IU i.m. on days 6–10). RU486 induced vaginal bleeding within 24–72 h after the initial administration in Groups 1–3. Animals of Group 4 presented luteal lengths ranging from 9–12 days. Progesterone concentrations at the onset of vaginal bleeding were $2.1 \pm 0.3$, $4.9 \pm 0.6$, $2.6 \pm 0.4$ and $11.2 \pm 1.5$ ng/ml ($x \pm$ SEM) for animals of Groups 1–4, respectively. Serum follicle stimulating hormone (FSH), luteinizing hormone (LH), $E_2$ and P levels were not altered during treatment. The availability of a compound such as RU486, that consistently induces vaginal bleeding due to its action at the target level (endometrium) without affecting the hormonal events of the menstrual cycle, opens a new approach to post-coital and interceptive contraception.

## INTRODUCTION

In recent decades, special effort has been dedicated to the development of methods that will interfere with normal luteal function, in order to induce a post-ovulatory form of contraception. Both basic and clinical research have focused on altering the function of the corpus luteum by inducing a deficiency of alleged leuteotropic substances, or by administering substances having luteolytic activity.

Various approaches directed toward neutralizing the secretion and/or activity of endogenous luteinizing hormone (LH) were experimentally developed and proved to be successful in inducing luteolysis [1–3]. However, recent information has cast doubt upon the need for LH in the maintenance of luteal function in primates and, therefore, on the above-mentioned approaches to inducing luteal insufficiency [4, 5].

Other approaches have been based on the administration of substances that inhibit luteal steroidogenesis, thus inducing progesterone (P) deficiencies and the subsequent early onset of mensus [6–8]. Prostaglandins have also been used to induce luteolysis in several animal species, including human and non-human primates [9–12]. However, neither of these last two approaches have proved to be a consistent, reproducible method of contraception, free of major side effects [13, 14].

More recently, a new method of luteal contraception, based on the interference of P activity at the endometrium by utilizing antiprogestogen substances with competitive binding to the P receptor, has been theorized [15]. The present study reports our experiences on the effects of a new synthetic antiprogestrogen steroid, 17$\beta$-hydroxy-11$\beta$-[4-dimethylaminophenyl]-17$\alpha$-[1-propynyl]estra-4,9-dien-3-one (RU486), on the luteal phase of the rhesus monkey [16].

## EXPERIMENTAL

Sexually adult female Rhesus monkeys (*Macaca mulatta*) experiencing regular menstrual cycles were selected for this study. The animals were individually caged and exposed to centrally controlled temperature ($23 \pm 1$°C), humidity (20%) and light–dark photoperiod (0600–2000–0600). Details on housing, feeding and general husbandry practices have been described elsewhere [4].

Ovulation was detected with an accuracy of $\pm 12$ h, using daily serum estradiol ($E_2$) levels and serial laparoscopies, as described previously (Fig. 1). [17]. Both blood drawing and surgical procedures were performed under sedation induced with ketamine HCl (5–7 mg/kg; Vetalar®, Parke–Davis, Morris Plains, NJ).

From the day of ovulation, blood (3 ml) was drawn on a daily basis (0800) from a femoral or saphenous vein for up to 17 days. Blood was centrifuged and the serum stored at −20°C until determination of follicle stimulating hormone (FSH), LH, $E_2$ and progesterone (P) by radioimmunoassays (RIAs) described previously [17, 18]. All samples were assayed

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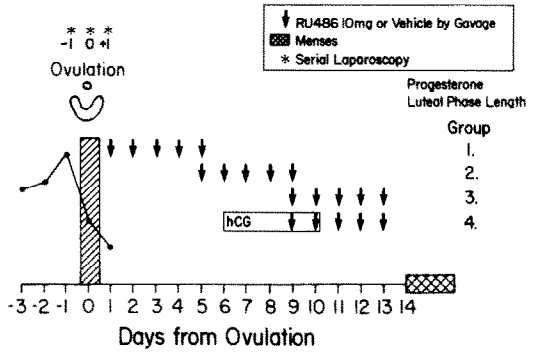


Fig. 1. Experimental design. Regularly cycling rhesus monkeys were divided into groups according to when RU486 or vehicle (controls) was administered. Ovulation was diagnosed using daily serum estradiol measurements (●——●) and serial laparoscopies.

in duplicate and in only one assay for each hormone to reduce variability in the determinations. Assay sensitivity was 800 ng/ml, 210 ng/ml, 20 pg/ml and 0.2 ng/ml for FSH, LH, $E_2$ and P, respectively. Intra-assay coefficients of correlation at a 70% maximum binding rate were 2.8, 5.3, 8.4 and 3.7% for FSH, LH, $E_2$ and P, respectively.

Animals were divided into four groups ($n = 5$/group) according to the schedule of administration of RU486, or RU486 plus human chorionic gonadotropin (hCG). RU486 was administered by gavage in daily doses of 10 mg in the following schedule (Fig. 1):

Group 1—From day 1 to day 5, postovulatory.
Group 2—From day 5 to day 9, postovulatory.
Group 3—From day 9 to day 13, postovulatory.
Group 4—From day 9 to day 13, postovulatory, plus increasing doses of hCG (30, 60, 90, 180 and 360 IU i.m. from day 6 to day 10, postovulatory) that mimic the early secretion of macaque chorionic gonadotropin (mCG) in the rhesus monkey gestation [19, 20].

Each group was compared to a control consisting of animals ($n = 5$/group) treated with vehicle (lactose) instead of RU486 in an experimental protocol identical to that explained above. Onset and duration of vaginal bleeding were determined by daily vaginal swabbings.

Comparison among groups in terms of length of the luteal phase and of hormone levels was carried out by Student's $t$-test and analysis of variance. Differences were considered statistically significant when less than $P = 0.05$.

## RESULTS

Table 1 shows the length of the luteal phase in animals from Groups 1 to 4 (treated with either vehicle only, RU486, or RU486 plus hCG). It is clear that, whereas vehicle appears to have no effect on the normal length of the luteal phase, the administration of RU486 significantly induced early onset of menses in all groups tested. Furthermore, the earlier in the cycle RU486 was administered, the earlier the onset of vaginal bleeding.

Table 1. Length of the luteal phase (days post-ovulation until the onset of vaginal bleeding $\bar{x} \pm$ SEM) in animals of Groups 1–4 treated with either vehicle or RU486

| | Vehicle | RU486 | $P$ |
|---|---|---|---|
| Group 1 | $13 \pm 2.0$ | $4 \pm 1.2$ | $<0.001$ |
| Group 2 | $14 \pm 1.5$ | $8 \pm 0.6$ | $<0.001$ |
| Group 3 | $15 \pm 2.1$ | $10 \pm 1.1$ | $<0.05$ |
| Group 4 | $17 \pm 3.3$ | $11 \pm 1.3$ | $<0.001$ |

Figures 2–4 show the hormone levels in animals of all groups, comparing the RU486-treated monkeys with the control animals. No significant changes were observed for FSH, LH, $E_2$ or P. Serum P concentrations at the onset of vaginal bleeding induced by RU486 ($\bar{x} \pm$ SEM) were $2.1 \pm 0.3$, $4.9 \pm 0.6$, $2.6 \pm 0.4$ and $11.2 \pm 1.5$ ng/ml for Groups 1–4, respectively.

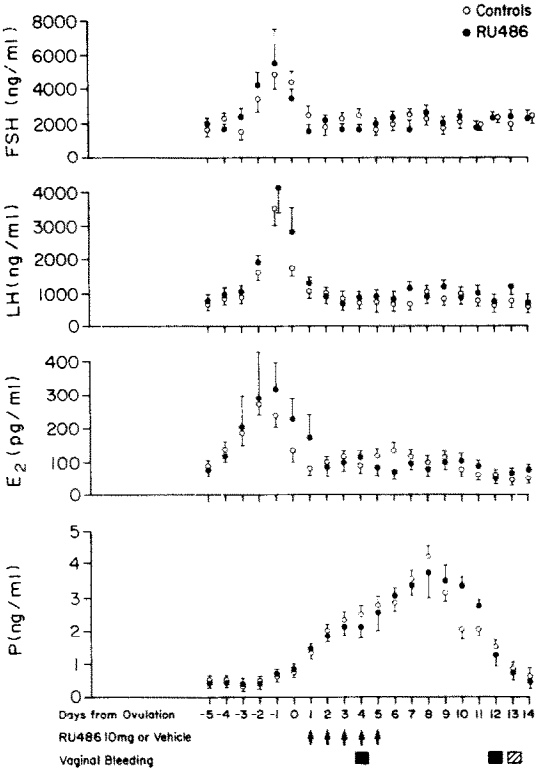


Fig. 2. Serum concentrations of FSH, LH, estradiol ($E_2$) and progesterone (P) in animals from Group 1 that received either RU486 or vehicle (controls) from day 1–5 post-ovulation. Onset of vaginal bleeding is depicted by ▨ in vehicle-treated animals and by ■ in RU486-treated animals.

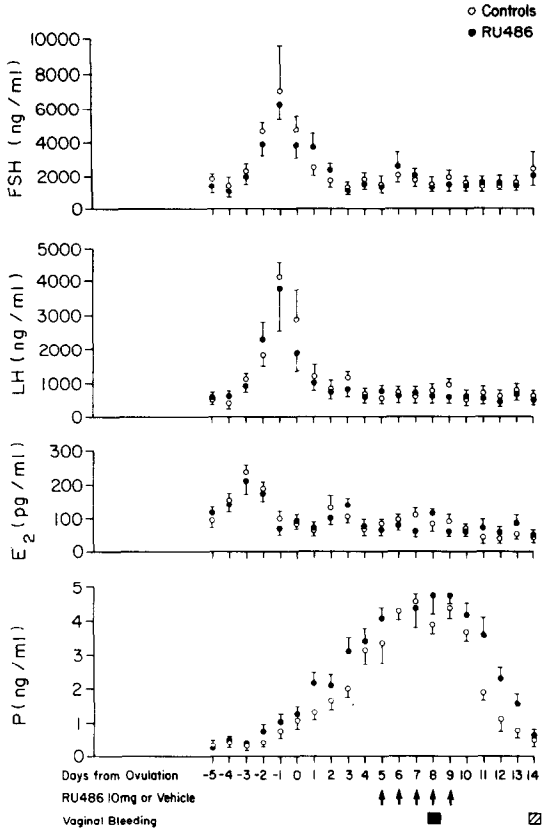


Fig. 3. Serum concentrations of FSH, LH, estradiol ($E_2$) and progesterone (P) in animals from Group 2 that received either RU486 or vehicle (controls) from day 5–9 post-ovulation. Onset of vaginal bleeding is depicted by ▨ in vehicle-treated animals and by ■ in RU486-treated animals.

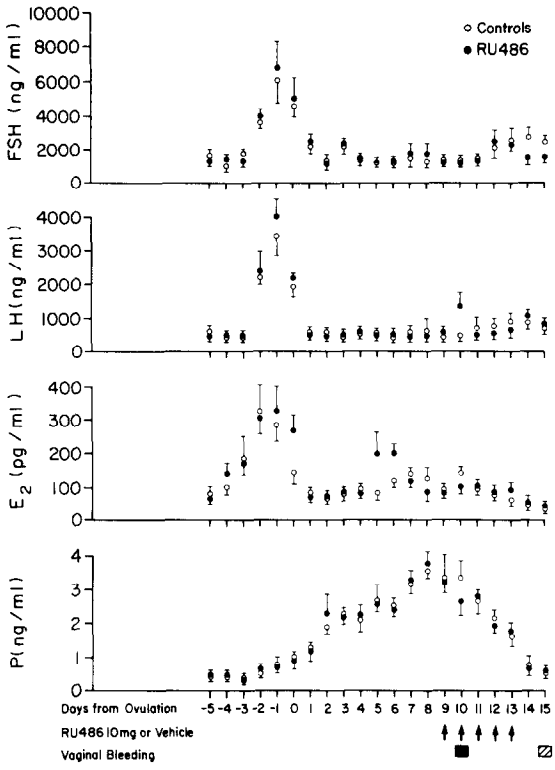


Fig. 4. Serum concentrations of FSH, LH, estradiol ($E_2$) and progesterone (P) in animals from Group 3 that received either RU486 or vehicle (controls) from day 9–13 post-ovulation. Onset of vaginal bleeding is depicted by ▨ in vehicle-treated animals and by ■ in RU486-treated animals.

In addition, Figs 2–4 show the average onset of vaginal bleeding for each group of animals. Of interest is that animals of Group 1 began to bleed approx 72 h after the initial dose of RU486, and continued to bleed for approx 2 days. After the cessation of bleeding, they started experiencing vaginal bleeding again on approximately day 12 of the postovulatory period. Animals of Groups 2–4 presented vaginal bleeding in an average of 72, 24 and 48 h, respectively, after the initiation of RU486 administration.

Figure 5 shows the serum P level in animals that received either RU486 or vehicle from day 9 to day 13, postovulation, in addition to increasing doses of hCG from day 6 to day 10, postovulation. It is clear that, even in the presence of hCG, RU486 was able to induce early onset of vaginal bleeding, despite high serum P levels. In addition, serum P levels did not differ among groups, suggesting a lack of effect of RU486 at the luteal level.

No side effects (e.g. loss of hair, vomiting, diarrhea, hypotension or changes in body weight, appetite or daily activity) were observed in any of the animals during drug administration.

## DISCUSSION

RU486 is an 11$\beta$-substituted 19-norsteroid synthesized from 3,3-ethylene-dioxy-estra(10),9(11)-dien-17-one, that displays potent antiprogesterone properties devoid of any agonist effects [16]. The

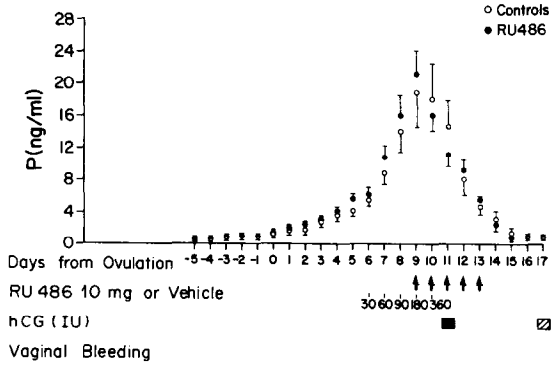


Fig. 5. Serum progesterone (P) concentrations in animals of Group 4 that received either RU486 or vehicle (controls) from day 9–13 postovulation and increasing doses of human chorionic gonadotropin (hCG) from day 6–10 post-ovulation. Onset of vaginal bleeding is depicted by ▨ in vehicle-treated animals and by ■ in RU486-treated animals.

present study reveals that RU486 consistently induced early onset of vaginal bleeding when administered during the luteal phase of regularly cycling adult rhesus monkeys. These experiments confirm and extend those of Healy *et al.* in another nonhuman species, cynomologus monkeys [21]. These authors administered RU486 to castrated, estrogen- and/or P-treated animals and observed consistently-induced vaginal bleeding within 48 h after drug administration.

Similarly, Herrmann and co-workers demonstrated that, in humans, oral administration of RU486 induced interruption of the luteal phase of the nonfertile menstrual cycle [15].

Our results conflict with those of Herrmann *et al.*[15], since serum concentrations of FSH and LH were not affected by drug administration, as compared to levels observed in vehicle-treated monkeys. The findings of this study suggest for the first time that RU486 exclusively exerts its effects on the luteal phase by a local action upon the endometrium, antagonizing the biological role of progesterone at the receptor level.

More importantly, the results of this study show that the administration of RU486 can induce early onset of vaginal bleeding in subjects that simultaneously receive doses of hCG that mimic early secretion of chorionic gonadotropin in the rhesus monkey. These findings, which confirm earlier observations of Hermann *et al.* in the early pregnancy of women, encourage the possible use of this antiprogestogen as a postcoital method of fertility control.

In summary, the present data reveal that the availability of a compound such as RU486, that consistently induces early onset of vaginal bleeding due to its action at the target level without affecting the hormonal events of the menstrual cycle or inducing major untoward effects, opens a new approach to post-coital and interceptive contraception.

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## REFERENCES

1. Moudgal N. R., MacDonald G. J. and Greep R. O.: Role of endogenous primate LH in maintaining corpus luteum function in the monkey. *J. clin. Endocr. Metab.* **35** (1972) 113–116.
2. Casper R. F. and Yen S. S. C.: Induction of luteolysis in the human with a long-acting analog of luteinizing hormone-releasing factor. *Science* **205** (1979) 408–410.
3. Asch R. H., Siler-Khodr T. M., Smith C. G. and Schally A. V.: Luteolytic effect of D-Trp$^6$-H-RH in the rhesus monkey (*Macaca mulatta*). *J. clin. Endocr. Metab.* **42** (1981) 565–571.
4. Asch R. H., Abou-Samra M., Braunstein G. D. and Pauerstein C. J.: Luteal function in hypophysectomized rhesus monkeys. *J. clin. Endocr. Metab.* **55** (1982) 154–161.
5. Balmaceda J. P., Borghi M. R., Coy D. H., Schally A. V. and Asch R. H.: Suppression of postovulatory gonadotropin levels does not affect corpus luteum function in rhesus monkeys. *J. clin. Endocr. Metab.* **57** (1983) 866–868.
6. Asch R. H., Fernandez E. O., Siler-Khodr T. M., Bartke A. and Pauerstein C. J.: Mechanism of induction of luteal phase defects by danazol. *Am. J. Obstet. Gynec.* **136** (1980) 932–937.
7. Schane H. P., Creange J. E., Anzalone A. J. and Potts G. O.: Interceptive activity of azastene in rhesus monkeys. *Fert. Steril.* **30** (1978) 343–347.
8. Asch R. H., Smith C. G., Siler-Khodr T. M. and Bartke A.: Luteolytic effect of azastene in the nonhuman primate. *Obstet. Gynec.* **59** (1982) 303–308.
9. Kirton K. T., Pharriss B. B. and Forbes A. D.: Luteolytic effects of prostaglandin $F_{2\alpha}$ in primates. *Proc. Soc. exp. Biol. Med.* **133** (1970) 314–316.
10. Balmaceda J. P., Valenzuela G. V., Eddy C. A. and Asch R. H.: Prostaglandin production by rhesus monkey corpora lutea *in vitro*: effects of estrogen administration. *Int. J. Gynec. Obstet.* **18** (1980) 15–18.
11. Auletta F. J., Agins H. and Scommegna A.: Prostaglandin F mediation of the inhibitory effect of estrogen on the corpus luteum of the rhesus monkey. *Endocrinology* **103** (1978) 1183–1189.
12. Butler R., Hotchkiss J. and Knobil E.: Functional luteolysis in the rhesus monkey: ovarian estrogen and progesterone during the luteal phase of the menstrual cycle. *Endocrinology* **96** (1975) 1509–1512.
13. Jones G. S. and Wentz A. C.: The effect of prostaglandin $E_{2\alpha}$ infusion on corpus luteum function. *Am. J. Obstet. Gynec.* **114** (1972) 393–401.
14. Lyneham R. C., Korda A. R., Shutt D. A., Smith I. D. and Shearman R. P.: The effect of intra-uterine prostaglandin $F_{2\alpha}$ on corpus luteum function in the human. *Prostaglandins* **9** (1975) 431–442.
15. Herrmann W., Wyss R., Riondel A., Philibert D., Teutsch G., Sakiz E. and Baulieu E.-E.: The effects of an antiprogesterone steroid on women: interruption of the menstrual cycle and of early pregnancy. *C.r. hebd. Séanc. Acad. Sci., Paris* (III) **294** (1982) 933–938.
16. Philibert D., Deraedt R., Tournemine C., Mary I. and Teutsch G.: RU 38486—A potent antiprogesterone. 6th International Congress of Hormonal Steroids, Jerusalem. *J. steroid Biochem.* **17** (1982) lxviii (Abstr. 204).
17. Pauerstein C. J., Eddy C., Croxatto H. D., Hess R., Siler-Khodr T. M. and Croxatto H. B.: Temporal relationships of estrogen, progesterone and luteinizing hormone levels to ovulation in women and infrahuman primates. *Am. J. Obstet. Gynec.* **130** (1978) 876–884.
18. Asch R. H., Balmaceda J. P., Eddy C. A., Siler-Khodr T. M., Coy D. H. and Schally A. V.: Inhibition of the postcastration rise of LH and FSH in female rhesus monkeys (*Macaca mulatta*) by the administration of LH-RH inhibitory analogue ([N-Ac-D-Trp$^{1,3}$,D-p Cl-phe$^2$,D-Phe$^6$,D-Ala$^{10}$]-LH-RH). *Fert. Steril.* **36** (1981) 388–391.
19. Wilks J. W., Noble A. S., Forbes A. D. and Forbes K. K.: Steroidogenic responsiveness of the monkey corpus luteum to chorionic gonadotropin. *Program for the 10th Annual Meeting of the Society for the Study of Reproduction*, Austin, Texas, p. 19 (Abstract No. 8), 1977.
20. Asch R. H., Smith C. G., Siler-Khodr T. M. and Pauerstein C. J.: Effects of $\Delta^9$tetrahydrocannabinol administration on gonadal steroidogenic activity *in vivo*. *Fert. Steril.* **32** (1979) 576–582.
21. Healy D. L., Baulieu E.-E. and Hodgen G. D.: Induction of menstruation by an antiproesterone steroid (RU486) in primates: site of action, dose-response relationships, and hormonal effects. *Fert. Steril.* **40** (1983) 253–257.