Blockade of Ovulation in Rabbits by RU-486, a Competitive Progesterone Antagonist

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(Received 14 December 1994/Accepted 8 November 1995)

ABSTRACT. The effect of an anti-progesterone compound, RU-486, administration (20–150 mg) on ovulation in rabbits was investigated. All of the seven control oil-treated rabbits ovulated, whereas, ovulation was blocked in one (12.5%) of eight rabbits treated with 20 mg RU-486, and in 4–6 (66.7–85.7%) of the 6 or 7 rabbits treated with 40–150 mg RU-486. Two successive injections of 10 mg progesterone (P) 4 and 8 hr after RU-486 injection did however recover ovulation in 3 (75.0%) out of 4 rabbits. These results indicate that P secreted from mature follicles during the ovulatory LH surge also play a significant role in follicular rupture of the rabbit, a copulatory ovulator.

— KEY WORDS: anti-progesterone, ovulation, RU-486.

J. Vet. Med. Sci. 58(3): 275–276, 1996

Successive injections of progesterone restore ovulation in acutely hypophysectomized proestrous rats [11]. It has also been reported that administration of anti-progesterone serum inhibits ovulation in immature rats treated with combined PMSG and hCG, and furthermore, that progesterone injected in combination with anti-progesterone serum eliminates the effect of the antiserum [6]. These findings indicate that progesterone secreted from mature follicles during the ovulatory LH surge plays a significant facilitative role, as an autocrine or paracrine effector, in the ovulation process of rats.

Recently, RU-486 (17 β -hydroxy-11 β -(4-dimethylaminophenyl)-17 α -(1-propynyl) oestra-4,9-dien-3-one) has been produced as an anti-progesterone compound. This synthetic steroid binds to progesterone receptors and acts as a competitive antagonist of progesterone [8, 10]. The compound has been reported to prevent implantation [5, 9] and to terminate pregnancy [3, 12]. The authors have already demonstrated that RU-486 has inhibitory effects on ovulation induced in mice [4], and in the present study, the effect of RU-486 administration on ovulation is examined in the rabbit, a copulatory ovulator.

Mature female Japanese white rabbits were purchased from Saitama Experimental Animal Supply Co., Ltd., Saitama, Japan. Rabbits weighing 2.8 to 3.2 kg were selected for the present study. These animals were housed individually in wire netting cages (68 × 82 × 70 cm; R3 type, Okazaki Sangyo Co., Ltd., Saitama, Japan) and kept in an air-conditioned room (23 ± 2°C; 55 ± 5% relative humidity) that was artificially illuminated, daily from 05:00 to 19:00 with 300 lux daylight fluorescent lamps. The rabbits were fed commercial pellet chow (Labo R Stock; Nihon Nosan Kogyo Co., Ltd., Yokohama, Japan) and tap water *ad libitum*. Prior to the experiments, each animal was isolated in a separate cage for at least 30 days.

An ethanol solution of RU-486 (Roussel Uclaf, Paris, France) was prepared at an appropriate concentration and mixed with sesame oil (Miyazawa Yakuhin Co., Ltd.,

Tokyo, Japan). The mixed solution was then placed in a boiling water bath, in order to remove the ethanol by evaporation, thus dissolving RU-486 in sesame oil at concentrations of 20, 40, 80, 100 and 150 mg per 7.5 ml.

Each female rabbit was given a single subcutaneous injection of 7.5 ml of sesame oil alone, or RU-486 (20–150 mg), just after copulation with proven fertile, mature males. The animals were euthanized by an intravenous overdose of pentobarbital sodium, Nembutal (Abbott Laboratories, North Chicago, IL, U.S.A.), 30 hr after treatment, and the number of ovulation points was counted in both ovaries. Oocytes released into the oviduct were recovered by perfusing the excised oviduct with TCM-199 medium (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan), containing 40% inactivated normal rabbit serum. The oocytes recovered from the oviducts were examined for their number and developmental stage under a stereoscopic microscope. Another group of copulating female rabbits, treated with 40 mg RU-486, were given an intramuscular injection of 10 mg progesterone (Luteogen; Sankyo Zoki Co., Ltd., Tokyo, Japan), 4 and 8 hr after the RU-486 administration and euthanized by Nembutal 30 hr after the injection of the progesterone antagonist.

Rabbits ovulate about 10 hr after copulation [2], and since the rabbits in this study were injected with RU-486 immediately after copulation, the RU-486 administration preceded ovulation by approximately 10 hr.

As shown in Table 1, all of the 7 control oil-treated rabbits ovulated. The mean numbers of ovulation points and tubal oocytes, of the control ovulating rabbits, were 9.1 and 8.4, respectively. Ovulation was blocked in 1 (12.5%) of the 8 rabbits treated with 20 mg RU-486, and in 4–6 (66.7–85.7%) of the 6 or 7 animals treated with the 40–150 mg RU-486, but was blocked in only 1 (25.0%) of the 4 rabbits treated with 40 mg RU-486 in combination with progesterone.

It has been reported that follicular protease activity increases during the preovulatory stage [1]. Additionally, it has also been indicated that ovarian follicular plasminogen activator activity, measured during the preovulatory LH surge, decreases when progesterone production is inhibited by the administration of cyanoketone, an inhibitor of 3 β -

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Table 1. The effect of RU-486 administration on ovulation in rabbits

Dose of RU-486 (mg)*	No. of rabbits examined	No. of ovulating rabbits	Total no. of ovulation points	Total no. of oocytes recovered	Total no. of oocytes fertilized	% of oocytes fertilized
0 (oil control)	7	7 (0)	64 [9.1 ± 1.2]	59 [8.4 ± 1.1]	55 <7.9 ± 0.9>	93.2
20	8	7 (1)	71 [10.1 ± 1.3]	61 [8.7 ± 1.2]	56 <8.0 ± 1.0>	91.8
40	7	1 (6)	9 [9]	9 [9]	9 <9>	100.0
60	6	1 (5)	12 [12]	9 [9]	9 <9>	100.0
80	6	2 (4)	13 [4, 9]	13 [4, 9]	11 <4, 7>	84.6
100	6	2 (4)	7 [3, 4]	7 [3, 4]	7 <3, 4>	100.0
150	6	1 (5)	7 [7]	7 [7]	7 <7>	100.0
40 + P**	4	3 (1)	34 [11.3 ± 0.9]	34 [11.3 ± 0.9]	34 <11.3 ± 0.9>	100.0

* Each rabbit was given a single subcutaneous injection of sesame oil or RU-486 (20–150 mg) immediately after copulation with proven fertile male rabbits.

** Each rabbit was given an intramuscular injection of 10 mg progesterone 4 and 8 hr after the injection of 40 mg RU-486.

( ) No. of unovulating rabbits

[ ] Mean no. of ovulation points per ovulating rabbit and standard error, or no. of ovulation points in individual ovulating rabbits

[ ] Mean no. of oocytes recovered per ovulating rabbit and standard error, or no. of oocytes recovered in individual ovulating rabbits

< > Mean no. of oocytes fertilized per ovulating rabbit and standard error, or no. of oocytes fertilized in individual ovulating rabbits

hydroxysteroid dehydrogenase [7]. In the present study with the rabbit, administration of RU-486, a competitive progesterone antagonist, right after copulation blocked ovulation in a dose-dependent fashion, and administration of a sufficient amount (40–150 mg) of RU-486 inhibited ovulation in the majority (66.7–85.7%) of the animals. Two successive injections of progesterone in combination with RU-486 did however result in an almost complete restoration of ovulation. It is, therefore, most plausible that the preovulatory increase in progesterone secretion, caused by the ovulatory LH surge, activates the follicular enzymes, including protease and plasminogen activator, and is causally related to the follicular rupture at the final step of the ovulatory process.

In the present study, the mean numbers of ovulation points and tubal oocytes per ovulating rabbit in RU-486-treated groups were nearly comparable to those in the control oil-treated group, although those values for the group treated with 100 mg RU-486 tended to be less. This finding might suggest that the progesterone antagonist prevents follicular rupture in an all-or-none fashion.

The tubal oocytes recovered 30 hr after copulation were morphologically normal in all RU-486-treated groups. Most (84.6–100%) of them were fertilized, and were at either 2-cell or 4-cell stage with the majority being at 4-cell stage. No differences in the developmental stage of the fertilized oocytes were evident among the groups. It thus appears that RU-486 has no adverse effects on the fertilization process in the oviduct.

ACKNOWLEDGEMENT. The authors wish to thank Roussel Uclaf, Paris, France, for supplying RU-486 for research use.

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