POSTCOITAL CONTRACEPTIVE EFFECTS OF MEDROXYPROGESTERONE ACETATE AND OESTRONE IN RATS

Z. DICKMANN

*Department of Gynecology and Obstetrics,
University of Kansas Medical Center,
Kansas City, Kansas 66103, U.S.A.*

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Summary. Pregnant rats were injected subcutaneously with either 1 μ g oestrone, 12.5 mg Depo-medroxyprogesterone acetate (MPA), or both. Oestrone injected on Day 3 had no effect on pregnancy. Injection of MPA on Day 1 caused delayed implantation, which was abolished when, in addition, oestrone was injected on Day 4. Injection of MPA on Day 1 followed by oestrone on Day 3 destroyed the eggs during the morula–blastocyst transformation period. The contraceptive effect of the latter treatment suggests a general guide-line for postcoital contraceptive trials in women.

INTRODUCTION

Knowledge that treatment with hormone A results in response a, and treatment with hormone B results in response b, is not an adequate basis for predicting the response that would result from simultaneous treatment with hormones A and B. The present study reports the fate of fertilized eggs in rats treated with medroxyprogesterone acetate (MPA) on Day 1 of pregnancy and oestrone on Day 3. The outcome of this treatment was not possible to predict on the basis of results obtained from pregnant rats treated with either MPA or oestrone alone, or MPA on Day 1 and oestrone on Day 4.

MATERIALS AND METHODS

Adult virgin female rats of the Holtzman strain, weighing 180 to 220 g, were mated with males of proven fertility. The morning of finding spermatozoa in the vagina was designated Day 1 of pregnancy. Mated females were assigned to various experimental groups, and were injected subcutaneously with (a) 1 μ g oestrone/0.1 ml sesame oil (Sigma, St. Louis), (b) 12.5 mg 6 α -methyl-17 α -hydroxyprogesterone acetate (Depo-MPA, Upjohn, Kalamazoo) suspended in 2 ml saline and injected into four subcutaneous sites, i.e. 0.5 ml/site, (c) oestrone and MPA. The treatment schedules are indicated in the 'Results' section and in Table 1. The rats were killed on various days of pregnancy (Table 1) to determine the fate of the fertilized eggs.

Table 1. The fate of fertilized eggs in rats injected with depo-MPA and oestrone

Exp. no.	No. of rats	Rats killed		No. of rats with fetuses	No. of fetuses		No. of resorption sites	
		Day	Hour		Total	Mean $\pm$ S.E.	Total	Mean $\pm$ S.E.
3	20	20	10.00	20	150	7.50 $\pm$ 0.75	21	1.05 $\pm$ 0.36
4	18	20	10.00	3	6	0.33 $\pm$ 0.22	7	0.38 $\pm$ 0.28
5	12	8	10.00	No. of rats with implantations 2	No. of implantations			
					Total	Mean $\pm$ S.E.	Total	Mean $\pm$ S.E.
					6	0.50 $\pm$ 0.41		
6	13	4	11.00	No. of rats with eggs 12	No. of eggs recovered			
					Total	Mean $\pm$ S.E.	Morula (% of total)	Degenerated (% of total)
					127	9.77 $\pm$ 1.11	112 (88)	15 (12)
					86	6.17 $\pm$ 1.05	50 (58)	36 (42)
					1	—	—	1 (100)
9*	13	5	10.00	1	6	—	—	—
								6

Depo-MPA (12.5 mg) was injected subcutaneously on Day 1 of pregnancy. Oestrone was injected on Day 4 (16.00 hours) in Exp. 3, and on Day 3 (16.00 hours) in all the other experiments.

* On the morning of Day 4, the cervical ends of the uteri were ligated to prevent possible expulsion of the eggs.

RESULTS

Experiment 1

Nine mated rats were injected with 1 μ g oestrone on Day 3 at 16.00 hours, and were killed on Day 18 when the numbers of fetuses and resorption sites (RS—sites where blastocysts had implanted, but sometime later the embryos had died and been resorbed) were recorded. The oestrone injection had no effect on pregnancy: the average number of fetuses was 10.75 ± 0.90 and that of RS, 0.75 ± 0.49 , figures which are within the normal range of untreated controls.

Experiment 2

Twenty mated rats were injected with 12.5 mg MPA on Day 1 at 08.30 hours, and were killed on Day 20, when the uteri were inspected. Two rats were not pregnant. In the remaining eighteen rats, there was delay of implantation which ranged from 1 to 15 days (as determined by the size of the conceptus or the weight of the embryo). In eight of the eighteen rats, implantation had apparently not started, since no implantations were seen, and flushing the uteri yielded blastocysts.

The results of Experiments 3 to 9 are recorded in Table 1.

Experiment 3

Twenty mated rats were injected with 12.5 mg MPA on Day 1 at 08.30 hours (as in Exp. 2) and 1 μ g oestrone on Day 4 at 16.00 hours. The rats were killed on Day 20. The oestrone treatment induced implantation at the normal time, thus abolishing the MPA effect: none of the rats showed delayed implantation.

There was a reduction in the average number of fetuses (7.5 ± 0.75), resulting from the low yield in some of the females.

Experiment 4

Eighteen mated rats were treated as in Exp. 3, but oestrone was injected on Day 3 at 16.00 hours. The rats were killed on Day 20 and only three of them had fetuses. There was a total of six fetuses and seven RS. The uteri of all the rats not showing implantations were flushed: no blastocysts were recovered.

The contraceptive effect of this particular MPA/oestrone treatment was unexpected. Thus, the next experiments aimed to determine when and how this treatment interrupted pregnancy.

Experiment 5

Twelve mated rats were treated as in Exp. 4, but were killed on Day 8. Two rats had a total of six implantations. All the uteri not showing implantations were flushed: the uteri of one rat yielded eleven completely degenerated eggs.

Experiment 6

Thirteen mated rats were treated as in Exp. 4, but were killed on Day 4 at 11.00 hours. Eggs were recovered according to the method recently described (Dickmann, 1971). One rat did not yield eggs. The remaining twelve rats yielded an average of 9.77 ± 1.11 eggs, which is within the normal range of untreated controls. The total number of eggs recovered was 127 of which 112 (88%) were morulae and 15 (12%) were degenerated eggs; the latter figure was significantly higher than that for untreated controls.

Experiment 7

Fourteen mated rats were treated as in Exp. 6, but were killed 6 hr later, i.e. on Day 4 at 17.00 hours. Two rats did not yield eggs. The remaining twelve rats yielded an average of 6.14 ± 0.05 eggs, which is significantly lower than the average in Exp. 6. The total number of eggs recovered was 86, of which 50 (58%) were morulae and 36 (42%) degenerated eggs, which constitutes a significant rise in the percentage of degenerated eggs.

Experiment 8

Twelve mated rats were treated as in Exp. 7, but were killed on Day 5 at 10.00 hours. Eleven rats did not yield any eggs. One rat yielded one degenerated egg.

To determine whether the eggs were expelled from the uteri, or were destroyed beyond recognition *in utero*, a further experiment was performed.

Experiment 9

Thirteen mated rats were treated as in Exp. 8, but in addition, the cervical ends of the uteri were ligated on the morning of Day 4 to prevent possible expulsion of the eggs. Twelve rats did not yield any eggs, whereas one rat yielded six blastocysts.

It was concluded that the eggs were destroyed *in utero* between the afternoon

of Day 4 and the morning of Day 5, which is the period when morula-blastocyst transformation occurs.

DISCUSSION

Many of the events during early pregnancy—for example, egg transport, preparation of the uterus for implantation, and loss of the zona pellucida—are known to be regulated by oestrogen/progesterone stimulation. Disturbance of the normal oestrogen/progesterone stimulation could lead to termination of pregnancy. Thus, preimplantation treatment of the intact rat with high doses of oestrogen interrupted pregnancy (Haddad & Ketchel, 1969); however, physiological doses of oestrogen (Haddad & Ketchel, 1969), and relatively high doses of progesterone (up to 16 mg/day—see Sammelwitz, Dziuk & Nalbandov, 1956; Cochrane & Meyer, 1957) did not have adverse effects on the pregnancy. Morulae degenerated subsequent to their transfer into the uteri of ovariectomized, progesterone (2 mg/day)-treated rats (Dickmann, 1970); apparently, progesterone unopposed by endogenous oestrogen has toxic effects (either direct, indirect, or both) on the morula.

The results of the present study demonstrate that slight hormonal changes during the preimplantation period can result in a variety of responses. Thus, injection of oestrone on Day 3 had no effect on pregnancy (Exp. 1); MPA on Day 1 induced delayed implantation (Exp. 2); oestrone on Day 4, superimposed on MPA, abolished the MPA effect thereby restoring pregnancy to normal (Exp. 3); oestrone on Day 3 superimposed on MPA, destroyed the embryo during the period when morula-blastocyst transformation occurs (Exps 4 to 9). The vulnerability of this stage of development has been discussed previously (Dickmann, 1970). It is not clear why treatment with MPA on Day 1 and oestrone on Day 3 destroyed the morulae. The cause was not related to MPA administered on Day 1, nor to oestrone on Day 3, nor to a general synergism between MPA and oestrone. The effect resulted from the combination of the two hormones administered at specific times. It should be noted that both hormones were given in physiological doses. Although 12.5 mg MPA may appear a large dose, this is misleading since this amount is absorbed over a period of 72 days on average (Z. Dickmann, unpublished work).

The present results reinforce the dictum that, as far as predicting the outcome of an untried hormonal treatment is concerned, endocrinology has not yet attained the status of a science. Nevertheless, the results do provide a general guide-line for human postcoital contraceptive trials.

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REFERENCES

- COCHRANE, R. L. & MEYER, R. K. (1957) Delayed nidation in the rat induced by progesterone. *Proc. Soc. exp. Biol. Med.* **96**, 155.

- DICKMANN, Z. (1970) Effects of progesterone on the development of the rat morula. *Fert. Steril.* **21**, 541.
- DICKMANN, Z. (1971) *Egg transfer*. In: *Methods in Mammalian Embryology*, p. 135. Ed. J. C. Daniel, Jr. W. H. Freeman and Company, San Francisco.
- HADDAD, V. & KETCHEL, M. M. (1969) Termination of pregnancy and occurrence of abnormalities following estrone administration during early pregnancy. *Int. J. Fert.* **14**, 56.
- SAMMELWITZ, P. H., DZIUK, P. J. & NALBANDOV, A. V. (1956) Effects of progesterone on embryonal mortality of rats and swine. *J. Anim. Sci.* **15**, 1211.