THE CONTRACEPTIVE ACTION OF IMPEDING OESTROGENS

II. POST-COITAL EFFECT OF OESTRIOL IN THE RAT

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Summary. High and low doses of oestriol (12.5 to 100 $\mu\text{g/day}$), when administered for about 4 days after mating, completely inhibit pregnancy in the rat. Inhibition of ovulation has been ruled out as the mechanism of action. Reversibility of the inhibition effect was demonstrated.

INTRODUCTION

A recent report from this laboratory (Scublinsky & Wotiz, 1971) showed that oestriol, as a representative 'impeding' oestrogen, was fully effective as a contraceptive in rats when administered in small doses.

The rationale for its action was based on the previously observed competition of oestriol for the uterine oestradiol receptor protein (Brecher & Wotiz, 1967) resulting in an altered endometrial milieu. The physiological end effect of this competition has been observed previously by several investigators (Hisaw, 1959; Huggins & Jensen, 1955; Wicks & Segal, 1956) through measurement of uterine weights.

It is well known that, in mammals, oestriol is produced endogenously and, particularly in humans, very large amounts are made during pregnancy by the foeto-placental unit. Few, if any, side reactions to its administration appear to occur and, thus, the use of small doses for contraceptive purposes may overcome the objections raised against those agents at present in use. It is also interesting to note that a possible preventive function with respect to breast tumour development has been suggested for oestriol (MacMahon & Cole, 1969). This activity is in contradistinction to the carcinogenic effect of oestradiol and other potent physiological oestrogens, which has been demonstrated in small animals, and suggested as a possible side-effect in humans.

The possibility that oestriol may interfere with the proper development of the endometrium and prevent implantation, even when the steroid is administered *post coitum*, would seem to be of considerable interest.

MATERIALS AND METHODS

All animals were Sprague-Dawley rats. Female weights averaged 200 g and male weights, 300 g.

Oestriol was obtained from Steraloids (Pawling, N.Y.). Its purity was ascertained by thin-layer chromatography and multiple gas chromatography, as described previously (Scublinsky & Wotiz, 1971).

The animals were checked with respect to the oestrous cycle and on the evening of pro-oestrus they were paired with a male. Examination of the next day's vaginal smear to ascertain the presence of spermatozoa was followed by injection of the properly selected substances according to the following schedule for different rats

<i>Numbers</i>	1 to 4 received 0.1 ml sesame oil containing	100 μg of oestriol
	5 to 8 received 0.1 ml sesame oil containing	50 μg of oestriol
	9 to 12 received 0.1 ml sesame oil containing	12.5 μg of oestriol
	13 to 16 received 0.1 ml sesame oil containing	6 μg of oestriol
	17 to 20 received 0.1 ml sesame oil containing	3 μg of oestriol
	21 to 24 received 0.1 ml sesame oil only.	

Injections were continued for 7 days and at Day 10, each animal was killed and the uteri were examined for the presence of implantation sites. In all non-pregnant animals, the uterine horns were washed with saline under a dissecting microscope.

In order to test the effect of varying the number of injections necessary to prevent implantation, the following experiment was carried out.

Twenty-eight female rats were mated at pro-oestrus, followed by confirmation of the event as before. Each group of four animals was injected with 12.5 μg of oestriol in 0.1 ml of sesame oil as follows:

- Group* 1, a single injection the day after mating
2, a single daily injection for 2 days
3, a single daily injection for 3 days
4, a single daily injection for 4 days
5, a single daily injection for 5 days
6, a single daily injection for 6 days
7, a single daily injection, of sesame oil only, for 7 days.

On the 10th day after proven mating, the animals were killed, the uteri were examined for implantation sites and the horns were washed with saline.

In order to determine whether ovulation was inhibited by the injection of oestriol, the following experiment was performed.

Twenty rats in five groups were checked by vaginal smears and on the day of metoestrus, four groups were treated with doses of oestriol from 6 to 100 μg for 4 days. The fifth group was a sesame-oil-treated control. On the 5th day, the animals were killed and the ovaries were examined under a dissecting microscope for corpora lutea (Long & Evans, 1922).

In order to test the reversibility of the anti-implantational effect of oestriol, two rats were treated with 25 μg of steroid/day for 11 days. After 30 days, the two animals were mated.

RESULTS

Table 1 shows the results from the experiment using varying doses of oestriol for 7 days after proven mating.

While all of the controls had implantations with an average density of 7.3 per horn, at concentrations from 100 to 12.5 μg oestradiol/day, no implantation sites were observed. Even at 6 and 3 μg /day, four out of five animals remained non-pregnant with a limited number of implants (one per horn) in the 6 μg -treated animals, and the normal number of implants (six per horn) in the 3

TABLE 1
POST-COITAL CONTRACEPTIVE EFFECT OF VARYING DOSES
OF OESTRIOL*

	μg oestriol/day					
	100	50	12.5	6.0	3.0	Control
No. of implantations/ uterine horn	0	0	0	0	6	7
	0	0	0	1	0	6
	0	0	0	0	0	8
	0	0	0	0	0	8
Average	0	0	0	0.25	1.5	7.3

* Injections were continued for 7 days following mating.

TABLE 2
EFFECT OF VARYING NUMBER OF OESTRIOL INJECTIONS ON
IMPLANTATIONS

No. of injections*	Injected material	Average no. of implantations/uterine horn				Average
		1	2	3	4	
1	Oestriol†	7	8	7.5	7	7.4
2	Oestriol†	1	2	1	1	1.25
3	Oestriol†	0	4	0	0	1.0
4	Oestriol†	0	0	0	0	0
5	Oestriol†	0	0	0	0	0
6	Oestriol†	0	0	0	0	0
7	Sesame oil‡	7	7	6	8	7.0

* Injections were made daily for the number of days indicated following successful mating.

† 12.5 μg .

‡ 0.1 ml.

μg -treated rat. The washings from the uterine horns of the non-pregnant animals never yielded any ova or blastocysts.

Table 2 shows the results obtained when the length of injection time was varied between 1 and 6 days, using the minimum effective dose of oestriol (12.5 μg). All control animals were pregnant, with an implantation density of 7.0 per horn. Group 1, which received a single injection of oestradiol showed no difference from the controls. In Group 2 also, all the animals were pregnant, but

the implantations were markedly reduced in number as well as in average diameter, while after three injections (Group 3), only one of the four animals became pregnant. In Groups 4 to 6, with a minimum of four daily injections, none of the animals became pregnant. In the last of these groups, no blastocysts or ova were observed in the washings from the uterine horns.

Table 3 records the data from an experiment to determine the effect of varying doses of oestriol on ovulation.

In those rats receiving 100 μg of oestriol/day for 3 days, two out of four

TABLE 3
EFFECT OF VARYING DOSES OF OESTRIOL ON
OVULATION*

<i>Rat no.</i>	<i>Oestriol†</i>	<i>Ovulation</i>	<i>Average</i>
1	100	20	7.5
2	100	0	
3	100	0	
4	100	10	
5	50	7	12.25
6	50	15	
7	50	15	
8	50	12	
9	12.5	15	15.25
10	12.5	15	
11	12.5	16	
12	12.5	15	
13	6	17	15.75
14	6	16	
15	6	15	
16	6	15	
17	0	20	17.5
18	0	17	
19	0	16	
20	0	17	

* As determined by counting the number of corpora lutea on each pair of ovaries.

† μg oestriol/day.

showed no corpora lutea. At a dose of 50 μg of oestriol/day, slightly fewer corpora lutea (average 12.25) were observed, while at doses below this, essentially the same number of ovulations were observed as for the control groups (average 17.5).

The reversibility of the inhibition was readily demonstrated. Two rats were treated with 25 μg of oestriol/day for 11 consecutive days, the animals were rested for 30 days and then mated on the day of pro-oestrus. Both had a normal litter consisting of fifteen young each.

DISCUSSION

The evidence obtained clearly demonstrates the low-dose effectiveness of

'impeding' oestrogens, such as oestriol, in preventing pregnancy in rats when administered *post coitum*. Even in doses of 3 µg/day, significant contraception occurred since blastocysts were only found in the uterine washings from one out of four animals. A fully effective dose of 12.5 µg/day coincides with the observations reported in an earlier paper (Scublinsky & Wotiz, 1971) where the same steroid was administered for several days before mating.

In order to attain full effectiveness, treatment must be continued for 4 days after mating although, in 75% of the animals, 3 days of treatment were sufficient to prevent implantation. This is not surprising since the rat is known to have an oestradiol surge on the 4th day, which is essential for subsequent implantation. It is interesting to point out that post-coital use of the strongly uterotropic oestradiol, when administered on the 4th day after mating, required 500 µg of steroid to achieve complete contraception (Emmens & Finn, 1962).

Of the three main avenues of contraception, prevention of implantation by altering the endometrial milieu is the most likely operative mechanism for oestriol (Scublinsky & Wotiz, 1971). The fact that both in the earlier experiments, where oestriol was used before mating and in the present study, an optimal dose of 12.5 µg was found, suggests that the mechanism involved is likely to be the same. Thus, the earlier work by Desaulles & Krähenbühl (1964), taken with the fact just mentioned, lends further support to the proposed mechanism of action based on the competition of oestriol for a uterine-specific receptor substance which prevents the preparation of the uterus for implantation by stimulation with oestradiol.

Further evidence that inhibition of ovulation was not involved in these experiments was also obtained. With doses at or near the optimal for contraception (6 to 50 µg), no significant effect on the number of corpora lutea in the ovary (i.e. ovulations) was observed. Only at very large doses (100 µg), was there a significant, but by no means complete, interference with ovulatory processes. This observation confirms that made earlier by Desaulles & Krähenbühl (1964). Thus, it is possible to rule out interruptions of ovulation as an active mechanism of contraception with low doses of oestriol.

Sperm capacitation, which may be affected when the steroid is administered before mating, can also probably be excluded as a result of the post-coital experiments, since it is likely that fertilization will have occurred before injection of the steroid.

Finally, it was important to determine the reversibility of the effect of oestriol. Administration of oestriol for 11 days appears to leave no readily observable residual detrimental effects since, at 30 days after treatment, both animals promptly became pregnant.

Thus, oestriol, as a typical 'impeding' oestrogen, appears to have complete contraceptive effects in the rat when administered in doses of 12.5 µg/day or more for at least 4 days *post coitum*.

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