Maintenance of Pregnancy in Ovariectomized Rats with Some Newer Progestins. (24397)

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In the rat, bilateral ovariectomy performed during the first half of pregnancy results in termination of gestation(1). When castration is performed during the second half of pregnancy, abortion occasionally does not occur, particularly when a high placenta:fetus ratio exists, because of the capacity of placentas to produce some progesterone and estrogen (2). Although abortion or resorption which generally follows castration after mid-term appears to be in part a result of trauma of surgery(3), it must be concluded that the principal cause for termination of pregnancy is inadequate placental production of progesterone and estrogen, and that the ovary is functional and necessary throughout normal pregnancy in the rat(1). It has been shown that pregnancy can be successfully maintained in rats castrated during the first half of pregnancy by administration of sufficient quantities of progesterone or progesterone and estrone(4). A number of new steroids have been defined as progestins on the basis of their ability to produce a secretory endometrium in the uterus of the estrogen-primed, castrate or immature rabbit. Although development of a secretory endometrium appears to be associated with the process of implantation(5), it cannot be assumed *a priori* that ability of a compound to produce secretory changes is directly related to its ability to maintain pregnancy after implantation has occurred. This communication reports studies on pregnancy maintaining activity of a number of steroids which cause a secretory proliferation of the endometrium, and draws particular attention to 6α-methyl-17α-hydroxyprogesterone acetate (*Provera*).* a recently reported compound(6) which shows unusual potency in production of a secretory endometrium and in maintenance of pregnancy.

Materials and methods. Mature, virgin, female Sprague-Dawley rats, 200-275 g, were caged with fertile males in the evening and vaginal smears were taken on the following morning to detect sperm. The day sperm were found, was considered day 1 of pregnancy. On 8th day of pregnancy, the rats were bilaterally ovariectomized if found pregnant upon examination of the uterus. Treatment was begun immediately after ovariectomy and continued through 20th day of pregnancy. At autopsy on 21st day, presence or absence of implantation sites and number of living young were recorded. The animals were also examined grossly for completeness of ovariectomy. Test progestins were administered subcutaneously once daily as suspensions in CMC† with one exception. In this group (Table II), the test compound was mixed with a powdered diet, and drug intake was calculated by measuring daily food intake. Estrone was administered once a day subcutaneously in cottonseed oil.

Results. Of 70 untreated rats found pregnant by palpation, 68 delivered an average of 10.3 live young each. The 70 animals contained an average of 11 implantation sites each. A "net success index" of 91 was calculated for this group as follows:

$$\frac{\text{Living young/group}}{\text{No. of mothers/group} \times 11 \text{ implantation sites}} \times 100.$$

A net success index of 100 would have been realized had the rats born an average of 11 living young each. Net success indices were similarly calculated for other groups (Table II). That indices be expressed as percentages of theoretical maximum success (one live fetus for each of 11 expected implantations), calculations were made using 11 sites as a constant. Fourteen rats ovariectomized on 8th day of pregnancy and either left un-

* Registered trade name, The Upjohn Co.

† Upjohn Sterile Vehicle No. 98, containing 10 mg carboxymethylcellulose, 4 mg polysorbate 80 and .42 mg propylparaben/milliliter.

TABLE I. Maintenance of Pregnancy in Rats Castrated on 8th Day of Pregnancy. Results obtained with compounds which failed to maintain live young at any dose employed.

Compound	Doses administered alone and concomitantly with 1 μg estrone,* mg/rat/day	Results (autopsy on 21st day of pregnancy)
17α-Ethinyltestosterone, 0.1(13)†	25, 10, 1, .1	No implantation sites remaining in any rat.
17α-Ethinyl-19-nortestosterone, 10(12)	50, 10, 1, .1	Implantation sites in all animals except rat administered .1 mg of compound alone; no evidence of pregnancy at this dose.
17α-Methyltestosterone, 0.05(13)	50, 10, 1, .1	Implantation sites in rats administered the 2 high doses with and without estrone; no evidence of pregnancy in remaining animals.
17α-Ethinyl-17-hydroxy-5(10)-estren-3-one,‡ 0.5(12)	10, 1, .1, .01	Implantation sites only in animal receiving high dose alone; no evidence of pregnancy in remaining animals; all animals lost weight.

* One rat/dose of compound or dose of compound plus estrone.

† Progestational activity × progesterone in production of secretory endometrium in estrogen-primed castrate or immature rabbit. Literature reference in parentheses.

‡ Contains the 3-methyl ether of ethinyl estradiol in sufficient quantity to give preparation estrogenic activity equal to 10% that of estrone.

treated or treated with 1 μg of estrone/day showed no fetuses, resorbing material or vestiges of implantation sites when autopsied on the 21st day of pregnancy. This constitutes evidence that successful maintenance of pregnancy in castrate progestin-treated rats is a consequence of the treatment.

All compounds were initially "spot tested" at a wide range of doses administered alone and concomitantly with 1 μg of estrone/day. One animal was employed/dose. If a compound was found capable of maintaining pregnancy in the spot test, additional doses were employed and number of animals/dose group increased to give a rough estimate of potency. Compounds which failed to maintain pregnancy at any dose employed appear in Table I. One steroid, 17α-ethinyltestosterone, failed to maintain even vestiges of implantation sites and was, therefore, equivalent to no treatment or to treatment with estrone alone. The remaining compounds, 17α-methyltestosterone, 17α-ethinyl-17-hydroxy-5(10)-estren-3-one‡ and 17α-ethinyl-19-nortestosterone§ maintained implantation sites at some doses even though they failed to maintain live young.

Compounds found capable of maintaining pregnancy (Table II) include progesterone, 17-hydroxyprogesterone acetate (*Prodox*),* 6α-methyl-17α-hydroxyprogesterone acetate,‖ 9α-bromo-11-ketoprogesterone,‖ 17α-ethyl-19-nortestosterone‡ and desoxycorticosterone acetate. With the exception of 17α-hydroxyprogesterone acetate, the compounds maintained pregnancy when administered alone, though for comparable success higher doses were generally required than when estrone was concomitantly administered. Though it is difficult to calculate potency ratios from these data, it can be seen that the most active compound was 6α-methyl-17α-hydroxyprogesterone acetate; the least active was 17α-hydroxyprogesterone acetate. The remaining steroids, progesterone, 9α-bromo-11-ketoprogesterone, 17α-ethyl-19-nortestosterone and desoxycorticosterone acetate possessed potencies intermediate between these 2 extremes. 6α-Methyl-17α-hydroxyprogesterone acetate, the only compound so tested, was also capable of maintaining pregnancy when administered orally in the diet (Table II).

‡Compounds supplied through courtesy of Dr. L. G. Hershberger, G. D. Searle & Co., Chicago, Ill.

§ Compound supplied through courtesy of Dr. Leon A. Sweet, Parke-Davis and Co., Detroit, Mich.

‖ Compounds prepared by Chemistry Dept., The Upjohn Co., Kalamazoo, Mich.

Discussion. The net success index of 91 obtained in intact, untreated rats expresses the fact that normally not all implantation sites develop into living young. Castration probably induces additional embryonic deaths even in adequately treated rats through generalized effects of surgical trauma(3) and through direct damage to embryos located

TABLE II. Maintenance of Pregnancy in Rats Castrated on 8th Day of Pregnancy. Results Obtained with compounds which maintained live young.*

Compound	Daily doses: Compound, mg	Daily doses: Estrone, μg	Successful pregnancies /No. of "tries"	Live young /successful pregnancy	Net success index
Progesterone	8		5/5	4	36
	4		4/5	2.5	18
	2		1/5	2	4
	8	1	4/5	5.5	40
	4	1	5/5	6.8	62
	2	1	5/5	3.6	33
	1	1	2/5	4.5	16
17α-Hydroxyprogesterone acetate, 6 (6)†	100		0/5	0	0
	100	1	2/3	2.5	15
	50	1	1/2	5	23
	20	1	0/2	0	0
6α-Methyl-17α-hydroxyprogesterone acetate, 50-60(6)	40		3/3	5.3	48
	20		3/3	9.3	85
	10		7/7	9	82
	5		4/4	9	82
	2.5		5/5	7.8	70
	1.25		4/5	5.8	42
	.625		4/4	7	64
	.313		3/3	4.7	42
	.150		5/5	4.4	39
	.075		3/5	1.3	7
	.040		0/3	0	0
	.040	1	2/3	1.5	9
	‡		3/6	4.3	20
9α-Bromo-11-ketoprogesterone, 0.5 (14)	10		2/2	7.5	68
	5		3/3	3.7	33
	1		1/3	3	9
	.1		0/1	0	0
	10	1	1/1	10	91
	1	1	3/3	5.7	52
	.5	1	2/2	7	64
	.1	1	0/3	0	0
17α-Ethyl-19-nortestosterone, 10(12)	10		4/4	1.7	16
	5		3/3	2.3	21
	1		1/4	2	5
	.1		0/1	0	0
	10	1	1/1	1	9
	1	1	3/4	3	20
	.5	1	1/3	1	3
	.1	1	0/4	0	0
Desoxycorticosterone acetate, 0.2(8)	50		1/1	5	45
	10		0/1	0	0
	2		0/1	0	0
	50	1	1/1	5	45
	10	1	1/1	2	18
	2	1	0/1	0	0

* Autopsy on 21st day of pregnancy.

† Progestational activity × progesterone in production of secretory endometrium in estrogen-primed castrate or immature rabbit. Literature reference in parentheses.

‡ 0.4 to 15.2 mg/day (avg 6.1) orally—in diet.

nearest the ovarian end of the uterus. Thus, with one exception,¶ all regimens resulted in success indices less than 91. However, the success indices were even higher in castrate groups than they probably would have been had the fetuses been required to undergo the risks of parturition as were those in the intact, untreated group.

Our results agree with the following reported findings. Progesterone alone can maintain pregnancy in castrate, hypophysectomized rats(4), or in castrate rabbits(7), but greater success is realized when estrogen is administered concomitantly with progesterone. Desoxycorticosterone acetate maintains pregnancy in castrate mice and rabbits(8), and 17α-ethyl-19-nortestosterone maintains pregnancy in castrate rabbits(9). 17α-ethinyltestosterone(10) and 17α-ethinyl-17-hydroxy-5(10)-estren-3-one(11) were found incapable of maintaining pregnancy.

Fair correlations exist between progestational potencies in rabbit endometrial secretory proliferation assays (Clauberg or McPhail) reported in the literature (Tables I and II) and ability to maintain pregnancy only for 6α-methyl-17α-hydroxyprogesterone acetate, 9α-bromo-11-ketoprogesterone and desoxycorticosterone acetate. 6α-methyl-17α-hydroprogesterone acetate is many times as potent as progesterone both in McPhail assays and in pregnancy maintenance. The potency of 9α-bromo-11-ketoprogesterone is slightly less than progesterone on the endometrium and slightly greater than progesterone in pregnancy maintenance. Desoxycorticosterone acetate is considerably less potent than progesterone in both respects. Poorer correlation exists between the 2 activities for 17α-ethyl-19-nortestosterone, reported 10 times progesterone in the Clauberg assay(12) and found to be only slightly more active than progesterone in maintenance of pregnancy. On the other hand, 17α-ethinyl-19-nortestosterone, also reported 10 times progesterone in the Clauberg test(12) was incapable of maintaining pregnancy in doses as high as 50 mg/day. It has, however, been reported active in allowing implantation to occur in rabbits castrated shortly after fertilization of the ova(12). This is probably a reflection of its ability to produce a secretory endometrium. No correlation between activity in McPhail or Clauberg tests and pregnancy maintenance activity exists for 17α-ethinyl-17-hydroxy-5(10)-estren-3-one, 17α-hydroxyprogesterone acetate, 17α-ethinyltestosterone and 17α-methyltestosterone.

It can be concluded that ability to maintain pregnancy in castrate rats cannot be predicted from ability to produce a secretory endometrium in uteri of estrogen-primed rabbits. It, therefore, appears unlikely that efficacy in the treatment of habitual or threatened abortion in humans can be predicted from a progestin's ability to produce endometrial secretory changes. The important pregnancy maintaining activity of progesterone has been postulated to be one of alteration of the impulse conducting capacity and hence reactivity of the myometrium(15). It remains to be seen if the ability of other progestins to maintain pregnancy will correlate with their ability to alter myometrial reactivity.

Pregnancy maintenance also involves biological activities other than those generally attributed to progestins. The pregnancy maintaining activity of the progestins studied here was uniformly improved by concomitant administration of estrone, a substance inactive by itself. Since 17α-hydroxyprogesterone acetate, unlike most other progestins, possesses no inherent estrogenic (uterotrophic) activity (unpublished data), it is attractive to postulate that lack of estrogenicity is responsible for failure of the steroid to maintain pregnancy when administered alone. It has been suggested that androgenic activity is also necessary for full gestational development(16), and shown that lower doses of progesterone can be employed for maintenance of pregnancy in castrate mice(17) or rabbits(7) when androgens are concomitantly administered. Since 17α-hydroxyprogesterone acetate is also nonandrogenic (unpublished data), we are investigating the possibility that concomitantly administered estrogen, androgen, and 17α-hydroxyprogesterone acetate may be more efficacious in maintaining preg-

¶ Ten live fetuses and hence a net success index of 91 was found in the one rat receiving 10 mg/day of 9α-bromo-11-ketoprogesterone.

nancy than concomitantly administered estrone and 17α-hydroxyprogesterone acetate.

Summary. Progestins found capable of maintaining pregnancy in rats castrated on the 8th day after insemination included progesterone, 17α-hydroxyprogesterone acetate, 17α-ethyl-19-nortestosterone, desoxycorticosterone acetate, 9α-bromo-11-ketoprogesterone and 6α-methyl-17α-hydroxyprogesterone acetate. The most potent compound in this respect was 6α-methyl-17α-hydroxyprogesterone acetate. Compounds which failed to maintain pregnancy include 17α-ethinyltestosterone, 17α-ethinyl-19-nortestosterone, 17α-ethinyl-17-hydroxy-5(10)-estren-3-one, and 17α-methyltestosterone. With the exception of 17α-hydroxyprogesterone acetate, all compounds which maintained pregnancy when administered concomitantly with estrone also maintained pregnancy when administered alone. Higher doses were required, however, for comparable results. Fair correlation between reported potency in the McPhail or Clauberg rabbit endometrium assays and pregnancy maintenance potency existed only for 6α-methyl-17α-hydroxyprogesterone acetate, 9α-bromo-11-ketoprogesterone and desoxycorticosterone acetate.

ADDENDUM: In experiments recently performed in this laboratory, it was found that pregnancy was maintained in 25/26 castrate rats fed 400 μg or more of 6α-methyl-17α-hydroxyprogesterone acetate/day. Pregnancy was not maintained in any of 10 castrate rats fed less than this amount/day.

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Fibrinolysis. I. Fibrinolytic Activity of Extracts from non-Pathogenic fungi.* (24398)

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Several proteolytic enzymes have been obtained from fungi. In particular, a crystalline protease was isolated by Crewther and Lennox (1) from a strain of *Aspergillus oryzae*. In an investigation to identify alternative sources of fibrinolytic agents, the additional observation was made that extracts of some non-pathogenic fungi are able to lyse purified fibrin and plasma clots of human and bovine source.

Materials and methods. Cultures of 160 fungi were obtained from many Institutions. In this article they are recognized by letters and numbers. The letter was arbitrarily assigned according to source, as shown below. The number which follows was either arbitrar-

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