Interruption of Pregnancy in the Rat by Administration of ACTH

W. H. YANG, W. P. YANG, AND L. L. LIN

Department of Reproductive Physiology and Obstetrics and Gynecology, Taipei Medical College, Taipei, Republic of China

ABSTRACT. Intraperitoneal injections in the rat of 12 IU ACTH on Days 1, 2 and 3 after mating resulted in a marked inhibition of both implantation and fetal development to 57 and 29%, respectively, of control values, but did not alter the rate of tubal transport or development of the ova. Following similar treatment with 3, 6 or 12 IU ACTH on Days 5, 6 and 7, both implantation and fetal development were reduced (to 80, 74 and 57% and 75, 61 and 39%, respectively). About 52% of the blastocysts transferred from donors receiving 12 IU ACTH on Days 1, 2 and 3, and 56% of the blastocysts from untreated donors developed into normal fetuses when transferred to the left or right uterine horns of recipients. Daily injections of 12 IU ACTH on Days 5, 6 and 7 with implants of 10 mg cortisone pellet on Day 5 resulted in a significantly high fetal mortality in intact females but not in females adrenalectomized on Day 5. In pregnant rats adrenalectomized on Day 5, a 10 mg cortisone pellet maintained pregnancy, whereas smaller (5 mg) or larger doses (20 or 40 mg) were less effective. It thus seems likely that the effect of ACTH on the embryos was chiefly an indirect effect mediated through the adrenal gland, and that the antifertility effect of ACTH was attributable to excessive production of corticosteroids by the adrenal. (*Endocrinology* 84: 1282, 1969)

IT HAS been demonstrated that the administration of a large amount of cortisone inhibited decidual development in the rat (1) and induced high fetal mortality in the rat (2–4) and rabbit (5). Similarly, ACTH was also shown to inhibit decidual development in the rat (1) and to provoke degenerative changes in the ovary of the mouse (6). However, the effect of ACTH during early pregnancy has not been clearly established.

Since the administration of ACTH results in increased secretion of corticosteroids (7–9), estrogens (10–12) and androgens (13, 14) in animals and humans, it is possible that following such treatment either tubal transport or implantation of ova can be affected.

The present study attempts to elucidate further the influence of ACTH during early pregnancy in the rat with special reference to its effects on tubal transport, implantation and development of embryos.

Materials and Methods

Long-Evans rats from NAMRU-2 (U. S. Naval Medical Research Unit No. 2 at Taipei, Republic of China) weighing 200–250 g were housed 5/cage and were kept in an air-conditioned room between 23 and 25 C under constant 12-hr artificial light (0500–1700). The animals were maintained on Prina Chow and water *ad lib.*

Proestrous females, selected on the basis of their vaginal smear, were caged with fertile males in the late afternoon. The presence of sperm in the vagina was verified on the following morning and this was designated as Day 1 of pregnancy. Daily doses of 12 IU ACTH in 0.6 ml buffer solution (Corticotrophine-Z and buffer solution, Organon Co.) were injected intraperitoneally daily at 1700 on Days 1, 2 and 3 of pregnancy. In another group of animals, doses of 3, 6 or 12 IU ACTH were given around the time of implantation on Days 5, 6 and 7. As controls, 0.6 ml buffer solution was injected either on Days 1, 2 and 3, or on Days 5, 6 and 7.

In an attempt to study the mode of action of exogenous ACTH, the following experiments were performed. Day-5 blastocysts from rats injected intraperitoneally with 12 IU ACTH on Days 1, 2 and 3 were transferred into the right uterine horn of pseudopregnant rats on Day 5. For comparison, blastocysts from normal rats were similarly transferred into the left uterine horn of the same recipients. Both implantation sites and living fetuses were examined 13 days after transfer of ova. In another experiment, a group of mated rats were adrenalectomized on Day 5 between 1700 and 1800 and implanted with 10 mg cortisone pellet (composed of 10 mg cortisone and the same amounts of calcium lactate and methylcellulose); daily injections of 12 IU ACTH were administered on Days 5, 6 and 7. For comparison, another group of mated females were similarly adrenalectomized and implanted with a cortisone pellet on Day 5, followed by daily injections of 12 IU ACTH on Days 5, 6 and 7. In other adrenalectomized controls, either 5, 10, 20 or 40 mg cortisone pellets were given, respectively, on Day 5 for the maintenance of pregnancy.

Animals were killed at 6-hr intervals beginning at 1800 on Day 4 for examination of tubal transport and development of ova in rats receiving ACTH injections during Days 1, 2 and 3 of pregnancy. Some females were killed at 0600 on Day 5 as donors

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TABLE 1. Transport and development of ova following daily intraperitoneal injections of 12 IU ACTH on Days 1, 2 and 3 of mated rats

Time of examination (day & hr)	ACTH-treated			Control		
	No. of rats	Ova recovered from		No. of rats	Ova recovered from	
		Tube[1] (stage of ova[2])	Uterus[1] (stage of ova[2])		Tube[1] (stage of ova[2])	Uterus[1] (stage of ova[2])
Day 4 1800	5	53 [5] (2, d; 1, 2c; 49, m)	0	5	50 [5] (3, d; 47, m)	"
Day 5 0000	5	27 [5] (2, d; 25, m)	24 [3] (1, d; 23, m)	5	28 [4] (1, d; 1, 2c; 26, m)	25 [4] (2, d; 23, m)
Day 5 0600	5	0	46 [5] (3, d; 43, b)	5	0	49 [5] (2, d; 2, 2c; 45, b)

[1] Figures in parentheses denote the number of animals that had ova in their tubes or in uteri.
[2] d = 1-celled or degenerating ova; 2c = 2-celled ova; 4c = 4-celled ova; m = morula; b = blastocyst.

for transfer of blastocysts into pseudopregnant rats. Most animals treated either on Days 1, 2 and 3 or on Days 5, 6 and 7 of pregnancy, and pseudopregnant recipients for transfer of ova, were sacrificed on Day 18 for determination of implantation sites and living fetuses.

Results

Following the daily intraperitoneal injection of 12 IU ACTH on Days 1, 2 and 3, tubal ova entered into the uterine cavity normally at the morula stage at the beginning of Day 5. The majority of uterine ova also developed normally from morula to blastocyst stage within 6 hr after their entrance into the uterine cavity (Table 1).

Nevertheless, following such treatment, the number of implantations and normal fetal development were markedly reduced to 57 and 29%, respectively, as compared with the control group. When 3, 6 or 12 IU ACTH was injected on Days 5, 6 and 7, the percentages of implantation and fetal development were reduced to 57 and 39%, respectively, of control values (Table 2).

When blastocysts from rats receiving 12 IU ACTH on Days 1, 2 and 3 were synchronously transferred into the right horn of normal pseudopregnant rats on Day 5, and blastocysts from normal rats into the left horn of the same recipients, similar percentages of implantations (58 *vs.* 63%) and living fetuses (52 *vs.* 56%) were obtained from the uterine horns (Table 3).

In a further experiment, daily injections of 12 IU ACTH on Days 5, 6 and 7 with subcutaneous implantation of 10 mg cortisone pellet on Day 5 in the pregnant rat resulted in reduction of the number of implantations and in living fetuses to, respectively, 83 and 16% of the adrenalectomized rats receiving similar treatments. In the additional controls, pregnancy in the adrenalectomized rat was maintained as in the controls by a pellet of 10 mg cortisone; whereas a dose of 5 or 20 to 40 mg was less effective for maintenance of normal pregnancy (Table 4).

TABLE 2. Effect of intraperitoneal injections of ACTH on pregnancy in mated rats (examined on Day 18)

Dose IU/rat/day	Days of inj.	No. of rats			Corp. luteum mean ± SE	Implant. sites mean ± SE (%)[1]	Live fetuses mean ± SE (%)[1]
		Mated	Having implant. sites	Having living fetuses			
12 IU	1-2-3	9	6	4	11.2±0.9	6.1±1.6 (56.5)	3.6±1.4 (29.1)
3 IU	5-6-7	7	6	5	13.0±0.6	8.0±2.8 (60.0)	7.0±2.2 (75.3)
6 IU	5-6-7	9	8	8	12.7±1.6	7.4±1.2 (74.0)	5.7±1.0 (61.3)
12 IU	5-6-7	9	6	5	13.6±0.8	5.7±1.6 (57.6)	3.6±1.5 (38.7)
Control, buffer solution							
0.6 ml	1-2-3	9	9	8	12.8±6.7	10.8±0.8 (100)	10.3±0.9 (100)
0.6 ml	5-6-7	9	9	9	12.3±1.0	10.0±1.0 (100)	9.3±0.8 (100)

[1] The percentage of implantation sites or live fetuses compared with controls.

TABLE 3. Development of transferred blastocysts (eggs recovered from ACTH-treated rats were transferred to the right uterus and eggs recovered from untreated rats were transferred to the left uterus of the same recipient)

Treatment of donors	No. of donors	No. of ova transferred	No. of recipients	Results	
				No. of implant. sites (%)	No. of live fetuses (%)
Untreated	10	104	10	65 (62.5)	58 (55.8)
12 IU ACTH on Days 1, 2, 3	10	101		59 (58.4)	52 (51.5)

Discussion

Following ACTH treatment a slight increase in estrogen secretion (10–12) and androgens occurs in conjunction with a large increase in production of corticosteroids (7–9) by the adrenal of animals or humans. Nevertheless, unlike the effect of estrogens (15–18), androgens (19) or gonadotropins (20) on either acceleration or inhibition of egg transport in the fallopian tube, treatment with ACTH on Days 1, 2 and 3 of normal pregnancy did not affect tubal function.

It has been observed on several occasions that, following early entry of tubal ova into the uterus either by hormonal treatments, or by mechanical means such as transfer of ova (21, 22), the development of young ova in the uterine environment is inhibited in the mouse, rabbit and rat. In the present experiment in the rat, following ACTH treatment, ova entered into the uterine cavity at the regular time and nearly all morulae subsequently developed into blastocysts. The high implantation loss and severe fetal mortality following such treatment suggested the possibility of endometrial dysfunction resulting from the administration of ACTH. A variable degree of implantation loss and fetal mortality brought about by different doses of ACTH treatments on Days 5, 6 and 7 after blastocyst formation in the rat also favored this hypothesis. Since blastocysts transferred on Day 5 either from the ACTH-treated group or from the normal pregnant group developed to the same extent in the contralateral uterine horns of the same recipients, the main reason for failure in either implantation or development was due to endometrial dysfunction induced by the treatment with ACTH. Furthermore, the effect of ACTH on the endometrium appeared to be mediated through the adrenal gland, since the effect of ACTH on the embryos was not found in the adrenalectomized rat treated with cortisone for maintenance of pregnancy.

It is apparent from previous investigations (3, 23), and also from the control experiment of this study, that a suitable amount of corticosteroids aids in the maintenance of preg-

TABLE 4. Effect of ACTH on pregnancy in cortisone-treated intact or adrenalectomized rats (adrenalectomized on Day 5 of pregnancy)

Groups	Dose/rat/day	Days of treatment	No. of rats			Corp. luteum mean ± SE	Implant. sites mean ± SE	Live fetuses mean ± SE
			Mated	Having implant. sites	Having living fetuses			
Intact	12 IU ACTH 10 mg C.P.[1]	5-6-7 5	12	8	7	13.5 ± 1.3	5.3 ± 1.3	0.9 ± 0.1[2]
Adrenalectomized	12 IU ACTH 10 mg C.P.[1]	5-6-7 5	12	8	8	13.0 ± 0.9	6.4 ± 1.3	5.8 ± 1.1[2]
Controls, various doses of cortisone pellet								
Adrenalectomized	5 mg C.P.[1]	5	10	10	8	12.8 ± 2.1	7.0 ± 1.7	2.8 ± 1.4
Adrenalectomized	10 mg C.P.[1]	5	9	7	7	11.1 ± 0.6	6.8 ± 1.4	5.6 ± 1.4
Adrenalectomized	20 mg C.P.[1]	5	8	5	3	10.3 ± 0.6	6.0 ± 1.8	2.0 ± 1.1
Adrenalectomized	40 mg C.P.[1]	5	7	5	2	10.6 ± 0.7	5.2 ± 1.5	1.3 ± 0.9

[1] Cortisone pellet composed of the designated weight of cortisone and equal amounts of calcium lactate and methylcellulose.
[2] Significant difference by *t*-test ($t = 3.11$, $p. < 0.01$).

nancy. In contrast, a relatively large amount of corticosteroids disturbs the normal function of the endometrium for implantation and embryonic development (2–4, 23). The results obtained in this study further illustrate the fact that the interruption of pregnancy may result from the secretion of an excessive amount of endogenous corticosteroids as the result of stimulation of the adrenal gland by a large amount of ACTH.

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