PREGNANCY-TERMINATING EFFECT OF HUMAN CHORIONIC GONADOTROPHIN IN RATS

U. K. BANIK

*Ayerst Research Laboratories,
Montreal, Canada*

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Summary. A single intramuscular dose of HCG (50 i.u. or more per rat) was able to induce fetal resorption and eventual termination of pregnancy when injected on Day 4 or between Days 7 and 11 of pregnancy. This dose was inactive when administered on Day 12 of pregnancy. A single large dose (500 i.u./rat) induced fetal resorption when administered even on Day 12 of pregnancy. In intact rats treated with HCG, daily doses of progesterone were unable to maintain normal implantations; in ovariectomized rats treated with HCG, daily progesterone did succeed in maintaining pregnancy in many of the animals. It is suggested that fetal resorption and the eventual pregnancy-terminating effects of HCG in rats are mediated through the alteration of normal ovarian steroidogenesis.

INTRODUCTION

Human chorionic gonadotrophin is synthesized by the syncytiotrophoblastic cells of the placenta. It has been highly purified and shown to contain two essential protein sub-units (Canfield, Morgan, Kammerman, Bell & Agosto, 1971). As yet, however, the physiological rôle of this hormone during early pregnancy is incompletely understood (Venning, 1968). Many investigators believe that HCG is essential for the maintenance of the functional life of the corpus luteum in early pregnancy (Bradbury, Brown & Gray, 1950; Lunenfeld, 1967; Hanson, Powell & Stevens, 1971). Despite the luteotrophic effect of HCG in some studies (Savard, Marsh & Rice, 1965; Armstrong, 1968), luteolytic effects have also been found (Stormshak & Casida, 1964; Spies, Coon & Gier, 1966; Moor, Rowson, Hay & Caldwell, 1968). The fetal mortality induced by single or multiple doses of HCG administered during the preimplantation and implantation periods of pregnancy in rats and hamsters has been reported by Yang & Chang (1968). In an extension of the above studies on the effect of HCG after implantation in rats, it was proposed that this hormone induces flow of the menses in primates (Banik, 1972). The purpose of this communication is to describe the pregnancy-terminating effect of a single dose of HCG administered to rats after implantation.

MATERIALS AND METHODS

All experiments were performed on adult (210 ± 25 g) Sprague-Dawley albino rats from the Canadian Breeding Laboratories, St Constant, Quebec. Standard (Purina) diet and water were given freely and animals were kept in uniform conditions with exposure to 12 hr light/12 hr dark.

The commercial preparation of HCG with an approximate potency of 3000 i.u./mg (A.P.L., Ayerst Laboratories, N.Y.) was dissolved and diluted in normal saline. Unless otherwise mentioned, the injection was given intramuscularly on the dorsal side of the neck at 15.00 hours on different days of pregnancy. Each dose was given in 0.2 ml vehicle to each rat and was not calculated on the basis of body weight. After placing females overnight with males known to be fertile, the day on which a vaginal plug was found or spermatozoa were seen in a vaginal smear was designated Day 1 of pregnancy. Progesterone was dissolved in sesame oil and administered intramuscularly twice daily at 09.00 hours and at 16.00 hours from Day 7 or 8 to Day 14 of pregnancy. Unless otherwise stated, animals were killed by chloroform on Day 15 of pregnancy and the numbers of normal and resorbing fetuses were counted. The results were tabulated on the basis of observed normal and resorbing implantations.

RESULTS

Effect of a single dose of HCG administered on Day 8 of pregnancy

The pregnancy-terminating effects of different doses of HCG administered to rats on Day 8 of pregnancy are presented in Table 1. Fetal resorption was observed in all the animals treated with HCG. Single doses of 50 or 100 i.u. induced 50% and 78% fetal resorption, respectively, and decreased the number of normal implantations compared to that in the control group. In another study of eight rats (not shown in Table 1), 100 i.u. HCG administered on Day 8 of pregnancy resulted in complete termination of pregnancy by Day 20; the uterine horns in these animals appeared empty and similar to those of non-pregnant rats. All rats treated with 250 or 500 i.u. HCG showed signs of resorption, with the exception of one rat which carried eight normal implantations.

Effect of a single dose of HCG administered on different days of pregnancy

A single dose of 50 i.u. HCG (Table 2) administered on Day 1, 2, 3, 5 or 6 was partly effective in interrupting pregnancy but was more effective on Day 4 of pregnancy. From Day 7 to Day 11 of pregnancy, this dose was consistently effective in inducing fetal wastage. The fetal resorption rate was pronounced following treatment between Days 9 and 11 of pregnancy. The same dose of HCG injected on Day 12 did not induce any fetal resorption. Daily administration from Days 1 to 12 of a preparation of HCG heated in boiling saline for 30 min was not active in terminating pregnancy, but 23% of implanted fetuses were resorbed. A dose of 50 i.u. of this 'boiled' preparation of HCG induced ovulation in five out of ten dioestrous mice (the results are not shown in Table 2), indicating that there was incomplete destruction of the HCG by this treatment.

Table 1. Termination of pregnancy in rats by single doses of HCG administered on Day 8 of pregnancy

HCG (i.u./rat)	No. of rats			Normal implantations/rat (Mean $\pm$ S.E.) [†]	Resorptions/rat (Mean $\pm$ S.E.)	Resorption (%) [*]
	With normal implantation	With total resorp- tion sites	With some resorption sites			
Control injected with saline	10/10	0/10	2/10	11.0 $\pm$ 0.5 (10) [†]	2.0 (2) [†]	3
50	1/11	4/11	6/11	5.0 $\pm$ 1.2 (7)	5.7 $\pm$ 1.3 (7)	50
100	0/7	4/7	3/7	4.0 $\pm$ 1.0 (3)	8.6 $\pm$ 2.1 (5)	78
250	0/6	6/6	0/6	0	13.8 $\pm$ 1.2 (6)	100
500	0/6	5/6	1/6	8 (1)	12.8 $\pm$ 1.3 (6)	90

* Resorption = $(r/r+i) \times 100$, where r = total no. of resorptions and i = total no. of normal implantations/group.[†] Figure in parentheses indicates the number of rats.

Table 2. Termination of pregnancy in rats by a single dose of HCG administered on different days of pregnancy

Treatment day of pregnancy	No. of rats			Normal implantations/rat (Mean $\pm$ S.E.)	Resorptions/rat (Mean $\pm$ S.E.)	Resorption (%) [*]
	With normal implantation	With total re-sorption sites	With some re-sorption sites			
Untreated control	10/10	0/10	0/10	11.0 $\pm$ 0.3 (10) [†]	0	0
Control treated with 0.2 ml saline						
1	6/6	0/6	1/6	10.5 $\pm$ 1.9 (6)	1.0 (1) [†]	1
2	6/7	0/7	5/7	5.5 $\pm$ 1.3 (6)	3.6 $\pm$ 1.0 (5)	35
3	1/7	0/7	6/7	9.8 $\pm$ 1.0 (7)	4.0 $\pm$ 1.0 (6)	26
4	2/8	0/8	5/8	9.3 $\pm$ 1.2 (7)	4.0 $\pm$ 1.3 (5)	23
5	0/6	3/6	1/6	11.0 (1)	8.5 $\pm$ 2.5 (4)	75
6	4/8	0/8	3/8	12.0 $\pm$ 0.6 (7)	2.0 $\pm$ 0.5 (3)	7
7	2/7	0/7	3/7	9.3 $\pm$ 2.0 (4)	1.5 $\pm$ 0.3 (4)	14
8	1/6	0/6	5/6	6.0 $\pm$ 1.5 (6)	8.0 $\pm$ 1.1 (5)	53
9	1/8	1/8	6/8	5.0 $\pm$ 1.2 (7)	5.7 $\pm$ 1.3 (7)	50
10	0/7	7/7	0/7	0	12.0 $\pm$ 0.6 (7)	100
11	0/9	3/9	4/9	2.5 $\pm$ 0.8 (4)	10.0 $\pm$ 0.6 (7)	87
12	1/7	6/7	1/7	13.0 (1)	12.0 $\pm$ 2.5 (6)	85
HCG boiled in saline for 30 min	7/7	0/7	0/7	12.7 $\pm$ 1.3 (7)	0	0
	10/10	0/10	8/10	9.2 $\pm$ 1.0 (10)	3.8 $\pm$ 1.0 (8)	23

Except for the controls, each rat received 50 i.u. HCG, injected daily at 15.00 hours from Days 1 to 12 of pregnancy.

^{*} See Table 1 for definition.

[†] Figure in parentheses indicates the number of rats.

Table 3. Pregnancy-terminating effect of a large dose of HCG administered on different days of pregnancy

Treatment day of pregnancy	No. of rats			Normal implantations/rat (Mean $\pm$ S.E.)	Resorptions/rat (Mean $\pm$ S.E.)	Resorption (%) [*]
	With normal implantation	With total re-sorption sites	With some re-sorption sites			
Control injected with saline (0.2 ml/rat on Day 1)						
1	6/6	0/6	0/6	10.5 $\pm$ 0.5 (6) [†]	0	0
4	6/6	0/6	0/6	6.6 $\pm$ 0.5 (6)	0	0
8	0/6	3/6	3/6	5.3 $\pm$ 0.8 (3)	5.7 $\pm$ 1.0 (6) [†]	68
12	0/6	5/6	1/6	5.0 (1)	8.9 $\pm$ 1.0 (6)	91
	0/5	5/5	0/5	0	7.4 $\pm$ 1.0 (5)	100

Except for the controls, each rat received 500 i.u. HCG.

^{*} See Table 1 for definition.

[†] Figure in parentheses indicates the number of rats.

Table 4. Effect of progesterone on intact rats treated with HCG

Treatment	No. of rats			Normal implantations/rat (Mean $\pm$ S.E.)	Resorptions/rat (Mean $\pm$ S.E.)	Resorption (%) [*]
	With normal implantation	With total resorption sites	With some resorption sites			
HCG† + oil§ (control)	0/6	5/6	1/6	1.0 (1)†	12.3 $\pm$ 0.5 (6)†	98
HCG† + progesterone§	0/6	5/6	1/6	4.0 (1)	12.0 $\pm$ 1.3 (6)	96
HCG¶ + oil§ (control)	0/8	1/8	7/8	5.4 $\pm$ 1.0 (7)	7.1 $\pm$ 1.0 (8)	60
HCG¶ + progesterone§	0/8	0/8	8/8	5.3 $\pm$ 1.1 (8)	5.9 $\pm$ 1.2 (8)	53
Untreated (control)	6/6	0/6	0/6	11.2 $\pm$ 0.6 (6)	0	0

* See Table 1 for definition.

† Figure in parentheses indicates the number of rats.

‡ 500 i.u. HCG was given intravenously on Day 8 of pregnancy.

§ 0.2 ml oil or 2 mg progesterone/rat intramuscularly was given twice daily from Days 8 to Day 14 of pregnancy.

¶ 50 i.u. HCG was given intramuscularly on Day 8 of pregnancy.

Table 5. Pregnancy-maintaining effect of progesterone in ovariectomized rats treated with HCG

Treatment	No. of rats			Normal implantations/rat (Mean $\pm$ S.E.)	Resorptions/rat (Mean $\pm$ S.E.)	Resorption (%)*
	With normal implantation	With total resorption sites	With some resorption sites			
Oil control (0.2 ml/rat)	0/6	6/6	0/6	0	0	0
HCG† + oil	0/6	6/6	0/6	0	0	0
Progesterone§ alone	6/6	0/6	6/6	8.5 $\pm$ 1.8 (6)†	5.5 $\pm$ 1.0 (6)†	38
HCG† + progesterone§	7/7	0/7	5/7	12.6 $\pm$ 1.7 (7)	2.6 $\pm$ 1.6 (5)	13

Rats were ovariectomized under ether anaesthesia at 09.00 hours on Day 8 of pregnancy.

* See Table 1 for definition.

† Figure in parentheses indicates the number of rats.

‡ A single dose of 50 i.u. on Day 8 of pregnancy.

§ Two doses of 2 mg per rat/day from Days 7 to 14 of pregnancy.

Pregnancy-terminating effect of a large dose of HCG administered on different days of pregnancy

The results shown in Table 3 demonstrate that a single large dose of 500 i.u. HCG on Day 1 of pregnancy was not completely effective in terminating pregnancy, since a number of normal implantations were observed on Day 15 and there were no resorbing fetuses. The same treatment on Day 4 of pregnancy was more effective in inducing resorption and the total number of normal implantations/group was reduced. When given on Day 8 or Day 12, the treatment was still more active in inducing fetal resorption.

Effect of progesterone on HCG-treated intact rats

Attempts were made to maintain pregnancy in rats treated with HCG by administering two doses of 2 mg progesterone daily as indicated in Table 4. In rats treated with 500 or 50 i.u. HCG, the progesterone failed to maintain pregnancy. The percentages of resorbed fetuses were similar to those in the oil-treated control groups.

Maintenance of pregnancy in HCG-treated ovariectomized rats with multiple doses of progesterone

Attempts were made to maintain fetal survival by daily treatment with progesterone in ovariectomized rats treated with 50 i.u. HCG.

These rats all resembled the oil-treated ovariectomized control group (Table 5) in failing to show any sign of normal or resorbing implantation. The uterine horns in these animals appeared clean and empty like those of non-pregnant rats. Two daily doses of 2 mg progesterone maintained 62% of normal implantations in ovariectomized rats, and 87% of normal implantations in HCG-treated ovariectomized rats.

DISCUSSION

Our studies showed that a single dose of HCG administered to rats after implantation resulted in fetal resorption and termination of pregnancy. These results are in general agreement with those of Yang & Chang (1968) though the experimental design with respect to dosage and duration of treatments was not similar. It was presumed that the susceptibility of the immediate postimplantation period to HCG was mediated through the alteration of normal ovarian steroidogenesis. It is known that HCG stimulates ovarian production of oestrogen (Gemzell, 1965; MacDonald, Armstrong & Greep, 1966), progesterone (Savard *et al.*, 1965; Armstrong, 1968), and the enzymatic reduction of progesterone to a metabolite, 20 α -hydroxypregn-4-en-3-one (20 α -OHP) (Wiest, Kidwell & Balogh, 1968). Although 20 α -OHP possesses some progestational activity in biological assays (Zander, Forbes, Von Münstermann & Neher, 1958), it failed to support pregnancy in ovariectomized rats (Talwalker, Krähenbühl & Desaulles, 1966). Since the blood levels of progesterone, 20 α -OHP or oestrogen were not measured in the present study, it is not known whether the levels of any of these steroids were affected following a single dose of HCG. In ovariectomized rats treated with HCG, daily doses of progesterone

were able to protect normal implantations which indicated the efficacy of this steroid in maintaining pregnancy under that experimental condition (Talwalker *et al.*, 1966). Progesterone failed to maintain pregnancy in HCG-treated intact rats; however, similar results were reported for PMSG-treated rats and hamsters by Yang & Chang (1968).

The pregnancy-terminating effect of HCG in rats is not surprising since it contains predominantly LH activity, which has been shown to have a luteolytic effect in the rat (Rothchild, 1965; MacDonald, Tashjian & Greep, 1970).

The possibility of enhanced production of oestrogen after a single dose of HCG during the preimplantation period (Yang & Chang, 1968) and expulsion of some of the unimplanted eggs cannot be excluded (Banik & Pincus, 1964). Dreisbach (1959) was successful in interrupting pregnancy in rats with a single subcutaneous dose of 0.4 mg oestrone/kg up to Day 11 *post coitum*. Alteration of physiological levels of endogenous LH, prolactin (Mantalenakis, 1968; MacDonald *et al.*, 1970; Raj & Moudgal, 1970) and increased production of intrauterine luteolytic substance(s) (see Fajer, 1972) after a single dose of HCG have also been suggested. Although the luteotrophic activity of HCG has been emphasized by many investigators (Bradbury *et al.*, 1950; Lunenfeld, 1967; Hanson *et al.*, 1971), the apparent relationship between HCG and the maintenance of the corpus luteum has been questioned by many investigators (Diczfalusy & Troen, 1961; Short, 1969; Strott, Yoshimi, Ross & Lipsett, 1969). In women, HCG may play an important rôle in controlling steroidogenesis in the corpus luteum during the first 4 weeks of pregnancy; after this period, the plasma levels of progesterone and 17-hydroxyprogesterone decline for a while, although HCG levels are still rising (Yoshimi, Strott, Marshall & Lipsett, 1969). Since, in animal experiments, HCG shows detrimental effects on the functional life of the corpus luteum (Stormshak & Casida, 1964; Spies *et al.*, 1966; Moor *et al.*, 1968) and terminates pregnancy in mares (Allen, 1974) and in rats, it would be interesting to determine if a large dose of this compound (e.g. 15,000 to 20,000 i.u.) would terminate human pregnancy in the early stages before the placenta takes over the pregnancy-maintaining steroidogenesis.

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