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EFFICACY OF HUMAN CHORIONIC GONADOTROPHIN (HCG) ON THE MAINTENANCE OF PREGNANCY IN BARBITURATE TREATED RATS

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INTRODUCTION

BARBITURATES block ovulation by inhibiting the preovulatory LH surge which can be prevented by the administration of progesterone, in adult cycling rats. As Pituitary LH is essential to stimulate the progesterone synthesis during day 8–12 of pregnancy, administration of phenobarbital or barbital sodium during this period interrupts gestation. Therefore, in this experiment, attempt has been made to maintain the pregnancy in barbiturate treated rats by the administration of HCG on day 8, 8 to 9 or 8 to 10 of pregnancy as HCG is having long LH-like activities. The results indicate that the foetal survival is reduced in these barbiturates treated HCG administered rats as the duration of HCG treatment is increased. This failure of pregnancy maintenance may be due to the luteolytic action of HCG, as high dose or prolonged treatment of HCG is known to be deleterious to gestation.

Administration of pheno- or pentobarbital prevents ovulation for one day if administered on any day of estrous cycle prior to so called 'critical period', in adult rats and also in pregnant mare serum gonadotrophin (PMSG) primed immature rats[1–5]. This blockade of ovulation is due to inhibition of preovulatory LH-surge which is responsible for the maintenance of progesterone levels necessary for ovulation. Therefore, barbiturate induced blockade of ovulation can be prevented by the administration of progesterone before the pheno- or pentobarbital treatment[2,6]. Administration of phenobarbital or barbital sodium from day 8–12 of pregnancy interrupts gestation which may be due to inhibition brought in the release of pituitary LH, as neutralization of endogenous LH or hypophysectomy during this period causes foetal resorption or abortion in rats[11–14]. Therefore the present investigation is taken up to test the efficacy of human chorionic gonadotrophin (HCG) on the maintenance of pregnancy in phenobarbital and barbital sodium treated rats, as HCG has a long acting LH-like activities[7–10].

MATERIALS AND METHODS

Nulliparous rats of Holtzman's strain weighing 140–180 g, 80–90 days old were caged with proven males at proestrus or estrus. The rats showing sperms in the vaginal smears on the subsequent day were selected for experimentation and the day was de-

signated as day 1 of pregnancy. All the pregnant rats were laparotomized on day 8 of pregnancy to note the number of implantations and treatment was started from the same day.

Experiment I

Earlier tested effective dose of phenobarbital (7.5 mg 100 g body weight)[12, 18] was administered in 0.5 ml saline twice a day from day 8 to 11 of pregnancy. 5 IU HCG (Ayerst, USA)/100 g body weight in 0.25 ml saline was injected to phenobarbital treated rats on day 8 only or from day 8 to 9 or from day 8 to 10.

Experiment II

Tested effective dose of barbital sodium (20 mg 100 g body weight)[12, 18] in 0.5 ml saline was administered twice a day from day 8 to 11 of pregnancy to these laparotomized rats. 5 IU HCG (Ayerst, USA), 100 g body weight was injected to these barbital sodium treated rats on day 8 only, or from day 8 to 9 or 8 to 10.

In both the experiments suitable controls treated with saline or barbiturates were maintained. Experimental rats were housed in individual cages at a room temperature of 27 ± 1°C with Hindustan Lever rat feed and water *ad libitum* with a lighting schedule of 12 hr light/12 hr darkness.

All rats were autopsied on day 20 of pregnancy. The number of implantations, live foetuses, placentomas and placental scars were counted. The ovaries were weighed to nearest mg, fixed in Bouin's fluid, embedded in paraffin and sectioned and stained in haematoxylin-eosin for histological observations.

RESULTS

Pregnancy Maintenance

The saline treated control rats maintain pregnancy with 97.4% foetal survival (table 1). But administration of 7.5 mg phenobarbital or 20 mg barbital sodium twice a day from day 8–11 fails to maintain the pregnancy as nil or 11.1% foetal survival is observed respectively (table 2). 5 IU HCG administered to phenobarbital or barbital sodium treated rats on day 8 only, has maintained pregnancy partially with respective 31.5 or 16.7% foetal survival. In these rats foetal loss is indicated by placentomas and rarely by placental scars indicating that pregnancy failure is mainly due to foetal resorption, rather than abortion. The administration of 5 IU HCG on day 8 and 9 to barbiturate treated rats fails to maintain the pregnancy as negligible (1.4–2.4%) foetal survival is seen. It is more interesting to note that administration of HCG from day 8 to 10 has least effect on the pregnancy maintenance as almost all rats have shown complete resorption of the embryos. Therefore, prolonged treatment of 5 IU HCG seems to be more deleterious for the maintenance of pregnancy in barbiturates treated rats. In almost all rats treated with barbiturates—administration of HCG could maintain only placentomas and not the foetuses. In HCG treated rats vaginal bleeding which is generally observed in mere barbiturate treated rats on day 12–13 is not seen, which again gives an evidence that foetal loss is due to resorption of the embryos.

Foetal Survival

97.4% foetal survival is observed in saline treated

Table 1 *Efficacy of HCG on the maintenance of pregnancy in phenobarbital treated rats*

Treatment	Phenobarbital – 7.5 mg/100 g BW 2 doses/day: Inplantations NT/T	Live foetuses NT/T	% Foetal survival	HCG – 5IU/100 g BW: Ovarian wt mg/100 g BW
Saline	(5) 38/7.60	37/7.40	97.4	39.89
Phenobarbital	(8) 60/7.50	–	–	31.07*
Phenobarbital + HCG				
day 8 only	(7) 54/7.71	17/2.97	31.5	67.01
day 8 & 9	(6) 42/7.08	1/0.17	2.4	50.49
day 8 to 10	(5) 35/7.00	–	–	61.08

NT = Total number; T = Mean.
Number in parenthesis denotes the number of rats.
* $P < 0.05$ in relation to saline treated controls.

Table 2 *Efficacy of HCG on the maintenance of pregnancy in barbital sodium treated rats*

Barbital Sal. – 20 mg/100 g BW 2 doses/day HCG – 5IU/100 g BW

Treatment	Inplant-ations NT/T	Live foetuses NT/T	% Foetal survival	Ovarian wt. mg/ 100 g BW
Saline	(5) 38/7.60	37.7440	97.4	39.89
Barbital sod.	(9) 63/7.00	7/0.78	11.1	25.59**
Barbital sod. + HCG				
day 8 only	(5) 36/7.20	7/1.4	16.7	56.56
day 8 & 9	(6) 58/9.67	1/0.16	1.4	68.00
day 8 to 10	(5) 40/8.00	–	0.6	60.09

NT = Total number; T = Mean
Number in parenthesis denotes the number of rats.
** $P < 0.01$ in relation to saline treated controls.

group because of loss of one implantation as 37 living foetuses are observed out of 38 implantation sites. Phenobarbital or barbital sodium treatment from day 8–11 exhibits nil or 11.1 % foetal survival respectively as 0 foetuses/60 implantations or 7 foetuses/63 implantations are observed at autopsy. HCG administration to phenobarbital treated rats on day 8 only has 31.5 % foetal survival as 17 foetuses/34 implantations are seen while in barbital sodium treated group 6 foetuses/36 implantations are seen with 16.7 % foetal survival. 2.4 % or 1.8 % foetal survival is observed when HCG is administered to phenobarbital or barbital sodium treated rats from day 8 to 9 as only one living foetus is observed in both the groups. HCG treatment for three days *i.e.* from day 8 to 10 has no foetal survival as almost all rats exhibit only placentomas and rarely placental scars but no foetuses.

Gravimetric and Histological changes of the ovary

The ovaries of the saline treated rats weigh 39.9 mg and show many large, well developed corpora lutea which is a characteristic of pregnancy. But phenobarbital or barbital sodium treatment reduces the ovarian weight significantly ($P < 0.05$ to 0.01) as the ovaries weigh 31.1 or 25.6 mg respectively. These ovaries show small corpora lutea and many developing follicles like those of non-pregnant rats as these rats return to estrus within 3–4 days after abortion. HCG treatment on day 8, 8 and 9 or 8 to 10 stimulates the ovarian growth, therefore; these ovaries are significantly heavier when compared to their respective controls ($P < 0.01$ to 0.001). The corpora lutea of these ovaries are though large enough, show luteolysis with prominent spaces inside. Therefore pregnancy failure in barbiturate treated rats even after HCG administration, may be because of this luteolytic action of corpora lutea.

DISCUSSION

Administration of LH to the hypophysectomized rats on day 8 or 9 of pregnancy maintains gestation successfully[11, 14]. It is also observed that treatment of LH-antiserum during this period terminates gestation[13]. Therefore, LH seems to be the main luteotrophin during day 8–11 of pregnancy in rats. As barbiturates block the pituitary LH surge and its tonic release, the interruption of pregnancy by barbiturates may be due to continued blockade of LH release during early part of gestation[1–5, 12]. Therefore this attempt to maintain pregnancy in barbiturate treated rats by HCG has been made, as HCG has a long acting LH-like activities stimulating the steroidogenesis[7–10].

In the present experiment, administration of HCG to phenobarbital treated rats on day 8, 8 and 9 or 8 to 10 has 31.5 and 2.5 % or nil foetal survival respectively, though the ovaries of these rats are significantly heavier with large corpora lutea. Similar results are obtained in barbital sodium treated rats also where the percent foetal survival is 16.7, 1.8 or nil respectively, with HCG administration on day 8, 8 and 9 or 8 to 10. Therefore prolonged treatment of HCG is detrimental to pregnancy, as low foetal survival is observed. Yang and Chang[15] have also observed that 100, 200, 400 or 800 IU HCG injected on day 5, 6 or 7 of pregnancy show 72.1, 81.9, 42.6 or 30.1 % respective foetal mortality[15].

These results with the present results suggest that increased dose or prolonged treatment of HCG is deleterious to gestation.

Yoshinaga *et al*[11] have shown that daily doses of 10 μg and 25 μg LH are found optimal for the maintenance of pregnancy, as higher or lower doses are less effective; higher doses of LH may stimulate the secretion of estrogen more than that of the progesterone, leading to resorption of foetuses[11]. Even in the present study, the foetal loss after HCG treatment may be because of high estrogen production leading to the resorption of the embryos, as no vaginal bleeding is observed. The persistentance of placentomas in the uterus observed on day 20 of pregnancy also supports that foetal loss is mainly due to resorption and not due to abortion. Though the ovaries of HCG administered, barbiturate treated rats are significantly heavier with large corpora lutea, they do not show normal histological appearance which may be partly due to the luteolytic activity of HCG and partly due to absence of placental gonadotrophins[16, 17]. However, exact dose and duration of the administration of HCG to maintain the pregnancy in barbiturate treated rats is to be studied.

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ANNOUNCEMENT

PANDIT JAWAHARLAL NEHRU AWARD FOR SCIENCES

The First Pandit Jawaharlal Nehru Award for Sciences has been awarded to Dr. Avtar Singh Paintal, Director, Sardar Patel Chest Institute, New Delhi presented by Mr. Zail Singh, the President of India.

The Award was instituted by the Madhya Pradesh government to promote excellence in sciences and to give recognition in outstanding contribution to sciences, the Award carries a cash prize of Rs. 1 lakh, a plaque and a citation.