

# Effect of maternal cigarette smoking on placental secretion *in vitro*

R Shurtz-Swirski<sup>2</sup>, ER Barnea<sup>1,2</sup>, R Korenblum<sup>2</sup>, JH Check<sup>1</sup>

<sup>1</sup>The University of Medicine/Dentistry of New Jersey Robert Wood Johnson Medical School at Camden Cooper Hospital/University Medical Center Department of Obstetrics/Gynecology Division of reproductive Endocrinology/infertility Camden, NJ, USA <sup>2</sup>Feto-Placental Endocrinology Unit, Technion, Haifa, Israel

Address all correspondence to: Eytan R. Barnea, M.D., 7447 Old York Road, Melrose Park, PA, 19126

## abstract

hCG secretion by superfused placental explants is spontaneously pulsatile and is modifiable by various factors. This pulsatility was evaluated in placentae obtained from women who smoke cigarettes in weeks 7 through 9 of pregnancy. Compared to the non-smokers there were significant changes in the pulse parameters as analyzed by the PULSAR programs in heavy smokers (those who smoked more than 20 cigarettes per day) there was a significant decrease in mean pulse amplitude and area under the curve. This was most evident with 60 cigarettes per day where pulse frequency decreased from 16 to 7 as well. In static cultures of explants incubated overnight from women smoking more than 20 cigarettes per day, a three or more fold lower level of hCG was secreted when compared to samples obtained from non-smoking women. *in vivo* exposure to environmental contaminants such as cigarette smoking affect markedly both the pattern and total hCG secretion by the placenta *in vitro* during the first trimester.

## Introduction

Cigarette smoking is a major cause of human morbidity / mortality (Heidelberger, 1975), that affects several aspects of fertility including pregnancy, causing spontaneous abortion, placental separation, and low birth weight (McIntoch, 1984, Pirani, 1978). Maternal smoking affects pregnancy as early as the first trimester of pregnancy.

The placenta is a multifunctional organ which separates the mother/fetus; its major marker in the first trimester is hCG. The precise mechanisms involved in control of hCG secretions have not been elucidated. Most studies involved the use of static culture models, at term (Kliman et al., 1986, Belisle et al., 1984, Zhou et al., 1987., Kato / Braunstein, 1990). In dynamic first trimester explant cultures, hCG patterns of secretion resemble hormone secretion *in vivo* (Barnea/Kaplan, 1989; Owens et al., 1981). hCG pulsatility in superfusion was modulated by gonadotropin releasing hormone (GnRH), (Barnea et al., 1991), progesterone (Barnea et al., 1991b), dynorphin (Barnea et al., 1991c), growth factors like epidermal growth factor (Barnea et al., 1990) and human embryonal organs (Barnea et al. 1989b).

In the present study hCG secretion was examined in static cultures and superfused placental explants. Heavy smoking reduces total hCG secretion as well as spontaneous hCG pulsatility.

### Materials / Methods

1. **Placental Material:** Twenty 7 to 9 week old placentas were studied. After obtaining appropriate consent, elective pregnancy terminations by vacuum curettage were performed. Patients were healthy and did not use any medication. Eleven patients were smokers and consumed 20 to 60 cigarettes per day. Only patients that consumed cigarettes all through pregnancy until the day of the procedure were used. Collected tissue was rinsed several times in cold 0.9% NaCl to remove all blood, followed by three further rinses with culture medium (Dulbecco's Modified Eagles Medium, DMEM, Beit Haemek, Israel) containing penicillin 5000U/ml, streptomycin 20 ug/ml, and amphotericin B 50 ug/ml.
2. **Explant Cultures:** Explants 50-70 mg wet weight were dissected out and rinsed in 1% antibiotic solution (Barnea et al., 1990, 1991b). For culture, explants from smokers and non-smokers were placed in dishes. At least three replicates per placenta were plated at 37°C in an atmosphere of 95% air and 5% CO<sub>2</sub>. After overnight incubation, the media were collected / stored at -20°C until assayed. The tissue was saved for protein analysis.
3. **Superfusion Studies:** A superfusion apparatus (Accusyst, Endotronics, St. Paul, MN) with a multichannel peristaltic pump and fraction collector (Model 272, ISCO, Durham, NC) was used to study the short-term dynamics of hCG secretion (Barnea et al., 1991; Barnea/Kaplan, 1989). The explants (200-300 mg wet weight) were placed into the culture chambers and a HEPES (18mM) DMEM solution was washed through in an atmosphere of 5% CO<sub>2</sub> and 95% air at 37°C. Experiments were conducted for 2-h; a 1 ml sample from the effluent was collected every 2.4 minute for hCG measurements. In each experiment, one channel served as control and five serves as experimental channels. Media collected were placed in -20°C until assayed.
4. **Assays:** bhCG radioimmunoassay was carried out (Barnea / Kaplan, 1989; Barnea et al., 1990) using hCG MAIA clone assay (Serono), with an intraassay variability of 1.7%. Placental tissue protein levels were measured by using bovine serum albumin as the standard (Lowry et al., 1951).
5. **Statistics:** Statistical analysis was performed by one-way ANOVA and Student's t-test, with  $p < 0.05$  considered statistically significant. For superfusion experiments, the area under the curve, mean peak amplitude, and peak frequency were calculated using the PULSAR program. Data for bhCG concentrations were expressed as mIU/mg protein. The data in superfusion experiments was representative of patterns of hCG secretion by explants of cigarette smokers or controls.

## Results

1. **Static Culture:** The total hCG secretion by placental explants from smoking mothers was evaluated following overnight incubation. There was at least a three-fold decrease in the concentration of hCG accumulated in the media following overnight incubation in samples from patients who consumed 30 or more cigarettes per day (Fig 1). The most pronounced effect was noted with 60 cigarettes a day. The levels of secreted hCG in non smokers was similar to that of those who smoked only 20 cigarettes per day.

2. **Superfusion:** The effect of maternal smoking upon the dynamic hCG secretion by superfused explants. Compared to non smokers the pattern of hormone secretion by placenta of heavy smokers was different. There was a dose dependent decrease in overall hCG secretion as well as a change in the pulse pattern with the increased number of cigarettes smoked. This was evidenced by some time intervals where hCG levels became barely detectable and pulsatility was absent. As expected the most noticeable loss of pulsating patterns was associated with the heaviest smoking. The effect of 20 cigarettes per day was, however, mild (Figure 2, Panel B). Statistical analysis of the pulsatile behavior using PULSAR revealed a clear dose response relationship between the number of cigarettes consumed per day and the mean peak amplitude (PA) and area under the curve (AUC). A significant decrease in the peak frequency was noted only with 60 cigarettes per day (Table 1).

**Table 1** PULSAR analysis of significant peaks, following superfusion of placental explants of cigarette smokers.

	PF	PA	AUC
Controls	16	47.3	2481
20 cig/day	17	53.6	3922
30 cig/day	17	3.3*	264*
40 cig/day	13	2.4*	153*
60 cig/day	7*	1.82*	32.8*

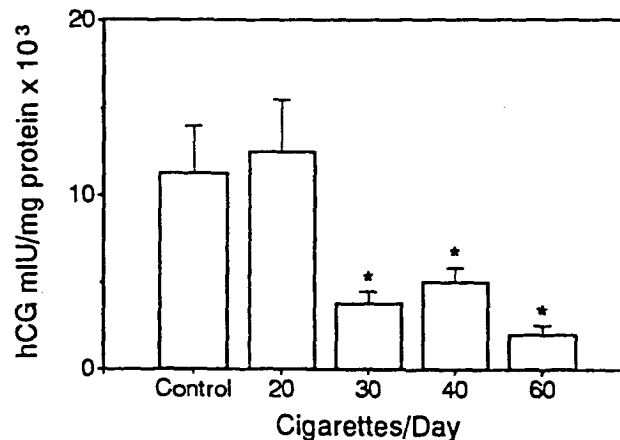
PF = peak frequency, PA = peak amplitude, AUC = area under the curve,  $p < 0.05$  vs control.

## Discussion

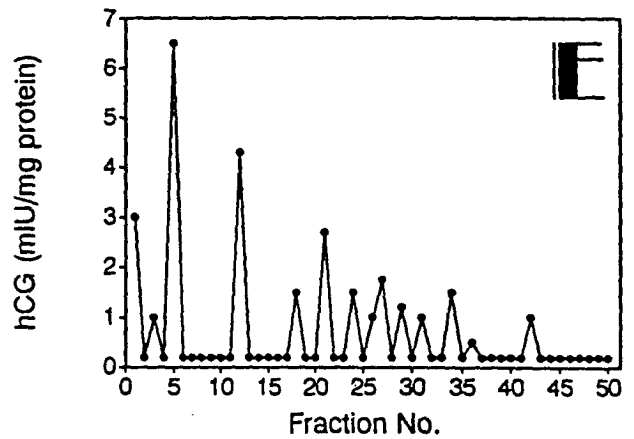
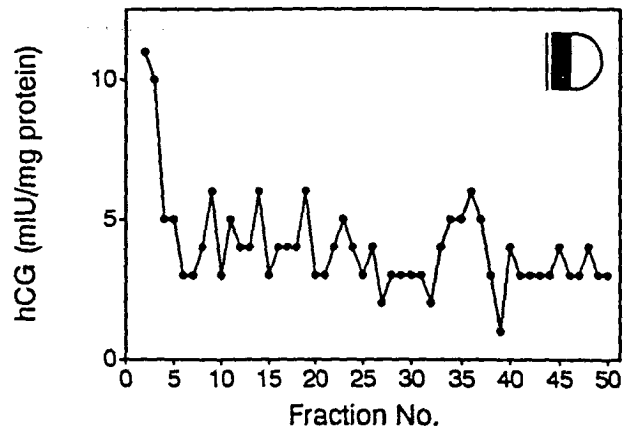
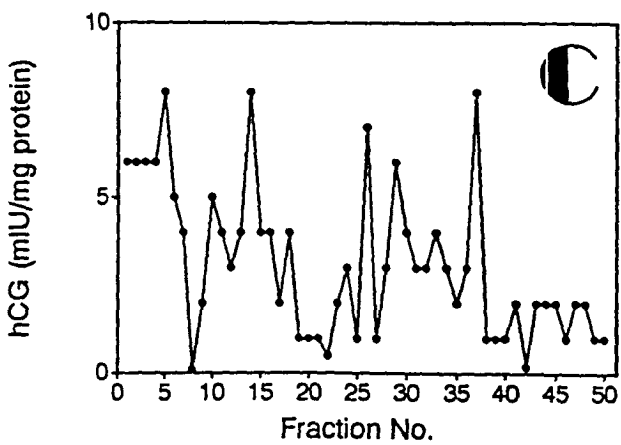
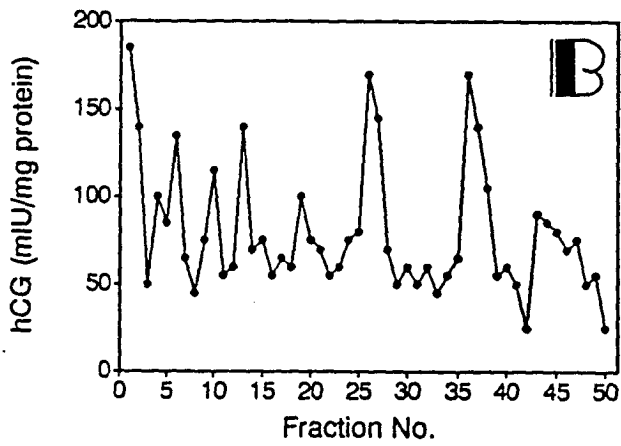
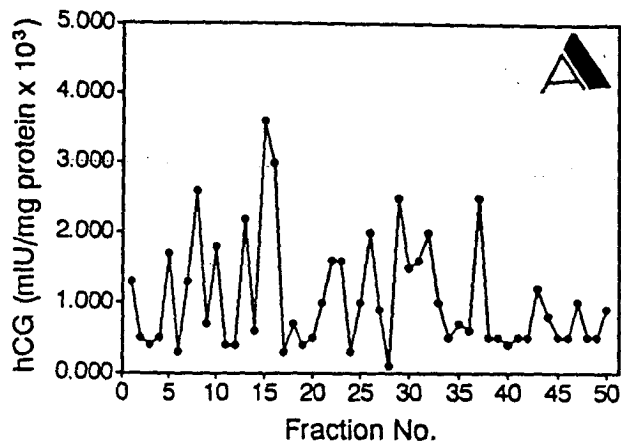
Maternal cigarette smoking affects placental hCG secretion in the first trimester. The hormone secretion *in vitro* was affected by maternal exposure *in vivo*. This change was noted mostly in heavy smokers < 20 cigarettes per day. The effect was dose-dependent since the pulse parameters PA and AUC decreased as cigarette consumption increased. The decrease was most pronounced when the woman smoked 60 cigarettes/day. In this case the pulse frequency was less than half of normal.

In static cultures, total secretion of hCG into the media is low in heavy smokers (30 cigarettes per day and above) when compared to controls and those who smoked only 20 cigarettes per day. This strengthens the data from the superfusion cultures where the same number of cigarettes smoked caused the pulse parameters to be affected. This suggests that there is some threshold of exposure below which placental hCG secretion is not affected by cigarette smoke.

The use of the two models illustrates hCG secretion can be studied in superfusion compared to static cultures where only the total output of hCG can be measured. Moreover, in static cultures there is feedback by the surrounding media of the explant. By contrast, in superfusion the placenta is free of such influences because of the unidirectional flow of the culture media which does not allow accumulation of effectors in explant surroundings.



**Figure 1** bhCG secretion by placental explants cultures obtained from women who smoked cigarettes during pregnancy. Data is expressed as mIU hCG/mg protein +/-SE. \* $p < 0.05$  compared to non-smoking controls.



**Figure 2** bhCG secretion by superfused placental explant cultures. Panel A-Non smoker, Panel B -20 cigarettes/day, Panel C -30 cigarettes/day, Panel D -40 cigarettes/day, Panel E -60 cigarettes/day.

This is the first research that shows a correlation between *in vivo* exposure to a common pollutant and hCG secretion *in vitro* in a quantifiable manner. How cigarette smoke affects hCG secretion is not clear since the smoke is composed of a large number of compounds. The effects of some isolated cigarette smoke derived compounds on hCG pulsatility *in vitro*. Benzo(a)pyrene, a major carcinogen, and cadmium, a toxic metal, increased hCG pulsatility in a dose-dependent manner following preincubation with the compounds for 24h. It would appear that there are differences between short term *in vitro* and long term *in vivo* type exposures which are likely to involve different mechanisms or to cause different effects. On the other hand, other compounds thus far untested like CO<sub>2</sub> and nicotine could have affected the stimulatory effect of benzo(a)pyrene or cadmium, thus decreasing hCG production and/or secretion. Benzo(a)pyrene also affects placental metabolism *in vitro* by stimulating aryl hydroxylase activity in the first trimester which activates these carcinogens (Barnea/Avigdor 1991b.)

The decrease in hCG pulse frequency is of interest. Specific storage granules for hCG are found in the placenta in the first trimester (Morrish et al., 1987). Reduction in the number of such storage granules and some form of inhibition of a local putative pacemaker, like endogenous GnRH for example, may have caused this reduction. It is rather rare to find an agent that reduces pulse frequency. Indeed, only progesterone and GnRH antagonist were effective in this regard (Barnea et al., 1991b). Thus, a reduction in the pulse frequency suggests that the effect of maternal smoking is both significant and specific, only with heavy smoking.

The effect of cigarette smoking is long-term and preserved even though there is no longer a direct contact with the smoke constituents. This suggests that some of the smoke-derived compounds have been incorporated in the placental tissue and that they continue to exert their influence throughout the incubation period. The exact steps involved in altered hCG secretion remain to be elucidated. Xenobiotic metabolizing enzyme activity aryl hydrocarbon hydroxylase is increased in term placentas of cigarette smokers, and specific DNA adducts were identified as well (Everson et al., 1986). Maternal cigarette smoking affects the placenta in the first trimester at the time of embryogenesis.

The use of the superfusion model enables the expression of xenobiotic-induced changes in pulsatile hCG secretion that are not detectable by static cultures (Barnea et al., 1990). The early placenta is vulnerable to environmental influences and this may explain, at least in part, the increased rate of spontaneous pregnancy loss which occurs in the early second trimester in heavy cigarette smokers.

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