

# In Vitro Study Cocaine Decreased Progesterone Synthesis in the Isolated Cytotrophoblast Cells and in Situ Study PGE<sub>2</sub> and PGF<sub>2A</sub> Levels Increased N the Amniotic Fluid in Cocaine Users in Humans

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## Abstract

In vitro study the capacity of isolated cytotrophoblast cells (placental cells) treated with cocaine hydrochloride ranging in dose level from 1-3.00  $\mu$ molar (0.25-75 mg/ml).to synthesize progesterone was examined. 25 hydroxycholesterol (OHC) or low density lipoprotein (LDL was added in the media as substrate to stimulate progesterone synthesis. In situ study, prostaglandins levels (PGE<sub>2</sub> and PGF<sub>2A</sub>) were measured in the aspirated amniotic fluid obtained from subjects who used cocaine throughout their pregnancy. The data show progesterone synthesis was significantly decreased ( $p<0.05$ ) in vitro study, in situ study prostaglandin (PGE<sub>2</sub> and PGF<sub>2A</sub>) levels in the aspirated amniotic fluid were significantly increased ( $p<0.01$ ). To determine whether cocaine effects cAMP synthesis in the isolated cytotrophoblast cells, cAMP level was measured in the presence of cocaine the data showed decreased cAMP level ( $p>0.01$ ). Data on birth weights of newborn and gestational age of mothers in cocaine users showed a significant decrease ( $p<0.05$ ) and the incidence of placental abruptio, a serious complications in pregnancy showed significant increase ( $p<0.001$ ) in cocaine users.

We conclude that cocaine induced decreased progesterone synthesis in the placental cells and an increased prostaglandins level in the amniotic fluid is the cause of early termination of pregnancy in subjects using cocaine during pregnancy.

**Keywords:** Placenta, Cocaine, Progesterone, Prostaglandins, Humans

## 1. Introduction

It is estimated that 50 million people in United States use cocaine on a regular basis and almost one million are women ranging in age from 18-34 years. (Das, 1990) The National Association of Perinatal Addiction Research and Education reports that almost 11percent of all pregnant women are substance abusers, cocaine accounting for most abuse (Smith and Asch 1987, Chavkin, and Kandall 1990). Exposure of cocaine during pregnancy contribute to several medical problems including placental previa, placental abruption (Acker *et al* 1983, Chasnoff *et al* 1992, and Botros, Atterbury and Groome, 1996) and premature births ( Chasnoff *et al* 1992) fetal growth retardation (Bingol 1987, Das 1994), numerous neurobehavioral deficits ( Hoyme *et al.*, 1990, Plessinger and Woods 1993, ). Even after variables such as race, age, smoking and poly drug use are controlled, there is a little doubt that the use of cocaine during pregnancy is significantly associated with adverse pregnancy outcome. (Little *et al*, 2003).

A question how does cocaine contribute to early termination of pregnancy in humans is not yet fully resolved. Several hypothesis have been presented and generally it is accepted that cocaine causes vasoconstriction of placental blood vessels (Moore *et al* 1986, Monga *et al* 1994) and thus decreasing the blood supply to the fetus and causing fetal anomalies. Although there is a strong evidence to support this hypothesis, it still does not explain fully the cause of commonly observed condition in cocaine users in humans, an early termination of pregnancy. (Botros, Atterbury and Groome 1996, Das 1994,. Studies were designed to examine whether cocaine affects fetal-placental endocrine milieu namely progesterone and prostaglandins synthesis, two vital biochemical events taking place preceding pregnancy termination in mammals including humans. The studies were conducted in vitro and in situ, in vitro study, effect of cocaine on progesterone synthesis was determined using isolated placental cells (cytotrophoblast cells), in situ study, prostaglandins (E<sub>2</sub> and F<sub>2A</sub>) level in the aspirated amniotic

fluid was determined in subjects who used cocaine on a regular basis throughout their pregnancy.

## 2. Materials

### Selection of Human Subjects

All participants in the study were volunteers and signed consent form which was approved by Institutional Review Board. The subjects came to Howard University Hospital for delivery. Medical history as well as substance abuse information was obtained from each subjects prior to their participation in the study. For in vitro study, each subject's history was reviewed and only those who provided negative history of drug abuse (licit and illicit) and tested negative for the presence of cocaine metabolites in the urine, free of infection, normal histology (microscopic examination) and morphology of the placenta. These subjects used no medication during pregnancy including over the counter medication in particular they had not taken aspirin or nonsteroidal anti-inflammatory medication in the weeks prior to delivery.

### Urine Test

Each subject was tested for the presence of benzoylecgonine (cocaine metabolite) in the urine (0.5ml) regardless of their medical history given to us using Synchron LX system. Drugs of Abuse Testing. The test is a qualitative determination it utilizes a homogenous enzyme immunoassay, it comprises specific antibodies which can detect benzoylecgonine in urine. Briefly, the principle of the assay is as follows. A drug labeled glucose-6-phosphate dehydrogenase conjugate competes with any free drug from the sample, the drug labeled G6PDH conjugate is bound by the specific antibody and enzyme activity is inhibited. This reaction creates a direct relationship between the presence of drug and enzyme activity G6PDH enzyme activity is determined spectrophotometrically by measuring its ability to convert nicotinamide adenine dinucleotide (NAD) to NADH (reduced form). The system can be calibrated from 0 ng/mL benzoylecgonone to 3000 ng/mL benzoylecgonine. The results are reported as positive or negative. A sample rate greater than or equal to cutoff rate is reported as positive (> 300 ng/mL). A negative test result indicates that cocaine metabolites are either not present, or are present at levels below the cutoff threshold of the test. Cross activity chart provided by the manufacturer indicate that none of the substance abuse has negative cross activity with this test. Inquiries into maternal drug use were made throughout the prenatal course and reported in the patient's chart is consistent with negative responses were considered reliable for this study. A resident physician explained the scope of the project and provided the participants with questionnaire. The selection of participants in the program was based on the following criteria: a) non drug users, essential or nonessential throughout pregnancy; b) no evidence of infection or known nutritional deficiencies at the time of delivery; 3) all had normal delivery with gestation age between 38-40 weeks.

The participants were of low socioeconomic status of indigent population and were in the age group between 20-25 years.

The duration of pregnancy was assessed by the date of the last menstrual period and by ultrasound examination.

For in situ study, subjects were tested for drugs and those who tested positive for cocaine and those who reported use of cocaine more than once per week throughout pregnancy were included in the study.

A total of 16 subjects were selected for the study. An equal number were used in the control group.. The groups were divided in sub groups of four (4). The differences between subgroups were not significant therefore all samples (sixteen) were pooled in each group and the data presented here are the in figures are the averages of sixteen subjects (16) in each group. The study was completed in two years period.

### Culture Medium and Fetal Calf Serum

Culture medium and fetal calf serum were purchased from Grand Island Biological Company (Grand Island, NY), culture dishes from Falcon Plastics (Cockeystown, MD), bovine serum albumin (Pentex fraction V) from Miles Laboratories (Kankakee, IL), co-factors (NADP,Glucose 6- phosphatase and G-6-dehydrogenase) from Sigma Chemical Co. (St.Louis, MO) 25- hydroxycholesterol was obtained from Sigma chemicals, cAMP assay kit was obtained from New England Nuclear. (Boston, Mass)

Cocaine (cocaine hydrochloride) was obtained from National Institute of Drug Abuse under a license.

### Cocaine hydrochloride:

Cocaine hydrochloride was acquired from NIDA in powder form and was dissolved in sterile water. Sterility was ensured by filtration through 0.22 um filter z(Millipore Corp, Bedford, MA) and verification with the litmus amebocytes lysate test (BioWhittaker Bioproducts, Walkersville, MD) for detection of Gram-negative bacterial endotoxins.

### Stock solution of cocaine

A stock solution of cocaine HCL was prepared by adding 5 mg of the compound to 10 ml of pH 7.4 Krebs-Ringer phosphate buffers. To one ml of stock solution was then added in 20 ml glass vial and Krebs-Ringer phosphate buffer pH 7.4 diluted to total of volume of 5 ml were pipetted into each of the 20 ml glass test tube. Varying level of cocaine concentration was added to the cells.

### Dose level of Cocaine

Several factors were taken into account for determining dose levels of cocaine in the media in vitro study. With the reported value in the blood of cocaine users being in the range of 1-2.5  $\mu\text{M}$  or 0.68 mg/L. We decided to use 1.0-3.0  $\mu\text{M}/\text{ml}$ . The dose level is consistent with plasma concentration similar to those of cocaine users (0.75 $\mu\text{g}/\text{ml}$ ). 10 to 40 mg is the usual intoxication dose in adults (Stead and Moffat 1983). Reported that concentration levels of cocaine in whole blood greater than 0.9 mg/L are reported to be associated with fatalities in both fetus and adults. Preliminary studies were conducted using 1.00, 1.5, 2.1, 2.8, and 3.00 $\mu\text{M}$  cocaine the controls were incubated with equivalent volume of media solution. Cocaine up to 1.5  $\mu\text{molar}$  had no effect on progesterone synthesis. Differences between 2.1 and 3.0  $\mu\text{molar}$  of cocaine were not significant. For all incubation 2.1  $\mu\text{molar}$  cocaine was used. All incubation was done using 6 hr. period using  $2 \times 10^6$  cytotrophoblast cells/ml.

Assay solution was prepared by combining 4 ml of 0.75  $\mu\text{g}/\text{ml}$  cocaine resulting final solution of 5 ml. This concentration of cocaine has been documented to be achievable through typical intranasal administration (Stead and Moffat 1983).

### Amniotic fluid Collection

At the time of birth, approximately 50 ml of amniotic fluid and 20 ml of umbilical arterial blood (fetal blood) were collected by an attending physician and precautions were taken not to contaminate the samples. Samples which were free of blood, mucus, or cell debris were chosen for the assays. All samples were stored at -20 until assayed. After the delivery, no follow up of the participants or their newborn was undertaken. The cord blood sample were used for another study to be published elsewhere.

Birth weights of newborn, gestation age and placenta abruption were recorded at the time of delivery.

### Collection of Placenta

Placenta was obtained aseptically upon delivery, and placental cells (cytotrophoblast) were isolated within 2 h of delivery. Only the healthy part of the placenta was used for the study and sections were obtained from various part of the placenta in order to represent the entire placenta and care was taken not to use the necrotic and calcified portion of the placenta.

All solutions and glassware used in the preparation were sterilized by autoclaving.

### Isolation of trophoblast cells (cytotrophoblast cells)

#### Procedure

The procedure involving the initial handing of placenta and preparation of cytotrophoblast cell isolation was accomplished according to our procedure published elsewhere (Ahluwalia *et al.*, 1992) with minor modification. Briefly the procedure is as follows: approximately 30-35 g of villous tissue was cut and washed several times with sterile saline solution and placed in sterile Petri dishes containing Hank's balanced salt solution (HBSS) without sodium bicarbonate (Gibco). The tissue was minced and centrifuged for 20 min at 1200g. The supernatant was discarded and the tissue was digested with trypsin solution and DNAase (Sigma) bovine deoxyribunuclease -1 (EC 3, 121.1) (1200 K at 37 C in a water bath shaking at 120 cycles/min.). After the incubation supernatant was aspirated off and filtered through a sterile NU gauze (Johnson and Johnson), 4x4.3 plyrayon /polyester) and 10%solution of fetal bovine serum (Gibco 200-6140 PG) was added. The filtered cell suspension was gently layered on the tubes and centrifuged for 20 min at 1200g. The supernatant was poured off and the pellet was re- suspended in RPMI to a total of 10 ml. A 4-ml sample was saved for loading into preformed Percoll gradient in a 50 ml conical centrifuge tube. The gradient was made by layering 14 solutions of 70 to 5% of Percoll (v/v) in 5% steps of 3 ml in 90% Percoll (18 ml Percoll and 2 ml HBSS 10x) in a 50 ml conical centrifuge tube. Cell suspension was gently layered and the contents were centrifuged at 200g for 20 min. The cytotrophoblasts were trapped in the layer that occurred between the 18-27 ml markings on the tube. This layer was aspirated and diluted up to 50 ml with RPMI, vortexed and centrifuged. The pellet was re- suspended in 10 ml of RPM containing 1 ml antibiotic/antimycotic (Gibco,1000x lyophilized penicillin G sodium, streptomycin, amphotericin. The cells were counted in a hematocytometer. Between  $40-50 \times 10^6$  cells were obtained from a

30-35 g of placental tissue.

#### **Characterization of cytotrophoblast cells and their cell viability**

The identification of cytotrophoblast cells was done according to the method described by Ahluwalia *et al.*, 1992.

#### **Isolation of Mitochondria and Microsomes**

Since steroid synthesis takes place in mitochondria and microsomes within the cellular system these organelles were isolated using differential gradient centrifugation (Ahluwalia *et al* 1992).

#### **Incubation conditions, number of cells used for incubation (cytotrophoblast cells) and dosage of cocaine**

A series of studies were conducted to determine optimum incubation period, approximate number of cells for incubation and dosages of cocaine added to the medium. Based on these results we choose 6 h as incubation time,  $2 \times 10^6$  numbers of cells and 2.1  $\mu\text{molar}$  concentration of cocaine to obtain measurable amount of progesterone synthesis. **Preparation of lipoprotein fraction (LDL).**

Preparation of LDL was done according to procedures described by Havel *et al.*, 1955 and modified by Ahluwalia *et al.*, 1992.

#### **Separation of Progesterone on column chromatography.**

A modified method described by Stone *et al.*, 1971 was used to separate progesterone on a celite column using benzene, methanol, and isoctane (5:10:85) as solvent system. This solvent system clearly separates progesterone from cholesterol. The recovery of progesterone was approximately 80-85%.

Progesterone values are expressed as nanogram progesterone per  $2 \times 10^6$  cells per 6-h incubation period. The extraction efficiency of progesterone assay was done according to the procedure described by Ahluwalia *et al* 1992.

#### **Use of LDL and 25-OH cholesterol (OHC) to stimulate progesterone synthesis**

LDL and OHC has been used as substrate to stimulate progesterone synthesis. Klinman 1986, and Hellig, *et al.*, 1970 reported that cytotrophoblast cells respond with an 8 fold increase in progesterone synthesis using LDL as substrate and to a lesser extent with OHC.

#### **Measurement of cAMP in the cytotrophoblast cells in the presence of cocaine**

Cytotrophoblast cells at a concentration of  $2 \times 10^6$  were pipetted into each of the 20 ml glass test tube Cell /ml with DMEM and 10  $\mu\text{l}$  of 25 -OH cholesterol (OHC) was added to each sample. Cocaine at 2.1  $\mu\text{molar}$  was added to each sample and samples were vortexed for 2 minutes and incubated for 2, 4, and 6 h at 37 °C in shaking water bath. At the end of incubation, the samples were stirred at -20°C until assayed. The samples were extracted and assayed using cAMP  $^{125}\text{I}$  RIA kit. The inter assay coefficient of variation ranged from 6.8-9.9% and intra assay coefficient of variation ranged from 6.8-15%.

#### **Prostaglandin's levels in amniotic fluid**

Prostaglandins were extracted using 6 volumes of acidified diethylether. The organic solvent was evaporated under nitrogen, and the residue was dissolved in phosphate buffer (pH 7.4). The concentration of prostaglandins was corrected for extraction efficiencies. PGF<sub>2α</sub> and PGE<sub>2</sub> were measured by tritium-labeled RIA kit (Advance Magnetics, Inc; Cambridge, MA). The cross activities with other prostaglandins were less than 1.0%. The sensitivity of the assay was 2.7 pg/ml and 50% binding for the standards was 50.5 pg. The extraction efficiency was 81.2%. Solvent blanks values were subtracted from measured values.

#### **Statistical analyses**

Data were analyzed using student's *t* test or analysis of variance followed by Mann-Whitney test for corrections.

### **3. Results**

#### **Preterm and low birth weight babies born to cocaine users**

Shown in Table 1 is the pregnancy outcome in cocaine users and controls (drug free). Although data presented here are on rather small number of subjects (sixteen subjects in each group), the results show that there was a significant decrease in gestation age and birth weights in cocaine users compared with control. Placenta abruptio was significantly increased in cocaine users 18% compared to 6 % in non drug users (control)

#### **Progesterone biosynthesis in the cytotrophoblast cells**

Shown on Figure 1 is progesterone synthesis in the cytotrophoblast cells. ( $2 \times 10^6$  /6 h incubation) with and without

exogenous substrate. The results showed that, in the control (without exogenous substrate) measurable amount of progesterone synthesis took place for up to 6 h suggesting that preformed cholesterol was present in the cell organelles. However, the amount of preformed cholesterol was limited, because incubation beyond 6 h did not increase progesterone synthesis. With OHC or LDL as a substrate in the medium, progesterone synthesis increased by several-folds compared to control, ( $p<0.01$ ). Differences in progesterone synthesis with LDL or OHC as substrate was not significant. When dibutyryl cAMP (0.20mM) was added in the medium along with OHC there was further increase in progesterone synthesis compared to LDL or OHC no cAMP in the medium suggesting the role of cAMP in progesterone synthesis. When cocaine (2.1  $\mu$ molar) was added in the medium in the presence of 25-OHC there was a significant decrease in progesterone synthesis ( $p<0.01$ )

Shown in the same Figure is the results of cAMP levels in the cytotrophoblast cells in the drug free (controls) and cocaine treated cells. The results showed significant decrease in the level of cAMP in the presence of cocaine suggesting the decrease in progesterone in the presence of cocaine is caused by decrease in cAMP levels. The decrease in cAMP level in the presence of cocaine is probably caused by inhibiting either the conversion of ATP →AMP or the breakdown of formed cAMP by phosphodiesterase.

Shown in Figure 2 are PGs levels in amniotic fluid. The data show that in cocaine users PGE<sub>2</sub> and PGF<sub>2</sub> alpha levels were increased significantly ( $p<0.01$ ) in the amniotic fluid. In cocaine users PGE<sub>2</sub> levels predominate. Shown in the same figure is prostaglandins synthesis in isolated fetal membranes in cocaine users and drug free subjects. Data show that PGF<sub>2 $\alpha$</sub>  and PGE<sub>2</sub> levels were significantly higher ( $p<0.01$ ) in cocaine users compared to drug free. Differences between PGF<sub>2 $\alpha$</sub>  and PGE<sub>2</sub> were not significant.

#### 4. Discussion

Some explanation is needed to interpret the data presented here. First, whether subjects who participated in the study can be relied upon their oral account of drug use and whether they had used drugs other than cocaine during pregnancy and second, does isolated cytotrophoblast cell preparation from placenta represents placental fetal endocrine functions.. To answer the first question, it should be noted that subjects who participated in the study tested positive for cocaine only and none other drugs confirmed their oral history of cocaine use as entered in their medical chart, furthermore, none of the subjects who denied using any drug during pregnancy tested positive for drugs tested in the study. There still remains a question, for how long and how much cocaine these subjects used during pregnancy. Based on common knowledge among obstetricians that pregnancy brings incentive to women to tell the truth we believe the truthfulness of subjects that they used cocaine and none other drugs throughout their pregnancy is believable. Still a possibility can't be ruled out that subjects on occasion may have used drugs other than cocaine for example alcohol, cigarette smoking. However, consistency of our data and the skill and trust of attending physicians towards their patients make us believe that drugs other than cocaine if used by the subjects occasionally has negligible effect on the results of our study. The answer to the second question is simple and the data presented here and elsewhere are compelling, isolated placental cells have been used routinely by several investigators to examine fetal placental endocrine functions (Hall *et al* 1977, Kliman., Feinman and Strauss 1987, Ahluwalia, 1992 ). In this study, the fact that isolated cytotrophoblast cells responded to stimuli such as LDL or 25 OHC by increasing progesterone synthesis strongly suggest that steroid producing enzyme system remains intact after their isolation and purification, moreover membrane receptors were not affected by several washings including the use of trypsin during the isolation procedure. A possibility that our preparation was contaminated with macrophages exist because human macrophages have been reported to possess LDL receptors (Brown *et al* 1980,), however, the methodology used in this study which includes several washings, percoll gradient separations, and no homogenization of the tissue rules out the macrophage contamination. Moreover the fact that isolated cytotrophoblast cells transformed to syncytiotrophoblast when cultured for 96 h argue against contamination with macrophages. We are also cognizant of the fact that pregnancy outcome in substance abusers can be compromised because of their poor dietary habits and likelihood of contacting infections. However, none of the participants in this study showed sign of infection or any known deficiency of key nutrients by the attending physicians.

The major objective of our study was to examine the two biochemical events in the fetal placental endocrine axis which play a vital in the maintenance of pregnancy and termination of pregnancy. (Speroff, Glass and Kase 1994). Termination of normal pregnancy in humans is signaled by decreasing progesterone synthesis in the placenta and increasing prostaglandins synthesis in the fetal membranes. The data presented here showing decreased progesterone synthesis in isolated cytotrophoblast cells (*in vitro* study) coupled with elevated prostaglandins levels in amniotic fluid (see Figure 2 and 3) in cocaine users (*in situ* study) presents a typical placental-fetal endocrine milieu preceding pregnancy termination in humans. It is well known that pharmacologic treatment with progesterone or synthetic progestational agents has a dramatic effect in the

prevention of premature labor in humans (Albrecht 1990). We believe that our data answers one of the important observations made by numerous investigators in cocaine users, the early termination of pregnancy in humans (Botros Atterbury and Groome 1996). How does cocaine effect progesterone synthesis is not known we offer the following explanation. Three possibilities exists 1) cocaine decreases the capacity of steroid producing enzyme in the placental cells 2) cocaine inhibits the entry of cholesterol into the steroid producing cells. 3) cocaine decreases the cAMP levels which is essential for steroid synthesis. The answer to the first question is negative because our preliminary data suggest that the capacity of steroid producing enzymes within the cells were not affected. Using labeled pregnenolone as a substrate we observed progesterone synthesis was not affected (unpublished ) in the presence of cocaine suggesting that steroid producing enzyme system remain intact, the second possibility that cocaine inhibits the entry of substrate into the cells also appear to be negative because when isolated cells when treated with cocaine prior or after the substrate was added in the media it had no effect on progesterone synthesis suggesting that cocaine did not effect the entry of substrate in the cells. In our previous study with alcohol we have shown that alcohol decreased progesterone synthesis in cytotrophoblast cells by preventing the entry of substrate into the cells. ( Ahluwalia *et al*, 1992 ) The third possibility that cocaine decreases the cAMP levels appears logical because cAMP is essential in the steroid biosynthesis. cAMP is required for synthesis of 20, 22dihydrocholesterol cholesterol from cholesterol in situ, an essential step in the synthesis of progesterone ( Albrecht and Pepe 1990). Cocaine induced decrease in cAMP in our study is contrary to several published which indicate elevated level of cAMP by cocaine in various transporter systems (Ganapathy *et al* 2000)). However, looking closely at the data it is a possibility that increase or decrease level of cAMP may be the same phenomenon in cellular system in situ because of transitory level of cAMP and its rapid degradation. Whether cocaine causes decrease conversion of ATP. cAMP or increases level of phosphodiesterase which degrades cAMP needs further study.

Prostaglandins are oxidized derivates of polyunsaturated fatty acids and eicosatetraenoic acid. Many PGs constrict arteries, induce platelet aggregation, increase membrane permeability, and produce ischemia. It is widely accepted that fetal membranes and decidua are the site of PGs synthesis. It has become increasingly evident that cAMP has marked effect on placental progesterone production in vitro. (Caritis *et al* 1983) reported a stimulation of progesterone secretion in human trophoblast culture by 8-bromo-cAMP, the  $B_2$  adrenergic receptor against terbutaline, and the  $B_1$  adrenergic receptor agonist dobutaline. It was suggested that  $B$ -adrenergic receptor activation increased adenyl cyclase, resulting in an increase in cAMP (Feinman *et al* (1986) Albright E Pepe GJ 1990). Increase level of prostaglandins (PGs) is interesting because there is a significant accumulation of cocaine in amnion and there is evidence that because of accumulation of cocaine in amniotic fluid the fetus is constantly exposed to cocaine via transport across chorion-amnion ( Thadani *et al* 2004).

The ability of placenta and fetal membranes to synthesize PGs has been demonstrated in several species including rat, rabbits and humans. PGF<sub>2α</sub> and PGE<sub>2</sub> are the major forms of PGs involved in the initiation of labor. Elevated PGs initiate parturition in humans and PGE<sub>2</sub> has been found in greater concentration at the time of parturition amniotic fluid. ((Speroff, Glass and Kase 1994).., How cocaine causes an increase in prostaglandins levels needs investigation. It could be the result of the increase in the release of precursors such as arachidonic acid or decrease in degradation of eicosanoids. Large variability in the placenta in the handling of cocaine has been presented. (Simone Cu *et al* 1994 Am J. Obstet Gynecol 170, 1404- 10.)

Data on gestation period, birth weight of newborn (see table 1) are consistent with the several published reports regarding the effect of cocaine during gestation in humans.

We conclude from these data that cocaine passes through the placenta and enters the cell organelles initiates a cascade of biochemical changes leading to decreased progesterone synthesis, increased prostaglandin synthesis leading to early termination of pregnancy.

## References

- Acker, D., Sachs, BP and Tracy, KJ. (1983). Abruptio placentas associated with cocaine users. *Am. J. Obstet Gynecol*, 145: 220-221.
- Ahluwalia, B., Smith, D., Adeyiga, O., Akbasak, B and Rajguru,S. (1992). Ethanol decreases progesterone synthesis in human placental cells. *Alcohol*, 9, 395-401,
- Albrecht, ED. And Pepe, Gerald, J. (1990). Placental Steroid hormone Biosynthesis in primate pregnancy *Endocrine Reviews*, 11 (1) 124-150, 1.
- Bingol N., Fushs, M., Diaz, V., Stone, RK., and Gromish DS. (1983). Teratogenicity of cocaine in humans. *J. Pediatric*, 110 95-96.

- Botros R, Atterbury JL.,and Groome, LJ. (1996). Reproductive risks of cocaine Human Reproduction Update 21, 43-55.
- Caritis, SN., Hirsch, RP., Zeleznik, AJ. (1983). Adrenergic stimulation of placenta Progesterone production. *J. Clin Endocrinol Metab*, 56: 969.
- Chasnoff, IJ., Griffith., Frier, C and Murray, J. (1992). Cocaine/polydrug use in Pregnancy: Two year follow -up. *Pediatrics*, 89-284-289.
- Chavkin, W., Kandall SR. (1990). Between a “rock” and a hard place: perinatal drug abuse. *Pediatrics*, 85, (2) 223-225.
- Das, G. (1990). Cocaine and cardiovascular system. *Can J Cardiol*, 6 (9) 461-483.
- Das, G. (1994). Cocaine and Reproduction Inter. *J. Clin Pharm Therap*, 32, 1 7-11.
- Feinman, MA., Kliman, HJ. Caltabiano, S., and Strauss, JF. (1986). 8 bromo 3,5 Adenosine monophosphate stimulates the endocrine activity in human Cytotrophoblast in culture. *J. Clin Endocrinology Metab*, 63, 1211,-1217.
- Ganapathy, V, Prasad. PD, Ganapathy, ME and Leibach, FH. (2000). Placental transporters relevant to drug distribution across maternal fetus interface. *Prespective Pharmacology*, 294, 2 413-420.
- Hall CSTG, James, T, Goodyer, C. Branchud, C, Guyda, H Giraoud, CJP. (1977). Short term culture of human mid term and term placenta parameters of hormognesis. *Steroids*, 30, 567-580.
- Havel, RJ., Eder, HA., Bragdon, JG. (1955). The distribution and chemical composition of ultracentrifugally separated lipoprotein in human serum. *J. Clin Invest*, 34: 1345-1350, 1955.
- Hoyme, EE., et al. (1990). Prenatal cocaine exposure and fetal vascular disruption *Pediatrics* 85, 743-747.
- Kliman, HJ., Feinman, MA., Strauss J.F. (1987). Differentiation of human cytotrophoblast into syncytiotrophoblast in culture. *Trophoblast Res*, 2: 409-421.
- Little, BB, Snell, LM, Van Beveren, Crowquell, RB, Traylor S, Johnson WL. (2003). Treatment of substance abuse during pregnancy and infant outcome *Am J. Perinatol*, 20255-20262.
- Monga, M, Chmielowice, MS, Andres, RL, Troyer, LR Parisi, VM 1994, Cocaine alters placental production of thromboxane and prostacyclin Am. *J. Obstet Gynecol*, 171, 965-969.
- Moore, TR., Sorg, J., Miller, L., Key, TC., and Resnik, R. (1986). Hemodynamic effects of intravenous cocaine on the pregnant ewe and fetus. *Am.J. Obstet Gynecol*, 155 (4) 883-888.
- Plessinger, MA, Woods, JA. (1993). Cocaine, A maternal, fetal and neonatal risk J. *Pediatric Health care*, 1, 120-127.
- Simone, CU, Derewelny, LO, Oskap, M, Knu, B, Koren G. (1994). Transfer of cocaine and benzoylecgonine *Am J. Obstetrics & Gynecology*, 170, 1404-1410.
- Smith CG., Asch, RH. (1987). Drug abuse and reproduction. *Fertil and Steril*, 48, 355-373.
- Speroff. L., Glass, RH., and Kase, NG. (1994). *Clinical Gynecologic Endocrinology and Infertility 5<sup>th</sup> edition Prostaglandins* pp308-311 Williams and Wilkins Baltimore, MD.
- Stead, AH., Moffat, AC. (1983). A collection of therapeutic, toxic, and fatal blood drug concentration in man, *Hum Toxicol*, 3: 437-464. 1983.
- Stone, S., Nakamura, RM., Michel, DR., Jr., Thorneycroft, IA. (1971). A modified technique for the assay of progesterone in blood using cellulose column chromatography. *Steroids*, 17: 411-422.
- Thadani, PV. (2004). National Institute on Drug Abuse Conference Report on Placental Proteins, Drug Transport and Fetal Development. *Am J. Obstetrics & Gynecology*, 191, 1858-1862.

Table 1.

Factors	Cocaine users	Control	pvalue
Maternal Age	25±4	26.3±7	NS
Birth Weight (g)	2250±495	2678±434	P<0.05*
Gestation Age (weeks)	33.5±4	38.4±3	P<0.05*
Apgar Score	6.3±2.0	7.8±3.0	NS**
Placental Abruptio	3/16	1/16	P<0.05

NS=Non Significant

Significant p>0.05.

Newborn weighing less than 2500 gm is considered low birth weight. Gestation age of 37 weeks and above is considered normal, 34 weeks or below is considered pre-term.

\*\*Apgar score of 7 and above is considered normal and below is border line

\*\*\* Non significant

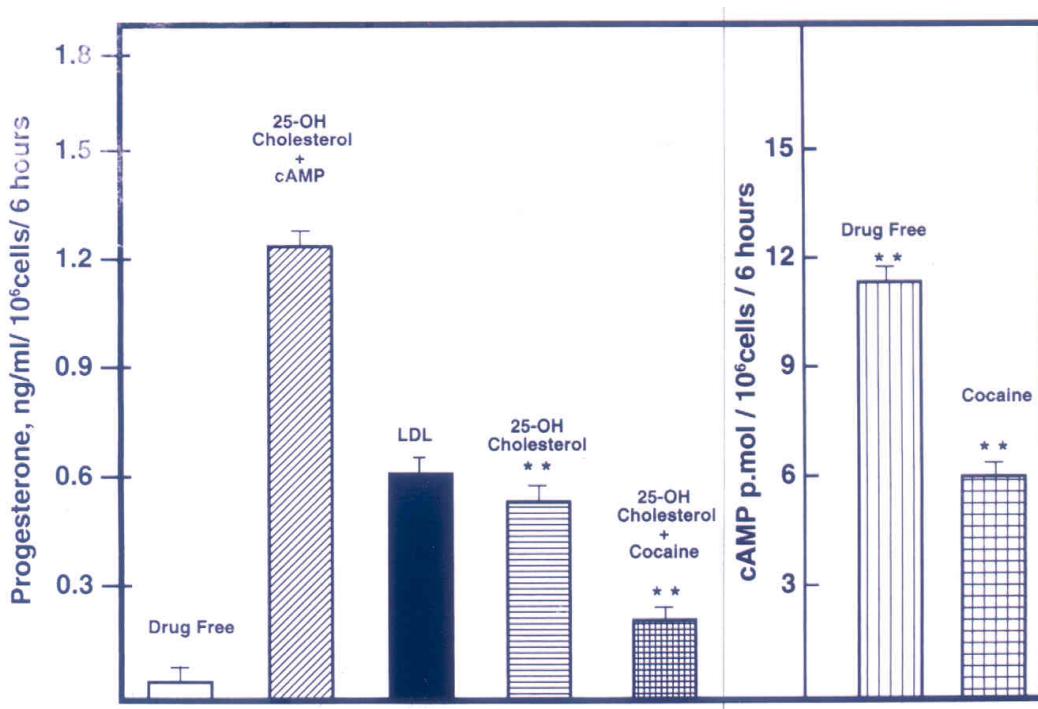


Figure 1.

Figure 1 Shows progesterone biosynthesis in isolated placental cells (cytotrophoblast cells).  $1 \times 10^6$  cells were incubated with cocaine ( $30 \mu\text{M}$ ) for 6 hr. at 37°C. Either of the substrate 25-OH cholesterol or LDL was added to the medium. Results show that either substrate progesterone synthesis increased significantly ( $p. <0.01$ ) compared with control. Differences between 25-OH and LDL were not significant. When cAMP was added in the media there was further increase in progesterone synthesis

Shown in the same Figure is the cAMP levels in cocaine treated and controls. CAMP levels were measured using radioimmunoassay. Results show that cAMP levels decreased significantly in cocaine treated cells.

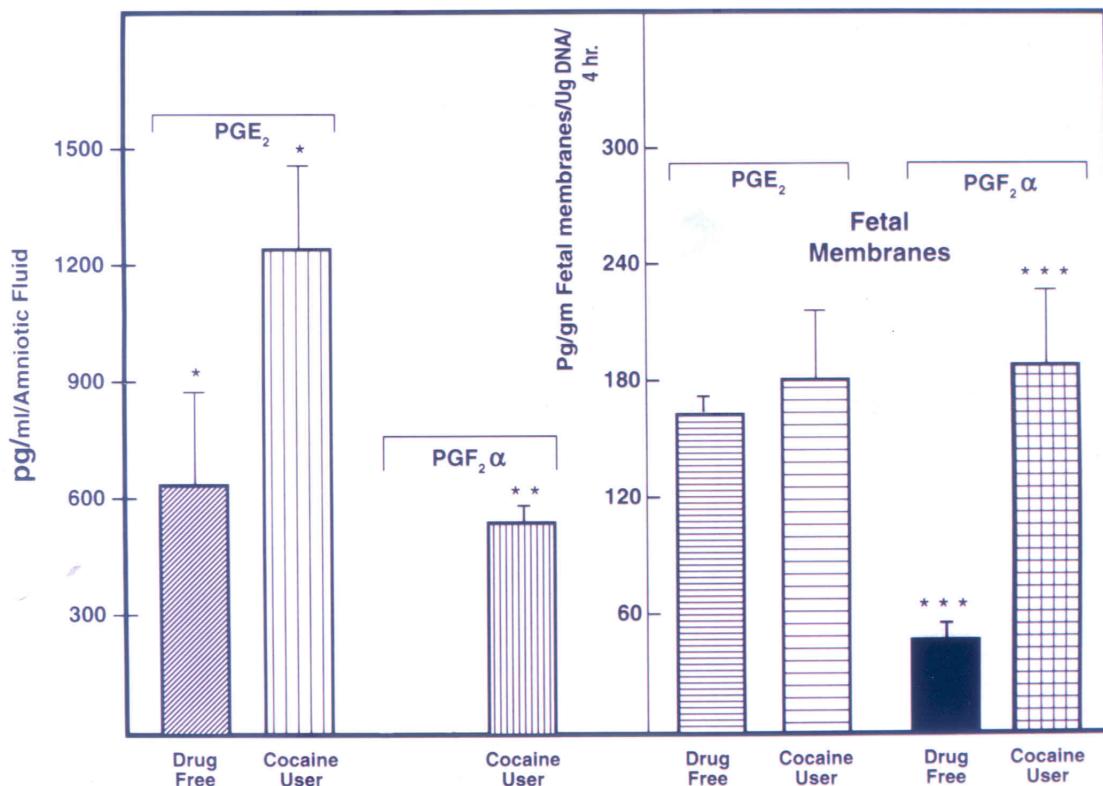


Figure 2.

Figure 2 shows PGs levels ( $\text{PGE}_2$  and  $\text{PGF}_{2\alpha}$ ) in amniotic fluid and isolated fetal membranes. Results show that  $\text{PGE}_2$  levels increased significantly ( $p < 0.001$ ) in cocaine users compared to controls. No significant differences were found in  $\text{PGF}_{2\alpha}$  and controls.

Shown in the same figure is PGs levels in the isolated fetal membranes. Results show that  $\text{PGE}_2$  and  $\text{PGF}_{2\alpha}$  levels increased significantly compared to controls. Differences between  $\text{PGE}_2$  and  $\text{PGF}_{2\alpha}$  were not significant.