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General Section

Methamphetamine, smoking, and gestational hypertension affect norepinephrine levels in umbilical cord tissues

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Summary

Background: These studies were undertaken to determine methamphetamine (METH) and smoking effects on umbilical vascular dynamics and pregnancy outcomes. *Materials and Methods:* Umbilical cords (54) were collected prospectively at birth, washed of blood, and stored at -80°C. Cords were thawed and lysates prepared, then catecholamine levels quantified with enzyme-linked immunosorbent assay (ELISA). *Results:* Catecholamine levels in umbilical cords were not associated with maternal or gestational age, gravidity, parity, neonatal or placental weight. Neither smoking nor METH affected dopamine or epinephrine. However, smoking (two-fold) and METH (four-fold) decreased norepinephrine and together a 60-fold reduction occurred ($p = 0.025$). Cesarean section and hypertension were both associated with lower norepinephrine levels ($p < 0.001$) regardless of drug status. In normotensive pregnancies, smoking and METH significantly decreased norepinephrine levels (two-fold and 3.5-fold each, respectively) with a 40-fold decrease for METH/smoking together. *Discussion:* Depletion of norephinephrine by METH and smoking likely contributes to pregnancy complications, including the higher incidence of respiratory distress and postpartum hemorrhage in cesarean section.

Key words: Drugs of abuse; Epinephrine; Norepinephrine; Dopamine.

Introduction

When drugs of abuse are ingested during pregnancy they can pass from the maternal circulation, through the placenta, and to the fetus. Blood exiting the placenta is delivered directly to the fetal heart by the umbilical vein, pumped around the fetal circulation, then returned to the placenta by the umbilical arteries [1]. Therefore, the umbilical artery and vein not only play vital roles in the delivery of oxygen and essential nutrients and removal of wastes, but also provide a major route for chemicals to reach the fetus. In addition, any substance with vasoactive properties may interfere with efficient placental and umbilical function and pose a risk to the fetus [1].

Commonly, women do not recognize that they are pregnant until the second missed menstrual cycle and may accidentally continue to use recreational or medical drugs that they would otherwise avoid [2]. However, after knowingly becoming pregnant some mothers either do not or cannot stop their ingestion of drugs and this is a hallmark of addiction. Methamphetamine (METH) addiction is associated with pre-term birth, intra-uterine growth restriction, small-for-gestational-age babies and neonatal dependence [3].

The drug METH is an indirect sympathomimetic that causes central euphoric effects through excessive release of catecholamines agonizing adrenergic receptors [4]. Outside of the central nervous system, METH's strongest effects occur in the cardiovascular system increasing systolic and diastolic blood pressure, and reflexive decreasing heart rate [4]. Eventually METH over-stimulation depletes catecholamines both centrally and peripherally. The cardiovascular sympathetic action is primarily mediated by norepinephrine at a- (vascular) and b- (heart and lung) adrenergic receptors, although dopamine effects on the renal system also contribute to blood pressure effects [5]. The umbilical cord vessels may provide a conduit for METH to directly affect fetal catecholamine signaling in the heart [6-8]. Additionally, maternal, and gestational tissues (placenta, uterus, and umbilical vein and arteries) also contain a- and b-receptors, hence the fact that METH may deplete catecholamines in the blood and interfere with sympathetic responses in these tissues is concerning [9, 10]. The primary effects of METH in the maternal-placental-fetal unit occur at a receptors in vascular smooth muscle and b-receptors in cardiac cells. Additionally, there is a distinct separation in dopamine receptor expression where umbilical arteries express D1 receptors [11], but D2, D3, and D4 receptors are only reported in the umbilical vein [12]. The functional consequences of dopamine signaling in the vessels in the umbilical cord, as compared to norepinephrine effects, are not well established and likely less critical.

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The potential for METH to affect adrenergic signaling in the umbilical cord vessels, as well as the fetal cardiovascular system prompted the present authors to undertake this study. The hypothesis was that METH and smoking alter normal catecholamine signaling thereby affecting umbilical vascular dynamics, and perhaps in the fetal cardiovascular system. Here they present data supporting this, including that METH and smoking alter constitutive (from cesarean section without labor) and parturition-mediated norepinephrine levels in the umbilical cord. They also demonstrate that hypertension is associated with lower umbilical tissue norepinephrine. They subsequently propose a link between METH effects on noradrenergic signaling, vascular effects in the umbilicus, and adverse reproductive outcomes.

Materials and Methods

Tissue collection and processing

Cord samples were collected by the Hawaii Biorepository, which banks de-identified placental and blood samples from women at delivery, and links these samples with a database of prenatal, delivery, and infant outcome information derived from medical record data. Exempt approval was obtained from the University of Hawaii Committee for Human Studies for the subproject and from the Western Institutional Review Board (WIRB) for the HiBR. Each piece of cord was coded with a study number, washed thoroughly, then stored at -80 ºC until use. All cords were used within 12 months of collection. It has been demonstrated that when stored at -80 ºC, human norepinephrine and epinephrine are stable for at least six months and probably much longer when stored in tissue or whole blood and do not show the degradation observed in urine or buffers [13].

A sample of 54 cords were used for this pilot study. For processing to lysates, cord pieces were thawed, wet weight recorded, then cords homogenized mechanically with a homogenizer at 6,500 RPM for 30 seconds each in a 1:4 (w:v) Tris-HCl buffer containing five mM $MgCl_2$ and two mM PMSF (pH 7.4). Total lysates were normalized to two mg/ml protein using the Bicinchoninic acid method, then aliquoted and frozen at -80 °C until use [14].

Enzyme-linked immunosorbent assay (ELISA) for catecholamines

The levels of epinephrine, norepinephrine, and dopamine were determined with a commercial 3-CAT ELISA from as per the manufacturer's instructions.

Data analysis and statistics

Quantification of catecholamines was performed blinded using only the study number for identification of samples. After ELISAs were completed and concentrations assigned, the study key was accessed and results were sorted into four groups: cords from non-smoking, non-METH patients (control), cords from METH using patients, cords from tobacco smoking patients, and cords from patients who both smoked tobacco and used METH.

Statistical analyses

Statistical analyses were performed using Prism 5.0 with significance set at $\alpha = 0.05$. Since these data are discrete, two-tailed student t-tests were used to assess differences between groups. Stratification and comparisons within groups were analyzed using multivariate analyses, ANOVA, and multiple regression for continuous data.

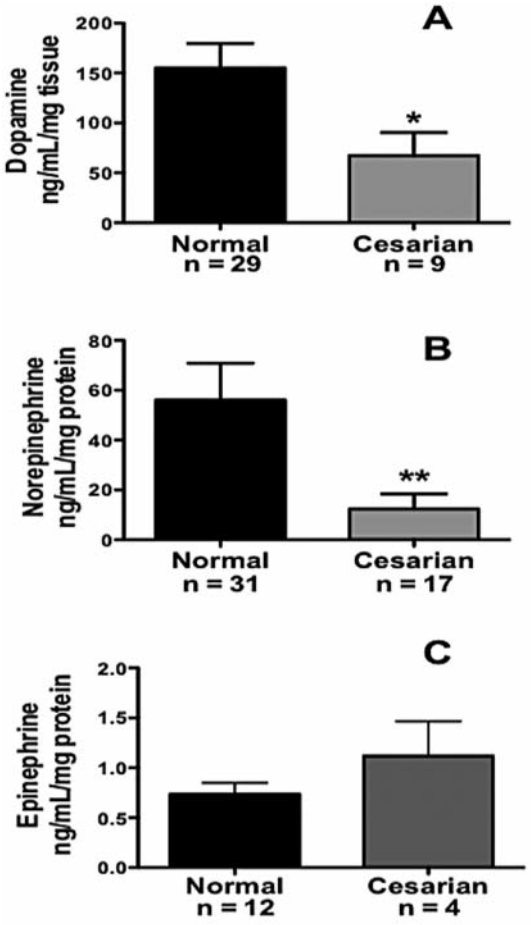

Figure 1. — The effects of cesarean section on umbilical catecholamine levels. (A) Cesarean section is associated with significantly lower dopamine levels in umbilical cords. (B) Cesarean section is associated with significantly lower norepinephrine levels in umbilical lysates. (C) No significant differences are observed for epinephrine with delivery method Bars are means ± SEM. * = $p < 0.05$, ** = $p < 0.01$, t-test.

Results

Of the 54 samples tested, dopamine was detected in 38 cord tissues, norepinephrine in 48 cords, and epinephrine in 16 cords. None of the catecholamines correlated with maternal age, gestational age, neonatal weight, placenta weight, gravidity, or parity. Cesarean section was associated with significantly decreased levels of dopamine and norepinephrine ($p < 0.05$ and $p < 0.001$, Figures 1A and 1B) but not epinephrine ($p = 0.18$, Figure 1C).

Neither METH nor smoking (alone or together) showed effects on umbilical cord levels of dopamine or epinephrine (Figure 2A and C). However, METH and smoking decreased umbilical norepinephrine levels alone and together ($p = 0.025$, ANOVA). Compared to drug free mothers, METH use by itself (in non-smokers) was associated with a four-fold decrease in umbilical norepinephrine levels,

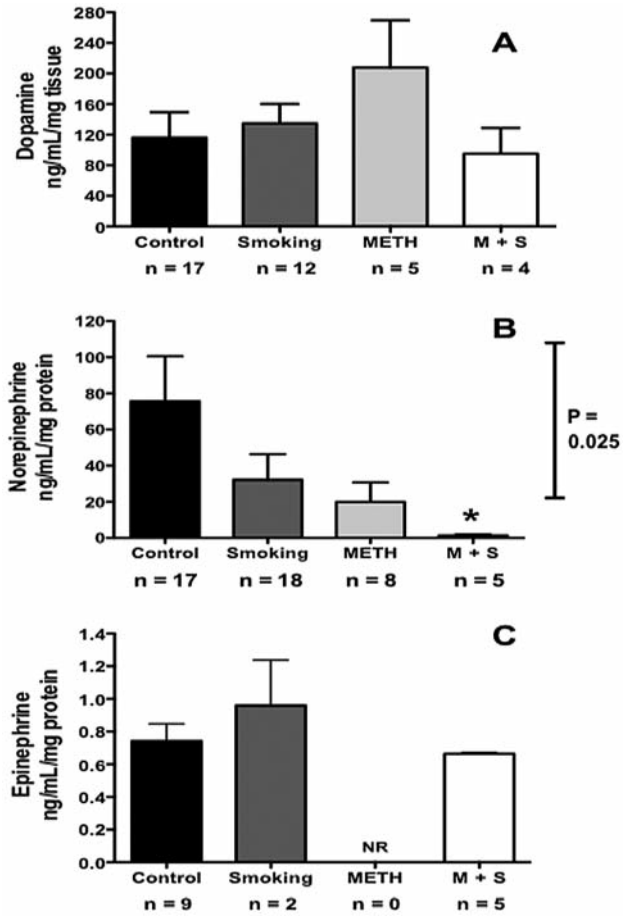

Figure 2. — Differential effects of METH and smoking on umbilical cord catecholamines. (A) Smoking and METH do not significantly alter cord dopamine levels. (B) METH and METH + smoking combined significantly deplete cord noradrenlaine levels. (C) No effects were observed on epinephrine. Bars are means ± SEM. * = $p < 0.01$ (Dunnet's multiple post hoc comparison), capped bar at end of graph indicates ANOVA results. METH = methamphetamine. M+S = METH and smoking together, NR = none recorded.

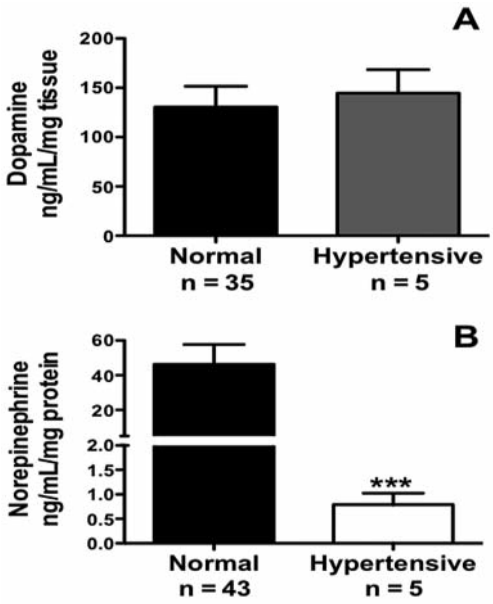

Figure 3. — The effects of hypertension (clinically diagnosed, any grade) on umbilical cord tissue catecholamine levels. (A) Dopamine levels are not changed by hypertension in pregnancy. (B) Norepinephrine was significantly reduced by hypertension. (C) Epinephrine was undetectable in umbilical cords from hypertensive patients. *** = $p < 0.001$, t-test. NR = none recorded.

smoking was associated with a two-fold decrease in norepinephrine levels and in women who both abused METH and smoked almost 60-fold decreases in norepinephrine were observed ($p < 0.05$, Dunnet's multiple comparison test, Figure 2B).

In the 38 samples where dopamine was detected, neurotransmitter levels were not associated with hypertension (Figure 3A). However, norepinephrine was significantly lower in umbilical cords from hypertensive pregnancies ($p < 0.001$, t-test, Figure 3B). Of the 16 cords where epinephrine was detected, no hypertensive cases were recorded.

While these drug effects are interesting, they must be further examined in light of the independent effects of delivery on dopamine and norepinephrine levels, as well as hypertension on norepinephrine levels. When stratified for delivery type and dopamine levels, drug ingestion has no effect on dopamine levels but cesarean section is consistently associated with higher dopamine levels in the umbilical cord vessels (Figure 4A). Vaginal birth is associated with higher levels of norepinephrine for all groups except smokers where there were no differences. Additionally, METH and METH + smoking were associated with successively lower norepinephrine levels compared to drug-free cords in both vaginal and cesarean births. These data demonstrate that both delivery type and drug ingestion independently alter norepinephrine levels in umbilical cords (Figure 4B). Finally, when the effects of hypertension and drug ingestion were stratified, two clear effects are identified. Firstly, in non-hypertensive cords, smoking and METH individually decrease norepinephrine levels. Control cord levels of norepinephrine were almost two-fold higher than in smokers and 3.5-fold higher than for METH users. Combined METH and smoking had a drastic effect on norepinephrine levels, lowering them 40-fold (Figure 4C). Secondly, in hypertensive pregnancies the levels of norepinephrine were drastically lowered in all samples tested with hypertensive cord norepinephrine levels being between 50 and 150-fold lower than drug free, non-hypertensive cords and with the norepinephrine levels within drug groups being between two- and 75-fold lower than the norepinephrine levels in the corresponding

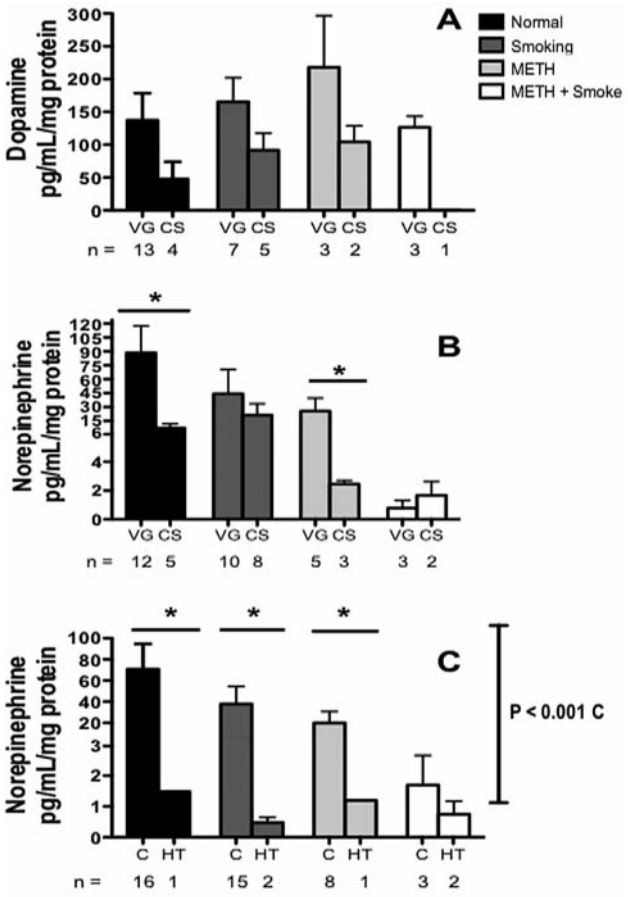

Figure 4. — Two-way stratification indicated that delivery effects on dopamine in umbilical cords are independent of drug ingestion but delivery effects on norepinephrine may be superseded by smoking. Furthermore, drugs, and hypertension independently affect norepinephrine levels in umbilical cords. (A) Drug ingestion has no effect on dopamine levels but cesarean section is consistently associated with higher dopamine levels in the umbilical cord. (B) For norepinephrine, vaginal birth is associated with higher levels of neurotransmitter in all groups, except smokers where there were no differences. Additionally, METH and METH + smoking were associated with successively lower norepinephrine levels compared to drug-free cords. (C) When the hypertension and drug ingestion are stratified, drug ingestion decreases norepinephrine levels in control cords, but hypertension itself decreases norepinephrine levels, above reductions caused by drugs with the exception of smoking and METH combined. Bars are means ± SEM. * = $p < 0.05$, Dunnets multiple post-hoc comparison. Capped bar at end indicates ANOVA significance. C = control, CS = cesarean section, HT = hypertension, METH = methamphetamine, VD = vaginal delivery.

drug-abusing, non-hypertensive groups (Figure 4C). There were no apparent effects of drugs in depleting norepinephrine further over the effect of hypertension. These latter data imply that hypertension is more strongly effective at depleting norepinephrine in the umbilical cord vessels than METH and smoking (alone or together). However, despite the visually arresting effects in the graphs, these latter data should be interpreted with caution due to the paucity of samples in the hypertensive group. Additionally, concurrent drug administration to the mothers may have affected these results.

Discussion

This study demonstrates that hypertension and drug ingestion (smoking and METH) can deplete norepinephrine levels in the umbilical cord tissues. The effects of the drugs are synergistic in normotensive pregnancies, but hypertension has a greater norepinephrine depletion effect than the drugs. Additionally, the authors demonstrate that delivery method (cesarean *vs.* vaginal) affects catecholamine levels in the umbilical tissues, with lower levels of catecholamines present after cesarean section than natural birth.

Similar to the present findings that the tissues of the umbilical cord from cesarean section had significantly lower dopamine and norepinephrine levels compared to those from vaginal deliveries, other investigators have reported this phenomenon in total cord blood [15], umbilical artery blood [16], and umbilical vein blood [17]. Here, using washed cords, the authors showed the same results in umbilical cord tissues, where the catecholamines are presumably derived from the bloodstream and either bound to receptors on the surface of vessels smooth muscle, or present in the cytosol of the smooth muscle due to transport by the uptake 2 transporter [4]. The physiological reason(s) for the results showing lower norepinephrine in cesarean section deliveries compared to labor are likely straightforward. It has been demonstrated that elevated norepinephrine causes transient pulmonary hypertension in term human fetuses [18] and that high doses of norepinephrine cause vasoconstriction of the placental vascular bed [19]. Hence, increases in umbilical norepinephrine during parturition may be advantageous for activating the fetal lungs at birth and are also certainly useful for uterine contractions and preventing post-partum bleeding. If confirmed, this could explain the increased incidence of respiratory distress and post-partum hemorrhage in cesarean deliveries.

Similarly, with respect to transporters, studies have shown that in addition to depleting neuronal norephinephrine stores, METH is a potent inhibitor of both serotonin and norepinephrine transporters in reproductive tissues, with the norepinephrine transporter being more affected [20]. This provides a good explanation for our drug-related results. Lower norepinephrine levels associated with METH use in umbilical vessel tissues may be caused by a combination of the classical mechanism for METH effects: depleted epinephrine release, less conversion to nor-epinephrine and lower circulating levels of the transmitter, but also by inhibition of norepinephrine uptake 2 transporters in reproductive tissues. Together, these mechanisms would combine to

produce lower levels of norepinephrine detected in umbilical cords. An alternative hypothesis, is that since the majority of systemic catecholamines circulate in sulfo-conjugated form, METH and/or smoking affect sulfation of catecholamines. A recent study in rats demonstrated that several sulfotransferases are upregulated by METH administration within 24 hours and this occurs in a time- and concentration-dependent manner [21], establishing in principle that these genes can be induced by METH. Since the placenta has high expression of sulfotransferases, lower levels of norepinephrine, and epinephrine in the tissues of the umbilical cord vessels due to increased sulfation across the placental vasculature is also a possibility.

The synergism reported herein for METH and smoking in lowering umbilical norepinephrine levels is of particular interest. A single report on this phenomenon has been previously published for humans where it was found that median epinephrine and norepinephrine concentrations in the cord blood of neonates were significantly lower in smokers compared with nonsmokers [22]. Based on the known physiological and pharmacological characteristics of both METH and smoking, it is sensible that their combined action would cause synergistically lower levels of catecholamines in circulating blood, as well as in other tissues including vascular smooth muscle.

Differences in umbilical tissue norepinephrine levels with hypertension are also intriguing. Hypertension in pregnancy is clinically diagnosed and can range from essential hypertension to preeclampsia – a disease characterized by abnormal vascular responses to placentation, increased systemic vascular resistance, and endothelial cell dysfunction [10]. The drastic hypertension-mediated depletion of norepinephrine levels observed in the umbilical cord (up to 150-fold), was stronger than METH-mediated norepinephrine depletion (up to 60-fold in normotensive pregnancies). Other authors have reported changes in umbilical cords with respect to hypertensive disorders in pregnancy, although these have primarily been ultrastructural [23] and/or involved alterations to blood flow [24, 25].

The authors acknowledge that this is a small study with only 54 samples and not all catecholamines were detected in all samples due to the limits of detection of the ELISA assay, given that largely intracellular levels of catecholamines were being detected, which are much lower than circulating levels in blood. Hence, with a small sample size of 54 individual cords, stratifying some of these data likely compromised statistical power. However, this is mitigated somewhat since non-detection of catecholamines in cords was not uniform. That is, no individual cords returned non-detection for all three catecholamines and no trends towards uniformly lower levels of any or all biogenic amines with collection, storage or processing were observed. Moreover, catecholamine levels were unlikely to be depleted due to storage conditions, since human norepinephrine and epinephrine are stable for at least six months (and probably much longer) when stored in tissue or whole blood, lacking the degradation observed in urine or buffers [13].

Furthermore, from a clinical perspective, the authors do not have data regarding medications used to treat maternal hypertension. In Hawaii, current practice encompasses the use of labetelol, hydralazine, and methyldopa (aldomet) for managing hypertension in pregnancy. Since labetalol acts directly at adrenergic receptors (mixed action) and hydralazine on smooth muscle (through a second messenger system), their effects would be expected to alter tissue responses to catecholamines [5]. Furthermore, the use of methyldopa is a potential confounder since it is a competitive inhibitor of DOPA decarboxylase which produces dopamine. Because dopamine is the precursor for norepinephrine and epinephrine, use of this drug would be expected to inhibit catecholamine production in peripheral tissues [5]. Despite this, the authors observed that although norepinephrine levels were lower in hypertension, dopamine levels were not, so confounding by methyldopa administration is not expected.

The authors believe that these results, particularly regarding the combined effects of METH and smoking on depleting norepinephrine in umbilical tissues, should be studied further. Rather than measuring blood levels of the catecholamines as a proxy, they measured tissue levels directly in washed umbilical cords. This means that although they only detected low levels of the molecules, these molecules are either directly bound to cell surfaces, or had been internalized into the cells of the umbilical vessels – representing the vasoactive fraction of the circulating transmitters. These studies also add to our understanding of the interplay between cesarean section and parturition signaling, since cesarean section was associated with much lower dopamine and norepinephrine levels than vaginal deliveries. This presents an avenue to investigate the mechanisms of well-known increases in respiratory distress and postpartum hemorrhage observed with cesarean deliveries. The authors have also provided a mechanistic direction that may elucidate how drugs of abuse ingested during pregnancy can affect pregnancy outcomes – namely through alterations to vasoactive signaling throughout pregnancy and at parturition. For mothers addicted to METH, future medical interventions to prevent adverse pregnancy outcomes could center on re-balancing appropriate catecholamine/vasoactive signaling in gestational tissues. For example, because beta blockers are contraindicated in pregnant METH users, determining the utility of other vasoactive drugs, such as the phosphodiesterase inhibitors, may prove useful. Although in its infancy, the authors hope that this research can assist to develop or repurpose existing pharmaceuticals and guide clinical practice to mitigate negative vascular effects of drug abuse and improve maternal and neonatal outcomes.

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