Review Article

Effects of Prenatal Cocaine Exposure on Human Pregnancy and Postpartum

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Abstract

During the early months of pregnancy, cocaine exposure may increase the risk of miscarriage. Later in pregnancy, cocaine use can cause placental abruption. Placental abruption can lead to severe bleeding, preterm birth, and fetal death. Women who use cocaine throughout their pregnancy have the risk of an increased chance of premature labor and birth defect. In addition babies may also have a smaller head and have their growth hindered. Babies who are exposed to cocaine later in pregnancy may be born dependent and suffer from withdrawal symptoms such as tremors, sleeplessness, muscle spasms, and feeding difficulties.

The effects of prenatal cocaine exposure (PCE) have been examined in infants and young children across multiple developmental domains (e.g., growth, intelligence, language, motor, attention, and neurophysiology). Studies revealed that in most domains, the neurobiological effects of PCE play a subtle role, with effects no greater than other known teratogens or environmental factors. Associations between PCE and negative developmental outcomes were typically attenuated when models included conditions that commonly co-occur with PCE (eg. tobacco or alcohol exposure, malnutrition, poor quality of care). Some investigations suggest that learning difficulties may result as the child gets older. Defects of the genitals, kidneys, and brain are also possible. The aim of this review is to provide information regarding the prenatal exposure and the associated impact on placental function and pregnancy outcomes.

Keywords: Illicit drug; Cocaine; Maternal exposure; Prenatal exposure; Human placenta; *Ex vivo* placenta perfusion model

Introduction

Substance abuse in pregnancy has increased over the past three decades in the United States [1]. A National survey on drug Use and Health from 2002-2003 has estimated that 4.3% of pregnant women aged 15-44 years reported illicit drug use within the month to being questioned [1,2]. Approximately 250,000 women in the United State, of whom 90% are of childbearing age with criteria for intravenous drug, resulting in approximately 225,000 infants yearly with prenatal exposure to illicit substances. Legal and illegal substances and their effect on pregnancy have recently reviewed include opiates, cocaine, alcohol, tobacco, marijuana, and amphetamines [3]. Illicit drug use during pregnancy is a major risk factor for maternal morbidity and neonatal complications.

Cocaine use in pregnancy can lead to spontaneous abortion, preterm births, placental abruption, and congenital anomalies. Neonatal issues include poor feeding, lethargy, and seizures. Mothers using cocaine require specialized prenatal care and the neonate may require extra supportive care.

The aim of this review is to provide information regarding the prenatal cocaine exposure (PCE) and the associated impact on placental function and pregnancy outcomes.

Effect of Cocaine on Preconception, Pregnancy and Postpartum

The information regarding the effect of cocaine on preconception, pregnancy and postpartum is driven from Keegan et al. [3].

Preconception

According to a 2005 government survey, approximately 4% of women use illicit drugs during their pregnancy, and cocaine is one of the most commonly abused drugs [4]. Prenatal cocaine use is commonly associated with poor pregnancy and adverse birth outcomes, and cocaine abuse particularly impacts measures of fetal growth and well-being. Low birth weight, intrauterine growth restriction, and decreased head circumference are all noted to be increased in newborns of mothers who use cocaine in pregnancy. In addition, cocaine use is frequently associated with inadequate prenatal care and the frequent concomitant use of tobacco and alcohol [5]. Moreover, cocaine use is associated with psychosocial, behavioral, and biomedical risk factors, such as poverty, poor nutrition, stress, depression, physical abuse, lack of social support, and sexually transmitted infections [5], all of which can greatly affect pregnancy outcome [6].

Pregnancy

Maternal cocaine use may have both direct and indirect effects on the fetus. Cocaine rapidly crosses the placenta and a higher concentration occurs in the fetus. There are many adverse outcomes associated with cocaine use during pregnancy.

Cocaine use during the early months of pregnancy can cause spontaneous abortion. Upto 38% of early pregnancies may result in miscarriage in cocaine-abusing mothers [5]. This increase in incidence

of spontaneous abortion is probably secondary to an increase in maternal plasma norepinephrine, which increases uterine contractility, constricts placental vessels, and decreases blood flow to the fetus. Placental abruption accounts for 2% to 15% of adverse effects of cocaine use during pregnancy. Abruption is thought to be caused by vasospasm and hypoxia of the placental bed, and it is more common with cocaine binging than with regular use. As a result of maternal cocaine use and placental abruption, the incidence of stillbirth in cocaine-abusing mothers is elevated 8% above the expected level when compared to the general population [5,7].

Cocaine stimulates uterine contractility through β-agonist action on the β2-receptors of the uterus. The consequence of this β-agonist property is an increased risk of premature preterm rupture of membranes, preterm labor, and preterm delivery. These adverse outcomes are observed in 17% to 29% of pregnancies of cocaine-abusing mothers. Intrauterine growth restriction (IUGR) and low birth weight can be observed in 22% to 34% of all infants exposed to cocaine in utero, secondary to the constriction of the uterine blood vessels, which leads to intermittent hypo-perfusion of the uterus and placenta [8]. Moreover, cocaine significantly suppresses maternal appetite which contributes to poor maternal and fetal nutrition [7,8]. Cocaine exposure can affect embryonic and fetal development. Congenital anomalies have been reported to occur in 7% to 40% of infants exposed to cocaine in utero. Evidence of brain malformation and cardiovascular abnormalities can occur in approximately 35% and 4% to 40% of exposed fetuses, respectively [9].

Postpartum

The mainstay of the management of the cocaine-addicted mother and newborn immediately following delivery is supportive care [7]. Although the symptoms of cocaine neurotoxicity are not often life threatening for the mother or the newborn, these symptoms are extremely unpleasant [7,10]. For the mother during the postpartum period, mood symptoms and, less commonly, hallucinations may require treatment with antipsychotic medications, particularly during the inpatient stay. From a social-focused and family-focused standpoint, the use of cocaine is extremely problematic. Cocaine use during pregnancy is considered a significant risk factor for infant neglect and abuse. Evidence of cocaine use in pregnancy often results in the removal of the in infant from maternal custody within the first 18 months of life [11]. Prospective studies have also indicated a strong link between cocaine-using mothers and child maltreatment, with high rates of care-giving disruption (43%) and child maltreatment by 2 years (9% to 23%) [11]. Finally, a stable and secure home environment helps reduce the stressors associated with cocaine addiction, so any intervention in this regard may be extremely helpful.

The effects of prenatal cocaine exposure (PCE) have been examined in infants and young children across multiple developmental domains (eg, growth, intelligence, language, motor, attention, and neurophysiology). A 2001 review of 36 peer-reviewed articles revealed that in most domains, the neurobiological effects of PCE play a subtle role, with effects no greater than other known teratogens or environmental factors [12]. Associations between PCE and negative developmental outcomes were typically attenuated when models included conditions that commonly co-occur with PCE (e.g., tobacco or alcohol exposure, malnutrition, poor quality of care). Little is known about the long-term effects of PCE. One possibility is that PCE has direct effects on brain structure or function, which may heighten children's vulnerability to negative developmental outcomes [13]. Another

possibility is that PCE is a marker for environmental risk factors and, therefore, must be considered in the context of other developmental threats, including poverty, insensitive parenting, maternal stress and depression, caregiver drug dependence, limited educational resources, unstable home environments, and high rates of domestic violence [14-16]. Both perspectives highlight the need to consider the long-term effects of PCE within an environmental and developmental context that includes brain and behavioral development. Over time, children face increasingly complex cognitive and social demands, requiring advances in aspects of executive control including sustained attention, working memory, planning, inhibitory control, and emotion regulation. Such higher-order processes are thought to underlie children's ability to engage in behavioral self-regulation, and preclinical models have suggested that PCE may target brain regions and pathways associated with the development of these capabilities.

Regions with strong dopaminergic innervation (eg, anterior cingulate cortex, prefrontal cortex, striatum) may be particularly susceptible to PCE [17]. Because these regions continue to develop throughout childhood and adolescence, the effects of PCE may not be evident until many years after the initial prenatal exposure. The effects of PCE are manifest in distinct ways at different ages. Investigations using longitudinal models with covariate controls can examine the differential effects of drug exposure over time. Studies that include parenting and environmental influences (eg, school, neighborhood, peers) are necessary to determine the amount of variance attributed to each level of influence.

Human Placenta Development

The placenta provides the direct link between mother and fetus, transferring nutrients for growth and development of the fetus as well as for its own growth and development. It plays an essential role in the growth and development of the fetus by performing a multitude of functions. The placenta and the chorion (outer membrane, trophoblast layer) are derived from the trophoectoderm cells of the blastocyst. Other extraembryonic tissues develop from the inner cell mass of the blastocyst. These include the amnion (inner membrane), the yolk sac, the allantois and the extraembryonic mesoderm. The umbilical cord and the blood vessels of the placenta are derived from the mesoderm. The placenta represents a significant, valuable and promising source of stem cells with variable potency. A unique function of the placenta is its role as an endocrine organ producing various steroid hormones (e.g., estrogens and progesterone) and polypeptide hormones (e.g., chorionic gonadotropin and placental lactogen) relevant to pregnancy. It functions as a nutritive organ by mediating the transfer of essential nutrients such as glucose, amino acids, fatty acids, minerals, and vitamins from mother to fetus. It is also responsible for the exchange of oxygen and carbon dioxide between the maternal and fetal circulations. In addition, it plays a critical role in the elimination of various metabolic waste products such as bilirubin from the fetus. In order to perform many of these functions, placenta expresses numerous transporters. In a manner similar to intestine and kidney, placenta is capable of vectorial transfer of nutrients and metabolic waste products. Thus, placenta can mediate the transfer of certain compounds from mother to fetus and certain other compounds from fetus to mother. This is made possible by the polarized nature of syncytiotrophoblast, the functional unit of the placenta. Placental syncytiotrophoblast is a multinucleated cell that is formed by the fusion of differentiating cytotrophoblasts. The plasma membrane of syncytiotrophoblast consists of two distinct regions: a brush border membrane that is facing the maternal side and a basal membrane

that is facing the fetal side. On the maternal side, there is no capillary network between the uterine arterioles and uterine venules because the invasion of the placental tissue into the uterine endometrium destroys the uterine blood vessels at the site of implantation. As a result, blood flows into the placental intervillous space and comes in direct contact with the brush border membrane of the syncytiotrophoblast without the intervening endothelium of the capillaries. The intraplacental fetal circulation is fully established at the end of the fifth week post-conception [18]. The complete fetal–placental– maternal circulation is not entirely established until around the tenth week of pregnancy, therefore substances present in the maternal blood until this time must be introduced to the embryo via diffusion through the extracellular fluid [19]. The fetal circulation ends in the villous trees and these are found in the vascular units (cotyledons) within the placenta. The full-term placenta contain between 10 and 40 cotyledons separated from each other by the placental septa. The transfer of any compound from the maternal blood into fetal blood across the syncytiotrophoblast has to involve transport across the brush border membrane followed by transport across the basal membrane. Similarly, the transfer of any compound from the fetal blood into maternal blood has to involve transport across the basal membrane followed by transport across the brush border membrane. To facilitate this process, the two membranes express various transporters in a differential manner. The fetal capillary endothelium forms an additional barrier for the maternal–fetal exchange of nutrients and metabolites and, accordingly, this cell also expresses a wide variety of transporters to accomplish the exchange process.

Placental Transport Mechanisms

In the human placenta the syncytiotrophoblast arises from the fusion of cytotrophoblast stem cells forming a syncytium over the surface of the placenta facing the maternal blood. The plasma membrane of the syncytiotrophoblast is polarized; the brush-border membrane in direct contact with maternal blood and the basal membrane facing the fetal circulation. The brush-border membrane possesses a microvillus structure that effectively amplifies the surface area, whereas the basal membrane lacks this structure. Transport proteins may exist in either or both the brush border and basal membrane with their location and orientation determining the direction of substrate transport [20,21].

The syncytiotrophoblast, the outermost layer of human the placenta is the main site of exchange for drugs and metabolites, nutrients, waste products and gases between maternal and fetal circulations [22]. Efficient transfer of nutrients, gasses, electrolytes and solutes across the placenta is essential for fetal growth and development. There are several mechanisms by which transfer occurs, and depending on the mechanism of transfer the direction may be toward maternal or fetal circulation [23].

Solvent drag

Solvent drag is the movement (bulk flow) of water in which drugs, solutes, gasses and nutrients are dissolved. Bulk flow has been demonstrated in the perfused human placental cotyledon in response to hydrostatic pressure changes. With this mechanism, transfer of a drug would be passive, in the sense of flow with water into or out of placental tissue. This mechanism is unlikely to represent an efficient mechanism for drug access to the placenta or fetus.

Simple diffusion

Simple diffusion is the passive transfer of solutes driven by concentration and electrical gradients. All solutes are transferred by diffusion, but the relative contribution is dependent on molecular properties, and presence of transport mechanisms which facilitate exchange between maternal and fetal circulations. As an example, lipophilic molecules, such as respiratory gases, are readily exchanged by simple diffusion. Many commonly used medications and abused agents observed during pregnancy cross the placenta by passive or simple diffusion were recently reviewed by Malek and Mattison, 2010 [24].

Determinants of passive diffusion across the placenta include the physicochemical properties of the molecule, as well as protein binding in maternal and fetal circulations and metabolism in the mother, placenta or fetus [19,25]. One determinant of passive diffusion is molecular weight of the chemical, with decreasing transfer as molecular weight increases. Hydrophobicity and ionizability also influence placental exchange, and may also influence the amount of the drug which remains sequestered, or bound within placental tissues. Protein binding in fetal and maternal circulations also influences transfer across the placenta as the concentration gradient driving diffusion is the free concentration difference. Consequently, total concentrations may be higher in maternal or fetal blood based on protein binding to albumin or alpha-1-acid glycoprotein but free concentrations similar [26-29].

Transcellular transfer

This type of transfer utilizes transport proteins in the microvillus, basal membranes of the syncytiotrophoblast or fetal capillary endothelium. There are three types:

Channels: These proteins form water-filled pores in the plasma membrane through which ions can diffuse down an electrochemical gradient. This allows transport of charged hydrophilic substances which are insoluble in lipids. Placental aquaporins and chloride channels are examples of channels that function in the transport of water and small molecules, and are essential for fetal development [30,31].

Facilitated diffusion: These transporters are saturable carrier proteins, which are independent of metabolic energy, so that transport occurs more rapidly than would occur for simple diffusion but will not occur against a concentration or other driving gradient. As an example, glucose is transported by the facilitated GLUT transporters. It has also been proposed that metformin is transported from fetal to maternal circulations by facilitated diffusion [32].

Carrier-mediated active transport: Primary active transport utilizes ATP to move solutes against a gradient, $Na+K+ATPase$ and $Ca2+ATPase$ are two examples. Secondary active transport utilizes concentration gradients across the cell that are set by the primary system, Na + amino-acid co-transport and the $Ca2 + / Na +$ exchanger are examples. Transport ATPases are known to be present in human placenta. These include the $Na+ K+pump$ ($Na+K+ATPase$), which is localized on the microvillus and basal membrane, and a high-affinity $Ca2+ATPase$ located on the basal membrane [19,33]. It is thought that these active transport proteins are dysregulated in fetal growth abnormalities [34,35]. The role these could play in transport of drugs or peptides into or out of the fetus remains to be described.

Endocytosis and exocytosis: During endocytosis, material is engulfed in extracellular fluid during invagination of the cell surface to form a fluid-filled vesicle. Exocytosis is the reverse of this process, vesicles fuse with the cell membrane to release their contents. This process can be receptor mediated, that is, it is triggered by a specific interaction between the solute and a receptor on the cell membrane.

Most drugs cross the human placenta by simple diffusion; however, any of the mechanisms described above may also be involved. Plasma membrane carriers, biotransforming enzymes, and efflux and influx pumps (transport proteins) may have a role in maternal-fetal exchange. Factors that affect transfer include molecular weight, degree of ionization, lipid solubility, protein binding, and fetal and maternal-placental blood flow and pH. Nonionized, nonprotein-bound lipid soluble drugs with molecular weight below 600 Da freely cross the placenta. High molecular weight drugs, such as insulin (6,000 Da), do not exchange between maternal and fetal circulations in significant amounts. One important placental function is transfer of antibodies (IgGs) from the mother to the fetus, which can occur when the mother is immunized during pregnancy. This passive immunization of the fetus requires the expression of placental FcRn receptor [36-40].

Transport Proteins

Within the placenta there are specific proteins, likely developed for endogenous substrates, which transport substances with high efficiency from maternal to fetal circulations (influx transporters) or fetal to maternal circulations (efflux transporters). Transport proteins may be expressed in the microvilli brush border or basal membrane of the syncytiotrophoblast or the endothelium of the fetal capillaries found in the villi. Many of these transport proteins are found in other organs including; gut, liver, brain and kidney where they perform similar functions. Recognizing the presence and substrate specificity of these transport proteins provides opportunity in drug development to target or exclude drug access to fetal or placental tissues [33,41]. Examples of efflux transporters in the placenta include; ATP binding cassette proteins (ABC), breast cancer resistant proteins (BCRP) and the multiple drug resistance associated proteins (MDRP). Examples of influx transporters include; organic cation transporters, dicarboxylate transporters, and the sodium/multivitamin transporters [42,19].

The expression of Permeability-glycoprotein (P-gp) on the maternal side of the placenta (eg, in the placental brush border of the syncytiotrophoblast facing the intervillous space) is encoded within the syncytiotrophoblast by the multidrug resistance gene. In some cells P-gp has physiological substrates (eg, estradiol-glucuronide, opioid neuropeptides) but also transports drugs (eg, digoxin and verapamil) out of the placenta and away from the fetal circulation. The function of this protein is to mediate active efflux of substrates from the cell with the driving force coming from ATP hydrolysis. P-gp-mediated active transport is unidirectional, facilitating efflux of substrates due to the asymmetrical membrane topology of the protein [20,21]. It appears that this efflux transporter may have evolved as a protective mechanism, being expressed not only in the placenta but also; GI tract, kidney, liver, and blood-brain barrier [20,21]. The plasma membranes of absorptive cells of the placenta, intestine, kidney, hepatocytes and endothelial cells of the blood-brain barrier exhibit polar expression of this protein.

Exploring Methods of Human Placental Function

Data from humans [43] and experimental animal studies have demonstrated that cocaine crosses the placenta [44-47]. Cocaine produces dose-related cardiac arrhythmias and death in pregnant ewes at lower doses than in nonpregnant ewes [46]. Plessinger and Woods (1990) [48] have indicated that pregnancy and progesterone enhance the maternal cardiovascular toxicity of cocaine in the pregnant sheep. For example, pregnant ewes exhibit a blood pressure response to cocaine two-fold greater than that of nonpregnant ewes given the identical dose [48]. In the Long Evans rat, pregnancy increases the

direct cardiotoxicity to cocaine, and progesterone may be responsible for this enhanced cocaine toxicity [49].

The study of exposure of preimplantation mouse embryos to cocaine *in vitro* has suggested that cocaine is capable of blocking preimplantation embryogenesis, particularly following exposure at the earliest stages of embryonic development [50]. Of interest, the authors suggest that this embryonic toxicity abates as cocaine is biotransformed to benzoylecogenine.

Administration of cocaine during pregnancy in the rabbit has shown similar adverse outcomes as seen in humans, such as stillbirth, maternal death, spontaneous abortion, and gross malformation [51]. Transplacental cardiotoxicity of cocaine during early pregnancy showed endocardial and myocardial damage and atrial walls thickening in the neonatal hamster [52] and causes atrial Purkinje fiber damage [53]. Administration of cocaine in the third-trimester to pregnant nonhuman primates have demonstrated not only cocaine's deleterious effects to the placental circulation, but also cocaine's direct pharmacological effect to the developing fetal brain [54].

Ex vivo Models of Human Placenta

In vivo, placental tissue maintains a highly active metabolism, with oxygen consumption similar to organs like brain, kidney or liver [55]. Although placental tissue is readily available after birth, this tissue has been exposed to the stress of parturition and to an ischemic period of 20-30 min before initiating experimental procedures, or beginning to prepare subcellular fractions. Despite these stresses, placental tissue shows a remarkable resistance to ischemic hypoxia, using adaptive mechanisms which allow tissue survival and maintenance of function [56,57].

The accessibility of human placental tissue together with its resistance to hypoxia or anoxia makes the human placenta particularly well suited for *in vitro* studies, with certain caveats, including; the tissue represents the structure and function of the mature placenta and may not reflect placental function earlier in gestation. Therefore multiple models such as; cell culture, tissue explants and *ex vivo* perfusion of human placental tissues have been utilized to explore a wide variety functions like cellular proliferation and differentiation as well as hormone production and endocrine function, permeability, transport, influx, efflux and metabolism.

Human placental cell culture

Cytotrophoblast cells can be isolated from human placenta and cultured, forming a syncytium which can be used to investigate drug uptake, metabolism, efflux or transfer across the syncytium. However, the method is complicated by the difficulty of preparing pure fractions of cytotrophoblast. Additionally, at the present time cytotrophoblast cultures are not viable for more than one week [58]. Other similar approaches include development of immortalized cell lines from human placental cytotrophoblast either after viral transformation or from choriocarcinomas [59].

Human placental explants

Recently, Miller et al. 2005 reviewed studies using placental explants [60], this research methodology has demonstrated improvement; providing additional tools for investigation of placental transport, metabolism, enzyme and endocrine function, and cellular proliferation and differentiation. Human placental explants have a lifetime of ~2 weeks, but users should carefully monitor explant integrity [60]. The culture of villous explants provides a model in which tissue structure is

partially maintained. Furthermore, this system allows experimentation using placental tissue from different stages of pregnancy [61].

Basic studies on placental villous explant viability; analysis of morphology, metabolic activity, glucose consumption, lactate production and protein expression have shown that placental explants remain functional for ~2 weeks following an initial degeneration with recovery after ~48 h of culture [62,63]. A detailed understanding of the extent to which placental explant integrity is comparable to the physiological, *in vivo* situation and how the process of post-ischemic recovery *in vitro* affects tissue integrity and viability is lacking [62-64]. In particular, the significance of the interval between delivery and initiation of explant culture is poorly understood. However, given the utility of this model across a broad range of gestational ages, the ability to explore influx, efflux, transport and metabolism, it is apparent that placental villous explants will be of substantial utility in drug development.

Ex vivo human placental perfusion

Placental perfusion for the study of tissue functions began in the 1960's [65-67]. Perfusion of the isolated human placental cotyledon was described in 1967 by Panigel [68], and in 1970 Nesbitt [69] introduced an apparatus for dual perfusion (both maternal and fetal circuits) which was later modified by other research groups enabling more systematic studies of placental synthetic, metabolic and transport functions [68-71]. Dual perfusion of the human placenta has been extremely useful in understanding transplacental pharmacokinetics and offers substantial opportunity to enhance drug development during pregnancy [41,57,72-74].

At the present time, and depending on the research question, placentae for perfusion are obtained from uncomplicated deliveries at term following normal pregnancies with an appropriately grown newborn [75]. Placentae from diseased states have also been utilized in perfusion experiments. Following delivery the placenta is taken to the laboratory and inspected for damage that would impair its use in a perfusion experiment. If the placenta appears suitable a peripheral cotyledon is identified for dual *ex vivo* perfusion; a chorionic artery and vein supplying the cotyledon are identified, cannulated and perfusion of the corresponding villous capillary system started (fetal compartment). Following cannulation the perfused cotyledon is fixed in a perfusion chamber maintained at 37°C. Blunt metal cannulae are introduced into the intervillous space by penetration of decidual tissue and connected to a second circuit for perfusion of the maternal compartment. The perfusate is generally tissue culture medium (eg, NCTC-135 or M199 with additional solutes as appropriate) and is continuously equilibrated with atmospheric oxygen and 5% carbon dioxide on the maternal and 95% nitrogen and 5% carbon dioxide on the fetal side. Integrity of the perfused tissue is monitored throughout the experiment [28].

To allow recovery and assure integrity of the isolated perfused cotyledon, experiment are preceded by a period of open perfusion (pre-phase) of both compartments [28]. Thereafter, experiments can be performed by using either closed (recirculating) or the open (non-recirculating) method. Recirculating studies imitate physiological conditions and can be used to study transplacental transfer and metabolism, in open perfusions drug clearance can be studied.

Antipyrine and creatinine are control references used to characterize membrane permeability and perfusion-perfusion overlap. Antipyrine diffuses rapidly, equilibrating between the two circuits, while creatinine, a hydrophilic molecule, diffuses more slowly across the placenta. The transport of antipyrine is flow limited [70-72,76], while creatinine transfer is limited by its hydrophilic property and transferred through extra-cellular pathways [76-78]. Antipyrine and creatinine are generally used to normalize data across multiple experiments [28].

In vivo information obtained through comparison of maternal and fetal (umbilical cord) concentrations of any medication (or other molecule), provides information on steady state concentrations without indicating the mechanism(s) involved in exchange between maternal and fetal compartments [79-81]. The *ex vivo* perfusion model allows studying placental transport, metabolism, influx or efflux as well as the kinetic profile and action of the chemical on placental tissue. This method has been used to study the transfer of many substances, such as nutrients, hormones, proteins, therapeutic agents and drugs of abuse, and offers an extremely useful tool for drug development [82].

Impact of Cocaine on Placental Function

In few studies the transport of cocaine and its effect on placental function under *ex vivo* conditions was investigated. In a first study using the *ex vivo* dually perfused human placentae with recirculation of both maternal and fetal perfusates, ^{3}H-cocaine and ^{14}C-inulin was added to the maternal circulation [83]. Inulin was used as reference marker for placental permeability. This study demonstrated that cocaine is taken up relatively quickly by the maternal tissue side of the placenta and transported to the fetal circulation. Steady state levels were achieved within 20 minutes in the fetal circulation, which were approximately 8% of the initial concentration of cocaine used in the maternal circulation. The level of cocaine in the fetal circulation remained higher than inulin. The permeability of inulin in the presence of cocaine was significantly reduced. The permeability measured for cocaine has indicated a similar permeability reduction as shown for inulin. Although the uptake of ^{3}H-cocaine was much higher than of inulin, the restriction of the transfer rate of cocaine compared to inulin as expected by simple diffusion is apparently due to the receptor binding site of cocaine in human placenta. In addition to the impact of cocaine on the placental permeability, there was an additional effect on placental hormone release function. After the addition of cocaine to the maternal circulation the released rate of human chorionic gonadotropin (HCG) into the maternal circulation was significantly reduced.

Similar to cocaine, opiate cross the placenta and cause intrauterine growth retardation (IUGR) and preterm deliveries. Methadone is the standard therapy for pregnant opioid-dependent women [84]. The positive effects of methadone are an increase in birth weight and prolongation of gestation [85,86]. Because co-consumption of methadone with other drugs such as cocaine and heroin is frequent, additional drugs may influence the placental transfer of methadone and other substances by different mechanisms. In case of inhibition of the P-glycoprotein (P-gp) function by other drugs, the placental barrier may disrupt, and P-gp substrates may increasingly transfer to fetal circulation [87,88]. The P-gp which is expressed in the brush-border of the placental syncytiotrophoblast and this syncytial layer is floating in the maternal blood. The P-gp is an efflux transporter meaning the protection of the placenta and the fetus against many drugs [87,88]. In a second perfusion study [89], the effect of the combined cocaine plus methadone on the placental function was investigated. All tested compounds were added to the maternal circulation. Under the conditions of the control experiments or the presence of methadone alone similar values were observed for metabolic function. While a slight

decrease in the placental permeability in the presence of methadone, a significant increase in the placental permeability was observed in the combined presence of the cocaine plus methadone. Consequently, more toxic substances or bacteria and viruses may cross the placenta and harm the fetus. Previous studies reported increased prevalence of infectious diagnoses in cocaine-exposed infants [89]. Under low oxygen (2%, hypoxic condition) there was an increased proliferation of the cytotrophoblast and lack of the fusion with syncytiotrophoblast, so shedding of MPs was predominantly the result of necrosis [91]. The already validated method of the *ex-vivo* placenta perfusion using 95% air on the maternal circuit was used [92]. In addition, the combined presence of cocaine plus methadone in the maternal circulation has induced the degradation of the syncytiotrophoblast [89], which was measured by the higher released fraction of the syncytial microparticles into the maternal circulation than those observed under control conditions, while methadone alone did not show similar effect. This observation suggests that cocaine may induce an oxidative stress on placental tissue causing the shedding of the syncytial microparticles seen in preeclampsia [92].

Conclusions

The impact of cocaine on human reproduction can be divided into three stages; preconception, pregnancy and postpartum. Approximately 4% of women use illicit drugs during their pregnancy, and cocaine is one of the most commonly abused drugs. Preconception and prenatal cocaine use is commonly associated with poor pregnancy outcomes with psychosocial, behavioral, and risk factors, such as poverty, poor nutrition, stress, depression, physical abuse, lake of social support, and sexually transmitted infection. Illicit drug use during pregnancy is a major risk factor for maternal morbidity and neonatal complications. Cocaine use during early pregnancy can cause spontaneous abortion, miscarriage, placental abortion and stillbirth. Beside cocaine effect on embryonic and fetal development, cocaine stimulates uterine contractility leading to an increased risk factor of preterm rupture of membranes, preterm labor, and preterm delivery. Moreover, congenital anomalies haven reported including brain malformation and cardiovascular abnormalities.

Supportive care should be provided for cocaine-addicted mother and newborn immediately following delivery. Cocaine use during pregnancy is considered a significant risk factor for infant neglect and abuse, which often results in the removal of the infant from maternal custody. The prenatal cocaine exposure has direct effect on brain structure or function, which heighten children´s vulnerability to negative developmental outcomes. The transport of cocaine and its effect on placental function were studied under *ex vivo* perfusion model of human placental tissues. This investigation demonstrated that cocaine is taken up relatively quickly by maternal tissue side of the placenta and transported to the fetal circulation. Moreover, cocaine induces an impact on placental permeability explaining the fetal nutritional reduction causing fetal growth reduction seen under *in vivo* conditions. There was an additional effect on placental hormone production and the degradation of the syncytiotrophoblast which is responsible for nutritional supply of the developing placenta and fetus.

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