PREDICTION OF PLACENTAL DRUG TRANSFER USING THE HUMAN PLACENTAL PERFUSION MODEL

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ABSTRACT

The placental perfusion model and a newly developed complementary computational model are reviewed. Examples are provided, where the computational model can be applied to adjust drug pharmacokinetic data obtained from the perfusion model to more closely resemble the *in vivo* placental transfer of therapeutic agents. After modelling the data, placental perfusion experiments can be used to predict placental drug transfer and can be useful for clinical assessment of the risks and benefits of drug therapy in pregnancy.

Key Words: *Placental perfusion, computational model, pharmacokinetics, pregnancy*

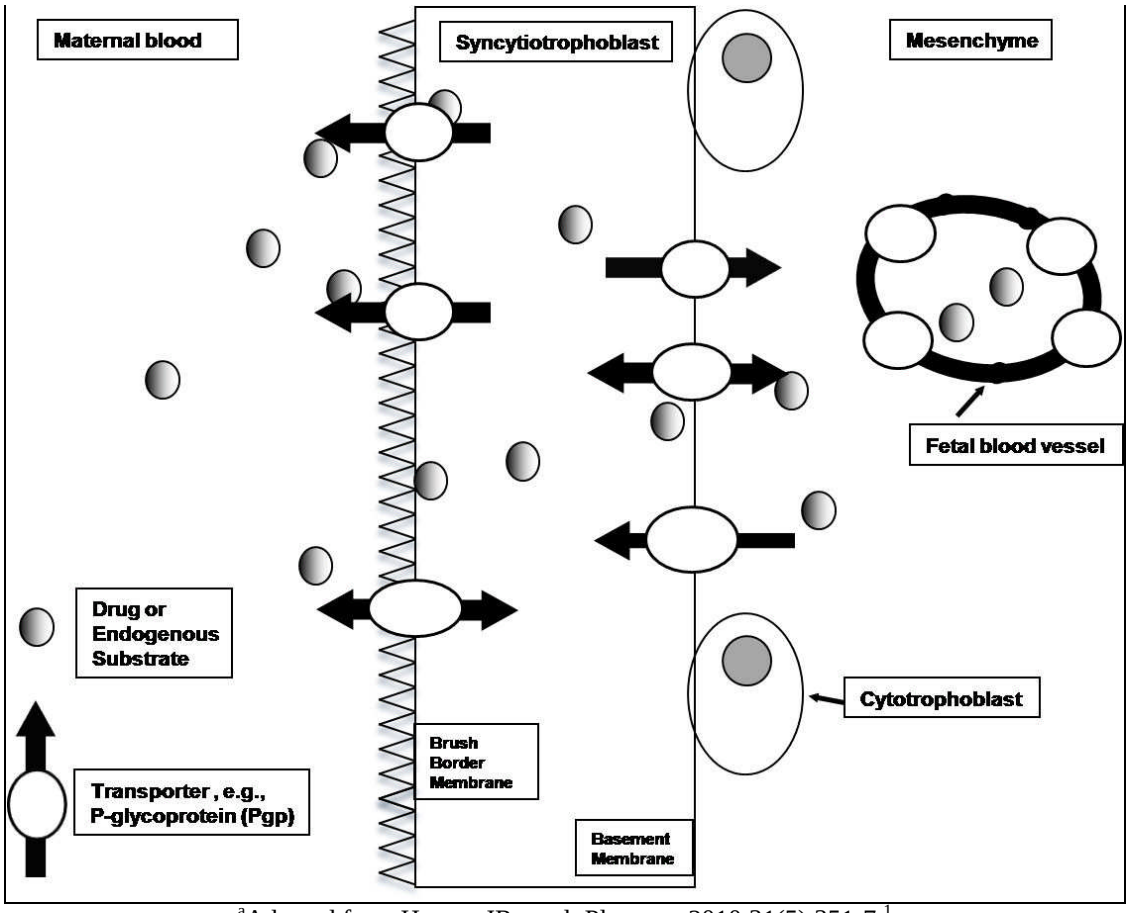
Introduction

In the basic science laboratory we have many models to look at the transfer of drugs across the placenta. However, one of the challenges of medical research is the translation of findings from the basic science laboratory into clinical practice. I will present an evaluation of the placental perfusion model and also a computational model, developed to adjust data obtained from the perfusion model to more closely resemble the *in vivo* placental transfer of therapeutic agents. After modelling the data, placental perfusion experiments can be used to predict placental drug transfer and can be useful for clinical assessment of the risks and benefits of drug therapy in pregnancy.

Background

The placenta separates the maternal and fetal circulations and performs many functions that support the maintenance of pregnancy and the normal development of the fetus. The cotyledon is the functional vascular unit of the placenta and each cotyledon contains highly branched villi suspended in the intervillous space. Maternal blood fills this intervillous space and is supplied by spiral arteries and carried away by uterine veins. Because there is virtually no basement membrane between the fetal endothelial cell and the syncytiotrophoblast, only 5 micrometres separate the fetal from the maternal blood. It is here that the rate-limiting step of drug transfer across the placenta occurs. Figure 1 shows a schematic of this interface, with the large middle rectangle representing the syncytiotrophoblast.

FIG. 1 Schematic of Fetal-Maternal Blood Interface in the Placenta[a]



[a]Adapted from Hutson JR, et al. Placenta. 2010;31(5):351-7.[1]

The syncytiotrophoblast has many membrane transport proteins that can efflux or facilitate transfer of drugs across the placenta. For example, P-glycoprotein (Pgp) on the maternal brush border membrane can prevent drugs from crossing the placenta by effluxing the drug back into the maternal circulation.

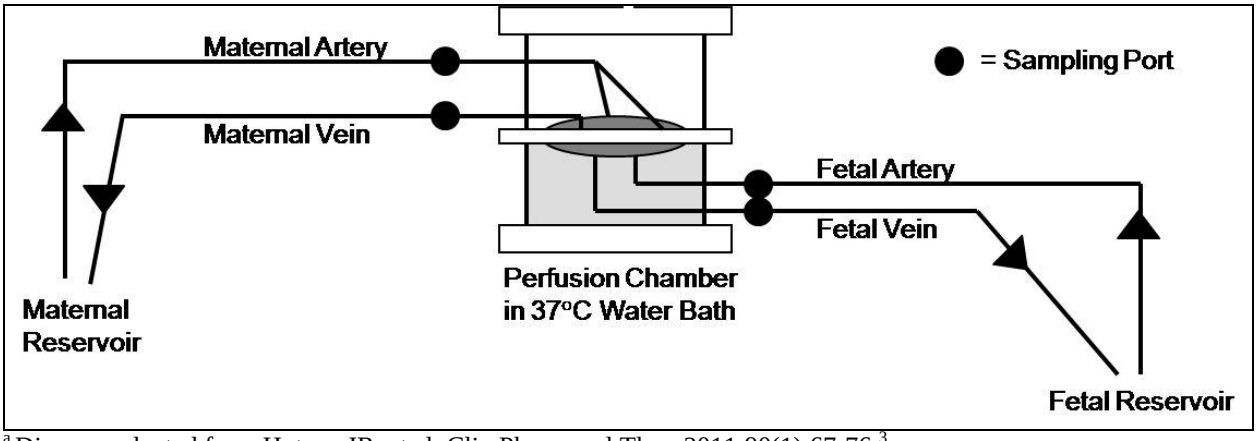
Various factors can influence placental transfer, including the physicochemical properties of a drug. These include the pK_a—*in vivo*, fetal blood pH is ~7.35 and maternal blood is ~7.4, which can lead to ion trapping of basic drugs due to the slightly more acidic fetal blood; molecular weight—drugs larger than 500 or 600 Da cannot cross the placenta unless transport is facilitated by a transport protein; and the higher the drug lipid solubility, the more drug will be transferred. Non-placental pharmacokinetic properties will also influence drug transfer, that is, protein binding in the maternal and the fetal circulations, suggested as the most important factor that can influence steady state distribution of drug across the placenta, and maternal and fetal drug distribution and elimination. Placental pharmacokinetic properties include drug transport proteins, placental drug metabolism, and binding to placental tissue, all of which can influence the rate or duration of transfer.

To study drug transfer across the placenta, various models are available (*see* Table 1), as it is often unethical to study this transfer directly in humans. The placental perfusion model should theoretically be best able to predict how drugs are transferred over time in humans.

TABLE 1 Models of Placental Drug Transfer

Type	Example	Notes
In vivo	Animal	The placenta is the most species-specific mammalian organ, therefore findings from animal studies cannot be generalized to humans with certainty.
	Termination of pregnancy	In older studies, the drug was given to the woman before termination of pregnancy. Such studies are generally no longer conducted for ethical reasons.
	Umbilical cord blood	The majority of human data originates from umbilical cord blood taken at the time of delivery. Almost always, only one time-point is collected, therefore no information is obtained about the rate of drug transfer or whether the maternal and fetal units are at steady state.
In vitro	Trophoblast cultures and tissue preparations	Membrane vesicles, placental explants, or trophoblast cell lines are used to determine whether a drug is a substrate for a placental drug transporter. This model is useful to determine the mechanism of transport, but does not provide information on the amount of drug transferred.
Computer Models	Physiologically based pharmacokinetic models (PBPK)	A computer model to estimate placental drug transfer may be developed, but it is difficult to determine how well it can be generalized to the human *in vivo*. Especially in pregnant women, caution needs to be exercised when attempting to generalize the results.
Ex vivo	Placental perfusion	This is the only experimental method that can be used to study human placental transfer of substances in organized placental tissue.

FIG. 2 A Schematic of the Placental Perfusion Model[b]



[a] Diagram adapted from Hutson JR, et al. Clin Pharmacol Ther. 2011;90(1):67-76.[3]

The Placental Perfusion Model

Perfusions are usually performed on placentae collected after caesarean section. The following procedure is performed in our laboratory. After tissue collection, a fetal vein/artery pair that supplies one cotyledon is found, usually around the periphery of the placenta. The fetal vein and artery are cannulated and the lobule is clamped into a plexiglass chamber, the maternal side facing up. The maternal and fetal circulations are maintained, as shown in Figure 2, and the chamber is kept at physiological temperature, 37°C.

The maternal and fetal circulations are independently controlled using roller-pumps, the physiological state being mimicked insofar as possible. The model can be open or closed. In the open model, the perfusate is not recycled, with fresh perfusate being constantly supplied to the placenta. In a closed model, the same perfusate is recirculated and used throughout the experiment.

Prior to our study, to our knowledge there had been no systematic evaluation of how well the perfusion model predicted fetal drug exposure. Only reviews of specific drug classes, such as antivirals, had been performed. Before the perfusion model can be used routinely to predict placental drug transfer in preclinical evaluation, careful validation of this model is needed.[2]

Evaluation of the Placental Perfusion Model

Our study, recently published in *Clinical Pharmacology and Therapeutics*,[3] had 3 primary objectives:

- To systematically evaluate the placental perfusion model in predicting placental drug transfer by comparing it to *in vivo* data.
- To construct a pharmacokinetic model that best allows prediction of the *in vivo* maternal-fetal drug distribution at steady state.
- To provide recommendations to improve the reliability of the predictions provided by the perfusion model.

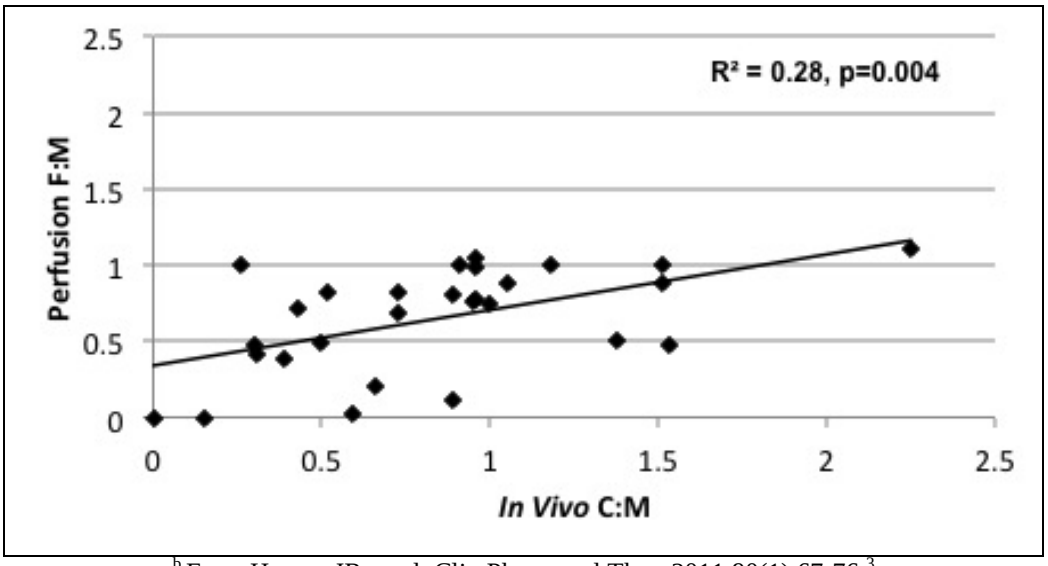
To evaluate how well the perfusion model predicts *in vivo* drug transfer, comparisons were made between fetal to maternal drug concentration ratios (F:M) from perfusion experiments and cord blood to maternal blood drug concentration ratios (C:M) at the time of delivery.

We performed a systematic search for papers evaluating placental transfer of therapeutic drugs using the perfusion model. Many drug classes have been investigated using the placental perfusion model. Most frequently studied have been antivirals and anaesthetic agents, with reports also for analgesics, antidepressants, antiepileptics, antimicrobials, antipsychotics, asthma medications, cardiac medications, chemotherapeutics, diabetic agents, endocrine agents, H_2-blockers, immunologic agents, and tocolytics. For our purposes, drugs were identified from the papers that met our inclusion criteria.[3] A subsequent search was performed, on each identified drug that was evaluated by the perfusion model, to locate papers reporting *in vivo* data, i.e., human cord blood and maternal blood concentrations at the time of delivery. F:M ratios from perfusion experiments were compared to C:M ratios, both qualitatively and quantitatively. From 1732 papers returned from the search for human placental perfusion, 147 full text articles were assessed for eligibility, resulting in 70 drugs to be compared qualitatively and 26 drugs quantitatively.

The 70 drugs compared qualitatively were classified as having limited transfer (F:M < 0.1), transfer (F:M = 0.1 to 1.0), or fetal accumulation (FM > 1.0). Forty-nine drugs showed placental transfer in both placental perfusion experiments and *in vivo*, and 9 drugs showed limited transfer in both placental perfusion and *in vivo*. It was found that any drug that showed limited transfer in the perfusion model, also had limited transfer *in vivo*. Of the 12 drugs that showed discrepancies, 5 had an F:M > 1.0 observed *in vivo*, but not in the model, and in 7, steady state was reached neither in perfusion nor *in vivo*.

Twenty-six drugs could be compared quantitatively (Figure 3). Of note, when accumulation in the fetal circulation was observed *in vivo* (C:M > 1), the perfusion model did not predict a F:M > 1 for all examples.

FIG. 3 Quantitative Comparison for 26 Drugs Using Original Data from Perfusion Experiments and Measurements[b]



[b] From Hutson JR, et al. Clin Pharmacol Ther. 2011;90(1):67-76.[3]

Adjusting the Placental Perfusion Results to Better Predict *In Vivo* Transfer

Where the placental perfusion model is excellent for investigating placental pharmacokinetics, it cannot incorporate maternal or fetal pharmacokinetic factors. These *in vivo* factors include maternal and fetal protein binding: the F:M ratio of albumin increases from 0.28 in the first trimester to 1.20 at term, and the F:M ratio of α_1- acid glycoprotein (AAG) increases from 0.09 in the first trimester to 0.37 at term. We therefore proposed a calculation model to adjust the perfusion results to better predict *in vivo* findings.

Our equation takes into account fetal and maternal protein binding, the pK_a of the drug in question, the difference in blood pH between fetus and mother, and the drug clearance in the fetus and in both mother to fetus and fetus to mother. Examples of the use of our equation to better predict *in vivo* disposition with the placental perfusion model are summarized below. Additional examples are published in our paper.[3]

$$F:M = \frac{\%\text{unbound}_M}{\%\text{unbound}_F} \times \frac{1+10^{pK_a-pH_F}}{1+10^{pK_a-pH_M}} \times \frac{CL_{MF}}{CL_{FM}+CL_F}$$

Valproic Acid

Two studies from closed perfusion experiments published steady state F:M ratios of 0.90 and 0.85.[4,5] Five *in vivo* studies showed fetal accumulation, with a weighted mean C:M ratio of 1.51 (n = 37).[6-10] Using our equation and taking *in vitro* protein binding values from the literature (maternal unbound drug = 15%, fetal unbound drug = 9.1%),[10] we arrive at an adjusted F:M ratio of 1.67, thus better estimating the *in vivo* value. F:M ratio of 1.0, using a closed perfusion model.[26]

Diazepam

Myllynen *et al*. reported a steady state F:M ratio of 0.55, using a closed perfusion model.[11] Thirteen *in vivo* studies provided a weighted mean C:M ratio of 1.27 (n = 255).[12-24] Taking this experimental ratio and *in vitro* protein binding values (maternal unbound drug = 3.24%, fetal unbound drug = 1.50%),[25] the adjusted F:M is 1.2. Again the equation better estimates the observed *in vivo* findings.

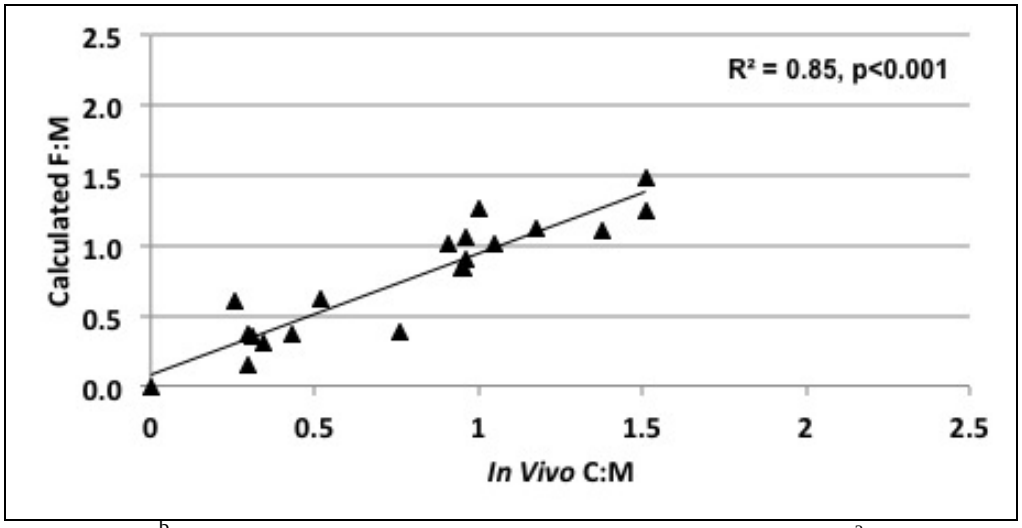
Propranolol

Here is an example where the perfusion experiment overestimates the *in vivo* drug transfer. Schneider *et al*. reported a steady state

However, Erkkola *et al*. measured the *in vivo* C:M to be 0.26 ± 0.62 in a sample of 8 patients.[27] Adjusting the perfusion results using our equation and *in vivo* protein binding data (maternal unbound drug = 21%, fetal unbound drug = 39%),[28] the adjusted F:M is 0.6 and is much closer to that observed *in vivo*.

We then calculated the F:M ratios for the 26 drugs for which we could perform quantitative analysis using our equation and replotted the data (Figure 4). This gave a better correlation between the two parameters and supports the use of the equation as an accurate way of determining placental drug transfer from perfusion experiments

FIG. 4 Quantitative Comparison for 26 Drugs after Adjusting the Placental Perfusion Results[b]



[b] From Hutson JR, et al. Clin Pharmacol Ther. 2011;90(1):67-76.[3]

Open vs. Closed Placental Perfusion Configuration

Perfusion model results and interpretations need to be considered with caution, as there are differences between the open and closed configurations. Normally, the open configuration, because of the constant supply of new perfusate containing the same drug concentration, underestimates the *in vivo* steady state C:M ratio or placental drug transfer. The results are calculated using only initial maternal drug concentration and the drug does not distribute between the maternal-placental-fetal compartments as it does *in vivo*. The open configuration is useful for calculating clearance calculations and not drug distribution. Caution should be exercised in comparing results to *in vivo* findings, as shown with alfentanil.

Alfentanil

Alfentanil is a weak base with a pK_a of 6.5, and is bound to AAG. The drug was perfused in open configuration with no protein in the perfusate.[29] At steady-state, the F:M ratio was 0.22. *In vivo* cord measurements gave a C:M ratio ranging from 0.29 to 0.35 in 4 studies (n = 45),[30-33] prompting the authors to note that their perfusion results closely estimated the *in vivo* findings. However, one study also reported a C:M ratio of about 1.0 for free drug levels (n = 31), which is what the perfusion results would be estimating, given there was no protein added to the perfusates.[30] Using our equation with the free drug data from the perfusion, the adjusted perfusion F:M ratio is 0.37: a ratio that represents total (free + bound) drug and is much closer to the *in vivo* observation.

Some investigators will add protein to either the maternal or the fetal perfusate, or to both, to more closely represent *in vivo* conditions. Albumin is commonly added to the perfusate; human plasma has also been used. The following example for bupivacaine is of a closed perfusion experiment with added protein.

Bupivacaine

In the two studies by Johnson *et al.*, 2% human serum albumin was added to both perfusates.[34-35] The findings were then F:M ratios of 0.81 and 0.74. By using human plasma on the maternal side and 4% human serum albumin on the fetal side, the F:M ratio was lower at 0.51 and 0.40, respectively.[13,14] *In vivo* observations from 3 studies (n = 51) gave a free C:M ratio of 0.73.[36-38] This closely resembles the perfusion findings where albumin was added to both sides, and represents the free drug equilibrating between the two circulations. With data from 16 studies (n = 232), a weighted mean C:M for total (bound + free) drug was calculated to be 0.30.[36,38-52] Our calculated F:M ratio, using clearance from the open placental perfusions, pK_a and protein binding data, is 0.28. By using the equation to estimate *in vivo* drug disposition, the addition of human plasma to the perfusate could be avoided. Furthermore, the results would be more accurate, and this proves to be a more practical approach to these experiments.

Limitations of the Model

When placental perfusions show limited transfer, our equation model cannot be applied. As mentioned earlier, 9 drugs from the literature search showed limited perfusion in both placental perfusion experiments and *in vivo*. In such cases, the agreement in results between the two experimental methods obviates the need to adjust the *in vitro* results using our equation. Indinavir is one such drug.

Indinavir

Two perfusion experiments were performed in open configuration with no protein added. At steady state, the F:M ratio was 0.04 and 0.06.[53,54] This matched well with what was observed *in vivo* in 2 studies (n = 25), where the C:M ratio ranged from below the limit of detection to 0.08.[55,56] If data from the perfusion model were used in the equation, the result would come to F:M = 0.26, an overestimation of the ratio.

Recommendations

As a result of reading and reviewing the papers that have been published on the subject of placental perfusion and *in vivo* fetal and maternal drug levels, we arrived at some suggestions for future studies. The details of our recommendations are published elsewhere,[3] but the key messages are:

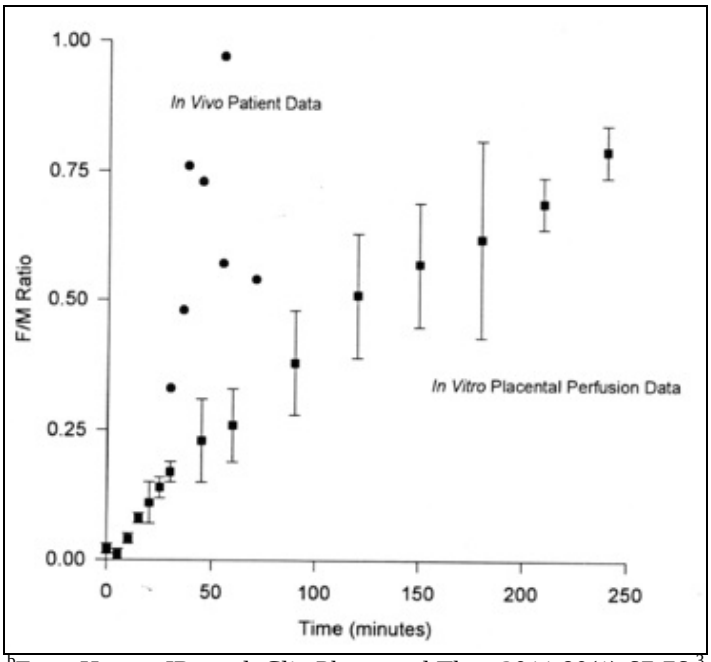
- Publication of perfusion results should report: the absolute drug concentrations, not only the F:M ratios (to facilitate secondary analyses); placental tissue binding, to

provide a fuller picture of the drug concentrations in the maternal, fetal and placental units; and the pH of maternal and fetal perfusates, so that it is clear whether physiological values are being mimicked.

- *In vitro* measurements of protein binding, conducted alongside the perfusion experiments, would enhance interpretation of perfusion results and could be used together with our equation.
- Authors need to state or show whether steady state was achieved, which would be useful for secondary analyses.

Comparison of *in vivo* timing to perfusion timing needs to be viewed with caution. The time to reach steady state *in vivo* can be very rapid, compared to perfusion experiments, as shown in Figure 5. This can be explained by the time it takes to circulate all the maternal blood in the body through the action of the heart pumping, i.e., about 1 minute. In the placental perfusion model, it takes approximately 25 minutes to pump the complete volume of maternal perfusate.

FIG. 5 *In Vivo* vs. *In Vitro* F:M of Morphine[b]



[b]From Hutson JR, et al. Clin Pharmacol Ther. 2011;90(1):67-76.[3]

SUMMARY

A systematic evaluation of the placental perfusion model shows that it is a suitable model to predict placental drug transfer. Using perfusion data together with data from *in vitro* protein binding experiments in maternal and cord blood would enhance interpretation of results from the placental perfusion model. The placental perfusion model, used appropriately, can be applied to help guide decisions regarding the benefits and risks of new medications that may be required during pregnancy.

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