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EFFETS DES INHIBITEURS SÉLECTIFS DE LA RECAPTURE DE LA SÉROTONINE SUR LE DÉVELOPPEMENT DU TROPHOBLASTE VILLEUX ET EXTRAVILLEUX PLACENTAIRE HUMAIN

Par

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RÉSUMÉ

Une femme sur cinq sera atteinte de dépression durant la grossesse, et 8-13 % d'entre elles seront traitées avec des inhibiteurs sélectifs de la recapture de la sérotonine (ISRS), la classe d'antidépresseurs la plus prescrite chez les femmes enceintes. Le bon déroulement d'une grossesse et du développement fœtal est tributaire de bons développement et fonctionnement placentaires. Les cellules trophoblastiques, cellules-clefs du placenta, se différencient selon deux phénotypes : le cytotrophoblaste villeux (CTBv) et le cytotrophoblaste extravilleux (CTBev). Les CTBv (mononucléées) fusionnent et se différencient pour former le syncytiotrophoblaste (STB; plurinucléé) ; c'est la syncytialisation. Les CTBev migrent et envahissent la muqueuse utérine, et sont indispensables à l'implantation et au remodelage des artères spiralées utérines maternelles. Un défaut d'invasion/migration ou de syncytialisation peut causer des complications importantes de grossesse, voire même une mortalité maternelle ou fœtale. Pourtant, l'effet des ISRS sur le développement du trophoblaste humain n'a jamais été étudié.

Basé sur la littérature scientifique, les hypothèses de recherche sont que les ISRS à des concentrations thérapeutiques (0.03 à 10 µM) : (1) n'ont pas d'effet sur la syncytialisation du CTBv, et (2) altèrent le processus d'implantation via l'inhibition de l'invasion, de la migration et de la prolifération des CTBev. Les objectifs spécifiques sont de déterminer l'effet des ISRS (fluoxétine, norfluoxétine, sertraline, venlafaxine, citalopram et paroxétine) sur : (1) la différenciation fonctionnelle (sécrétion de l'hormone gonadotrophine chorionique humaine (hCG)) et morphologique (fusion), et l'apoptose du trophoblaste villeux, dans les cellules de choriocarcinome BeWo et les primocultures de CTBv de placentas de grossesse normale à terme ; et (2) l'invasion, la migration et la prolifération des CTBev, dans les lignées JEG-3 (choriocarcinome) et HIPEC (dérivée de primocultures humaines de trophoblastes extravilleux). Les résultats démontrent qu'aucun de ces ISRS, aux concentrations retrouvées dans le sang maternel et dans le sang de cordon, n'a d'effets cytotoxiques, mais qu'ils ont des effets concentration- et structure-dépendants sur l'une ou l'autre des caractéristiques étudiées (fusion, différenciation biochimique, invasion, migration). Les résultats montrent également une

différence de sensibilité aux ISRS entre les CTBv et les CTBev (prolifération, cycle cellulaire), ainsi qu'entre les lignées et les primocultures. Cette étude permet de mieux comprendre les effets des antidépresseurs de type ISRS sur le développement placentaire. Elle permet d'avoir une meilleure idée de quel ISRS a le plus d'impact sur les trophoblastes villeux et extravilleux (e.g. sertraline) et fournit de nouvelles connaissances pour éclairer la communauté scientifique et les praticiens sur le bien-fondé de l'utilisation de ISRS pour le traitement des femmes enceintes dépressives.

Mots clefs : antidépresseur, grossesse, placenta, fusion, différenciation, invasion, migration, prolifération, métalloprotéase matricielle

ABSTRACT

One in five women will develop depression during pregnancy, and 8-13% of them will be treated with selective serotonin reuptake inhibitors (SSRIs), which is the most commonly prescribed antidepressant class during pregnancy. Sucessful pregnancy and healthy fetal development depends on the quality of placental development and function. Trophoblasts, key placental cells, differentiate into two phenotypes: villous cytotrophoblasts (vCTB) and extravillous cytotrophoblasts (evCTB). vCTBs (mononucleated) fuse and differentiate to form the syncytiotrophoblast (STB, plurinucleated); a process called syncytialization. evCTBs migrate and invade the maternal uterus, and are crucial for embryo implantation and for the adaptation of maternal spiral arteries. A defect of invasion / migration or syncytialization can cause significant pregnancy complications, and even maternal or fetal mortality. However, the effect of SSRIs on the human trophoblast development has never been studied.

Based on the scientific literature, the research hypotheses are that the SSRIs at therapeutic concentrations (0.03 to 10 µM): (1) have no effect on the syncytialization of vCTBs, and (2) alter the implantation process via inhibition of invasion, migration and proliferation of evCTBs. The specific objectives are to determine the effects of SSRIs (fluoxetine, norfluoxetine, sertraline, venlafaxine, citalopram and paroxetine) on: (1) functional differentiation (human chorionic gonadotropin (hCG) secretion), morphological differentiation (fusion) and villous trophoblast apoptosis, in BeWo choriocarcinoma cells and human placental trophoblast cells from normal term pregnancy placentas; and (2) invasion, migration and proliferation of CTBev, in JEG-3 (choriocarcinoma) and HIPEC (derived from human primary extravillous trophoblast cells) lines. The results demonstrate that none of these SSRIs, at concentrations found in maternal blood and cord blood, have cytotoxic effects, but that they have concentration- and structure-dependent effects on one or the other studied characteristics (fusion, biochemical differentiation, invasion, migration). The results also show a sensitivity difference to SSRIs between CTBv and CTBev (proliferation, cell cycle), as well as between cell lines and primary cells. This study provides insights into the effects of SSRIs on placental development. It provides a better idea of which SSRIs

have the greatest impact on villous and extravillous trophoblasts (e.g. sertraline) and provides new insights to inform the scientific community and practitioners about the appropriateness of using SSRIs for the treatment of depressed pregnant women.

Key words: antidepressant, pregnancy, placenta, fusion, differentiation, invasion, migration, proliferation, matrix metalloproteinase

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LISTE DES ABRÉVIATIONS

5-HIAA : 5-hydroxyindole acétique

5-HT : 5-hydroxytryptamine ou sérotonine

5-HTP : L-5-hydroxytryptophane

AADC : L-aromatique amino-acide décarboxylase

AANAT : aralkylamine N-acétyltransférase

ABC : *ATP binding cassette*

AC : adénylate cyclase

ADAM : *a disintegrin and metalloproteinase*

ADN : acide désoxyribonucléique

AGP : alpha-1 glycoprotéine acide

Alpha-1 : récepteur alpha-1 adrénergique

AMPc : adenosine monophosphate cyclique

ARC : *actin-related protein*

ARN : acide ribonucléique

ASCT2 : *anti-neutral amino acid transporter*

BDNF : *brain-derived neurotrophic factor*

BHE : barrière hémato-encéphalique

CASP : caspase ou *cysteinyl aspartate-specific proteinases*

CGB : *chorionic gonadotropin beta*

CKS1 : *cyclin dependent kinase subunit 1*

Col : collagénase

CSF-1 : *colony stimulating factor 1*

CSH : *chorionic somatomammotropin hormone*

CTB : cytotrophoblaste

CTBev : cytotrophoblaste extravilleux

CTBv : cytotrophoblaste villeux

Cx 43 : connexine 43

CYP : cytochrome P450

CYP : cytochrome P450

DAT : transporteur de la dopamine

DDIT : *DNA damage inducible transcript*

ddPCR : Doplet Digital© PCR

DPP4 : dipeptidyl peptidase-4

EGF : *epidermal growth factor*

EGFR : *epidermal growth factor receptor*

EIF2AK3 : *eukaryotic translation initiation factor 2 alpha kinase 3*

ERK1/2 : *extracellular signal-regulated kinase 1/2*

ERVFRD-1 : *endogenous retrovirus group FRD member 1*

ERVW-1 : *endogenous retrovirus group W member 1*

EV : explants villeux

FBS : sérum fœtal bovin

FOS : *FBJ murine osteosarcoma*

GABA : acide gamma-aminobutyrique

GCM : *glial cells missing*

GJA1 : *gap junction protein alpha 1*

GM-CSF : *granulocyte-macrophage colony-stimulating factor*

hCG : hormone gonadotrophine chorionique humaine

hCG-H : hormone gonadotrophine chorionique humaine hyperglycosylée

HERV-W : rétrovirus endogènes humains W

HIF-1 : *hypoxia inducible factor* 1

HIOMT : hydroxyindol O-méthyltransférase

HIPEC : *human invasive, proliferative extravillous cytotrophoblast*

hPL : lactogène placentaire

HSPA : *heat shock protein family A*

Ig: immunoglobuline

IGF : *insulin-like growth factor*

IL : interleukine

IMAO : inhibiteur de monoamine oxydase

IMAO : Inhibiteurs de la monoamine oxydase

IRS : *insulin receptor substrate*

ISRN: inhibiteurs de la recapture de la sérotonine et de la noradrénaline

ISRS : inhibiteurs sélectifs de recapture de la sérotonine

KCNJ : *potassium inwardly-rectifying channel, subfamily J*

LIF : *leukemia inhibitory factor*

L-Trp : L-tryptophane

MA : récepteur muscarinique

MAO : monoamine oxydase

MAP4K4 : *mitogen-actived protein kinase kinase kinase kinase 4*

MAPK : *mitogen-activated protein kinase*

MASH-2 : *magnesium for aneurysmal subarachnoid haemorrhage 2*

MEC : matrice extracellulaire

MMP : métalloprotéase matricielle

NET : *norepinephrine transporter* ou transporteur de la noradrénaline

NK : *natural killer*

NMDA : N-methyl-D-aspartate

PAI-1 : *plasminogen activator inhibitor-1*

PAPP-A : *pregnancy-associated plasma protein-A*

PECAM1 : *platelet endothelial cell adhesion molecule*

pGH : hormone de croissance placentaire

P-gp : P-glycoprotéine

PKA : *protéine kinase A*

PLA2G4A : *phospholipase A2, group IVA*

PLGF : *placental growth factor*

PRL : prolactine

PS : phosphatidylsérine

PUMP-1 : *putative metalloproteiase-1*

R 5-HT2 : récepteur sérotoninergique de type 2

RCIU : restriction de croissance intra-utérine

R-D2 : récepteur dopaminergique D2

R-H1 : récepteur histamininique H1

R-MA1 : récepteur muscarinique de type 1

R-α-1A : récepteur alpha-1 adrénergique

SERT : transporteurs membranaires de la 5-HT

SLC6A4 : *solute carrier family 6 member 4*

SNC : système nerveux central

STAT3 *:signal transducers and activators of transcription 3*

STB : syncytiotrophoblastes

$t_{1/2}$: temps de demi-vie

TAAR : *trace amine associated receptor*

TCA : tricyclique

TGF : *transforming growth factor*

TIMP-1 *: tissue inhibitor of metalloproteinase-1*

TNFα : *tumor necrosis factor alpha*

TPH : tryptophane hydroxylase.

uPA : *urokinase plasminogen activator*

VCAM-1: *vascular cell adhesion protein 1*

VEGF : *vascular endothelial growth factor*

VMAT : *vesicular monoamine transporter*

1 CHAPITRE 1 : CADRE CONCEPTUEL

1.1 Placenta humain

1.1.1 Généralités

Le placenta humain, organe transitoire et autonome, joue un rôle capital dans le bon déroulement de la grossesse. Étant à l'interface entre le fœtus et les tissus maternels, on peut parler de barrière placentaire (Figure 1.1). Cependant, cet organe pluripotent possède aussi de nombreuses fonctions : de nutrition, de filtration, d'épuration ainsi qu'un rôle de respiration pour le fœtus (Malassine, 2001, Schaaps *et al.*, 1998). Organe endocrine, il produit des hormones stéroïdes et peptidiques spécifiques à la grossesse (Vaillancourt *et al.*, 2009). Il a également un rôle immunologique en participant à la tolérance des antigènes fœtaux par le système immunitaire maternel (Le Bouteiller, 2001).

Le placenta humain comporte des villosités choriales (unités fonctionnelles) composées d'une partie flottante dans l'espace intervilleux (villosité flottante) et d'une partie ancrée à l'endomètre maternel (villosité crampon) (Challier *et al.*, 2003) (Figure 1.1). Il est de type hémomonochorial, c'est-à-dire que le trophoblaste villeux, situé en périphérie des villosités placentaires, entre directement en contact avec le sang maternel au niveau de la chambre intervilleuse (Aplin, 1991, Malassine, 2001). Le sang maternel et le sang fœtal ne se mélangent donc pas. Ainsi, les circulations maternelle et fœtale sont séparées par une structure d'origine fœtale, la « barrière placentaire » formée par l'endothélium des capillaires placentaires, le mésenchyme qui les entoure, et le trophoblaste, les cytotrophoblastes (couche discontinue en 3^{e} trimestre de grossesse) et le syncytiotrophoblaste.

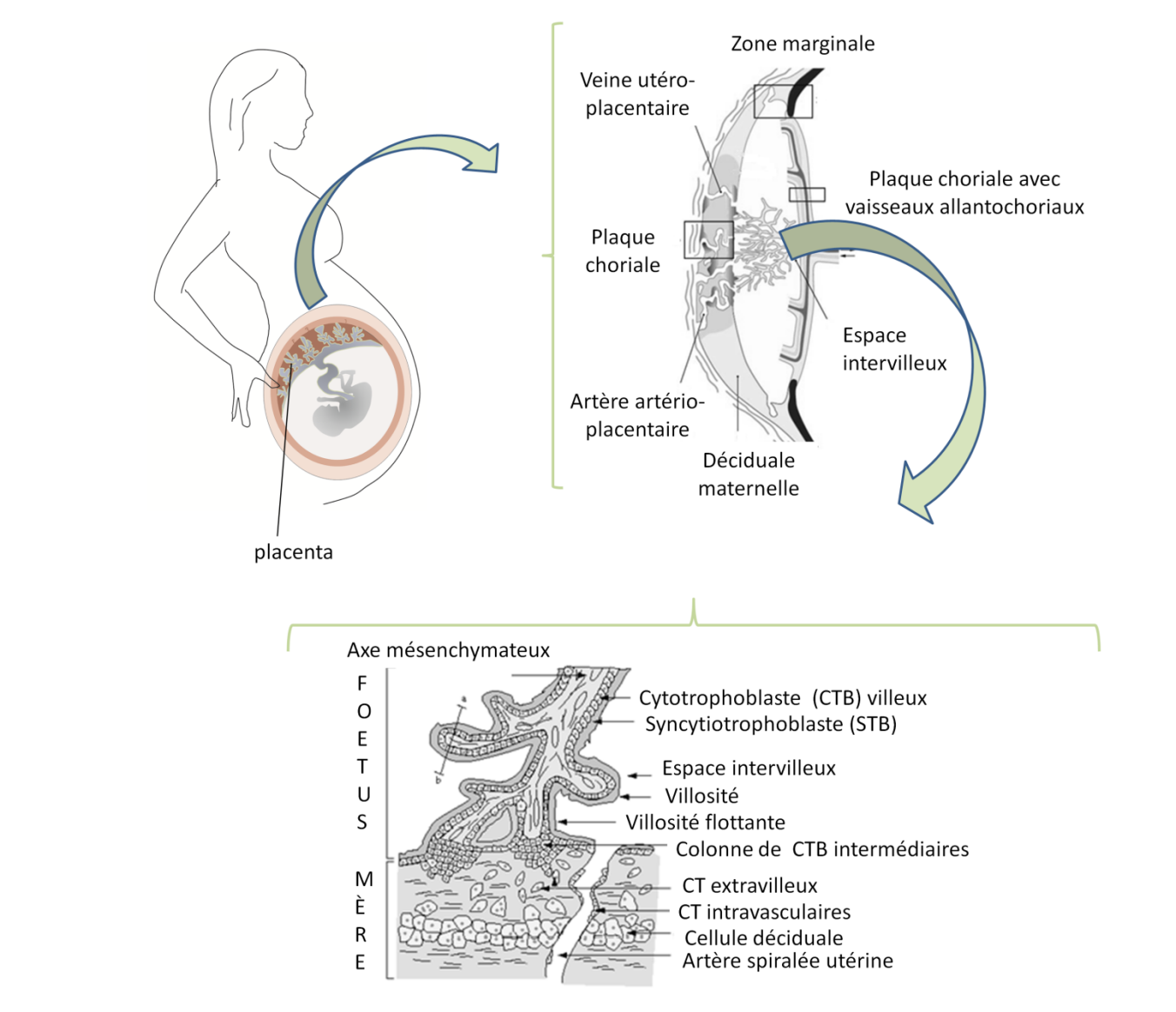

Figure 1.1 **Schéma de la structure du placenta humain.** Vue générale en haut à gauche et coupe macroscopique d'un placenta mature en haut à droite. En bas, sont présentées, des coupes longitudinales et transversales d'une villosité choriale à terme. CTB : cytotrophoblaste, STB : syncytiotrophoblaste, cellule de Hofbauer : macrophage placentaire. Adapté d'après : (Alsat et al., 1999a, Schaaps et al., 1998).

1.1.2 Différenciation du cytotrophoblaste placentaire

Les cytotrophoblastes se différencient selon deux grandes voies : la voie villeuse et la voie extravilleuse. Le cytotrophoblaste villeux (CTBv), cellule mononucléée, fusionne avec le syncytiotrophoblaste (STB) sous-jacent. Le STB est une cellule plurinucléé polarisée, qui constitue la couche cellulaire externe de la villosité chorionique (Figure 1.1) (Alsat *et al.*, 1999). Ce phénomène de fusion est appelé syncytialisation. Il s'agit d'un évènement rare qui n'est retrouvé que dans trois types cellulaires : le trophoblaste villeux placentaire, les cellules musculaires squelettiques ainsi que les ostéoclastes (Huppertz *et al.*, 2001, Vignery, 2000).

Le cytotrophoblaste extravilleux (CTBev) est primordial dans l'implantation de l'embryon et du placenta dans l'utérus maternel, ainsi que dans le remodelage des artères spiralées utérines maternelles. Le CTBev prolifère, puis devient invasif et colonise la décidue (muqueuse utérine maternelle) et le myomètre : c'est le CTBev interstitiel. Par la suite, certains CTBev migrent dans les vaisseaux maternels (CTBev endovasculaire) alors que d'autres CTBev se différencient en cellules géantes multinucléées en migrant dans la décidue (Figure 1.2) (Tsatsaris *et al.*, 2006).

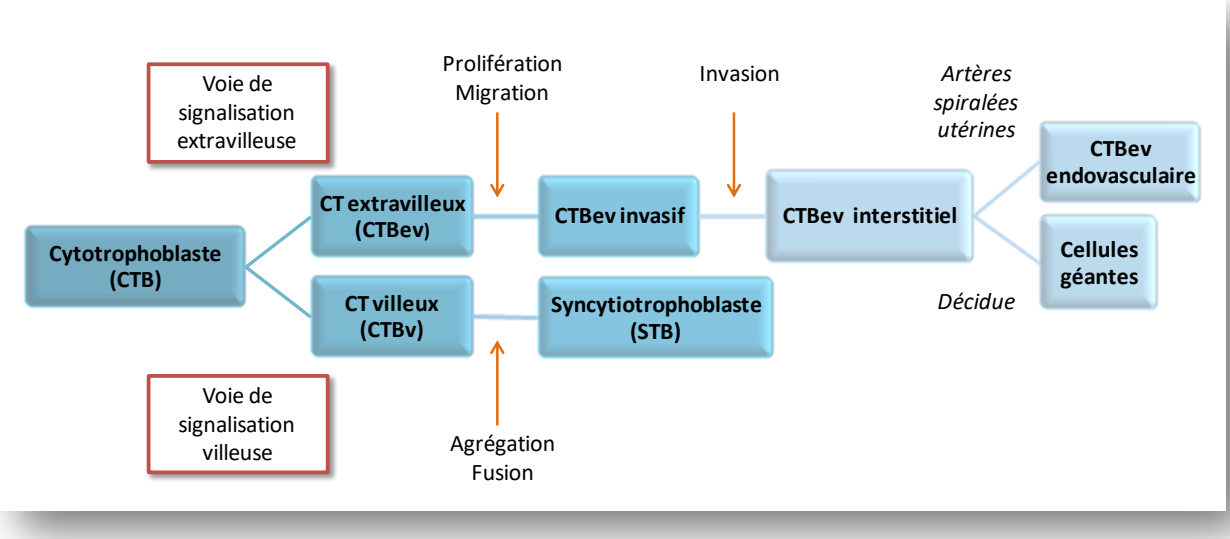

Figure 1.2 **Schéma des voies de différenciation du cytotrophoblaste.** Adapté d'après (Tsatsaris *et al.*, 2006) et (Vaillancourt *et al.*, 2009).

1.1.3 Du cytotrophoblaste villeux au syncytiotrophoblaste : la différenciation morphologique (fusion) et fonctionnelle (biochimique)

1.1.3.1 Généralités

La différenciation du trophoblaste villeux est un processus biologique complexe se produisant autant *in vivo* qu'*in vitro*. Les premiers indices de la fusion trophoblastique en un syncytium, *in vivo*, ont été rapportés par Richart en 1961, grâce à des études d'incorporation de thymidine tritiée ([^{3}H]-thymidine) (Richart, 1961a, Richart, 1961b). Les connaissances sur les mécanismes cellulaires impliqués dans la différenciation du trophoblaste villeux ont été grandement améliorées depuis qu'il est possible d'isoler et de maintenir les CTBv en culture primaire (Kliman *et al.*, 1986) (voir annexe 1). De plus, des études sur la synthèse de l'ADN par incorporation de [^{3}H]-thymidine, ont montré qu'il n'y a pas de réplication au niveau des noyaux dans la cellule de syncytium (Richart, 1961b). Pour le maintien du syncytium et la préservation des molécules qui le composent (lipides, protéines, acides nucléiques, organites, etc.), il y a donc nécessité d'une incorporation constante de CTBv par fusion durant toute la grossesse (Lanoix *et al.*, 2012a, Le Bellego *et al.*, 2009). Pour conserver l'homéostasie, ce phénomène est contrebalancé par l'exocytose de matériel apoptotique dans le sang maternel (Huppertz *et al.*, 2011, Lanoix *et al.*, 2012a).

La syncytialisation est caractérisée par une différenciation morphologique et fonctionnelle (biochimique) (Kliman *et al.*, 1986, Morrish *et al.*, 1987). La première se définit par la fusion des CTBv en un syncytium, tandis que la seconde se caractérise par la production d'hormones telles que l'hormone gonadotrophine chorionique humaine (hCG), et la lactogène placentaire humaine (hPL), l'hormone de croissance placentaire (pGH), le neuropeptide Y et la leptine (Jacquemin *et al.*, 1996, Kliman *et al.*, 1986, Morrish *et al.*, 1987, Senaris *et al.*, 1997) (revue de littérature par Costa : (Costa, 2016)). L'hCG et l'hPL sont deux hormones couramment dosées pour l'étude de la différenciation fonctionnelle du cytotrophoblaste villeux (Figure 1.3).

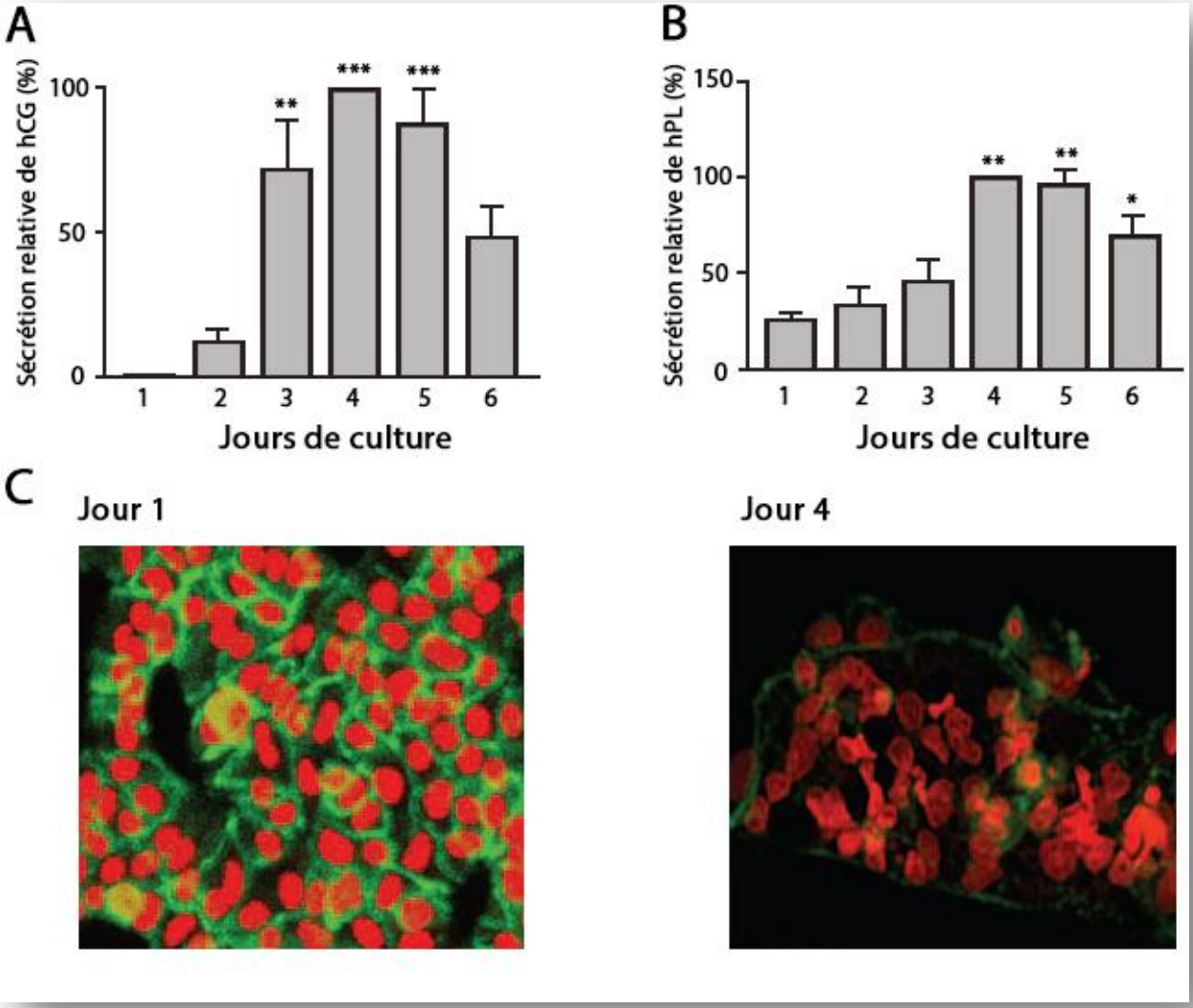

Figure 1.3 **Différenciation de primoculture de cytotrophoblastes villeux isolés de placentas humains à terme.** A et B : Différenciation biochimique (fonctionnelle) déterminée par mesure de la sécrétion d'hCG (A) et de hPL (B) du jour 1 au jour 6 de culture. C : Différenciation morphologique observée après coloration avec de l'iodure de propidium (rouge) pour les noyaux et avec un anti-desmosome (vert) pour les membranes. Tiré de (Lanoix *et al.*, 2008).

1.1.3.2 Facteurs régulant la syncytialisation du trophoblaste villeux

Les mécanismes impliqués dans la différenciation des CTBv sont des processus complexes qui, à l'heure actuelle, ne sont pas encore tous bien connus (Evain-Brion, 2001, Ji *et al.*, 2012). Néanmoins, ces dernières années, certains grands mécanismes

et facteurs stimulant ou inhibant les processus de fusion et de différenciation fonctionnelle ont pu être identifiés, tels que des facteurs de croissance, des kinases ou particules rétrovirales (Tableau 1.1).

Différents facteurs de croissance (*epidermal growth factor* (EGF), *colony stimulating factor* (CSF), *granulocyte-macrophage colony-stimulating factor* (GM-CSF), *transforming growth factor alpha* (TGFα) et *vascular endothelial growth factor* (VEGF)) (Crocker *et al.*, 2001, Garcia-Lloret *et al.*, 1994, Morrish *et al.*, 1987) et cytokines (facteur d'inhibition de la leucémie (LIF)) (Nachtigall *et al.*, 1996), d'origine autant maternelle que fœtale, sont impliqués dans la syncytialisation. La hCG, sécrétée par le STB et biomarqueur de la différenciation fonctionnelle, stimule la syncytialisation de façon autocrine par rétroaction positive (Alsat *et al.*, 1999, Shi *et al.*, 1993). L'estradiol stimule également la fusion des CTBv et la différenciation fonctionnelle (Cronier *et al.*, 1999). L'*extracellular signal-regulated kinase 1/*2 (ERK*1/*2) et p38 sont deux protéines kinases impliquées dans l'initiation de la différenciation des cellules trophoblastiques (Roux *et al.*, 2004, Vaillancourt *et al.*, 2009). L'adénosine monophosphate cyclique (AMPc), via l'activation des protéines kinases A (PKA), induit la fusion et la différenciation fonctionnelle des cytotrophoblastes *in vitro* (Gerbaud *et al.*, 2011, Knerr *et al.*, 2005, Orendi *et al.*, 2010). La présence de l'ion calcium, dans le milieu extracellulaire et aux concentrations physiologiques, est indispensable à la fusion des membranes (Le Bellego *et al.*, 2009, Rote, 2005). Le *transforming growth factor beta* (TGF-β) et le facteur de nécrose tumorale alpha (TNF-α) inhibent la syncytialisation *in vitro* ainsi que la sécrétion d'hCG et de hPL (Leisser *et al.*, 2006, Morrish *et al.*, 1991). L'oxygène joue également un rôle crucial dans la syncytialisation. En condition hypoxique, on observe une inhibition de la fusion et de la différenciation fonctionnelle (diminution de sécrétion d'hCG et de hPL), aussi bien sur les cellules BeWo que sur les cytotrophoblastes villeux primaires (Alsat *et al.*, 1996a, Alsat *et al.*, 1996b). En outre, Hu et Jiang et al. ont montré qu'en condition hypoxique *in vitro*, l'expression de nombreux facteurs de transcription est modulée, comme la kératine 1, la galectine 3 ou la Mash-2 (Hu *et al.*, 2007, Jiang *et al.*, 2000).

Les membranes cellulaires sont constituées d'une bicouche lipidique asymétrique. Les phosphatidylsérines (PS), des phospholipides chargés négativement, sont

majoritairement retrouvés du côté intracellulaire de la membrane. Lors de la fusion des cellules trophoblastiques, les PS basculent (« *flippent* ») de la membrane interne vers la membrane externe, ce qui favoriserait la fusion (Huppertz *et al.*, 1998a, Huppertz *et al.*, 2011). D'autre part, les protéines rétrovirales syncytine-1 et syncytine-2, et leurs récepteurs retrouvés, au niveau des membranes de trophoblastes, participent au phénomène de fusion trophoblastique (Blond *et al.*, 2000, Larsson *et al.*, 2008). Dès la fin des années 1980, des protéines et des particules rétrovirales ont été retrouvées au nivaux des membranes de trophoblastes (Levy, 1993). Ces particules ayant des propriétés fusogènes, leur participation dans le phénomène de fusion trophoblastique a longtemps été fortement suggérée (Lower *et al.*, 1996). Ces particules ont désormais été identifiées comme étant les protéines de syncytine-1 et de syncytine-2 (Blond *et al.*, 2000, Larsson *et al.*, 2008). La syncytine-1 est une protéine transmembranaire codée par un gène d'enveloppe de la famille des rétrovirus endogènes humains W (HERV-W) (Mi *et al.*, 2000). Frendo *et al.* ont mis en évidence, lors d'une stimulation de cytotrophoblaste primaire par de l'AMPc (un inducteur de fusion), une augmentation de l'expression de la syncytine-1 ; alors que dans des cellules trophoblastiques n'exprimant pas le gène de la syncytine-1, ils ont observé une inhibition de la syncytialisation (Jean-Louis Frendo *et al.*, 2003b). Outre l'AMPc, l'estradiol peut également réguler l'expression de cette protéine (Carino *et al.*, 2003). La syncytine-1 possède deux récepteurs : les *sodium-dependent neutral amino acid transporter* de type 1 et 2 (ASCT1 et ASCT2) (Marin *et al.*, 2003). ASCT1 n'est que faiblement exprimé au niveau placentaire (Jansson, 2001). La syncytine-2, dont l'expression placentaire a été plus récemment découverte, est codée par un gène d'enveloppe de rétrovirus endogènes de la famille de HERV-FRD (Blaise *et al.*, 2003). Cette protéine a des propriétés fusogènes et immunosuppressives (Ji *et al.*, 2012). La connexine 43 (Cx 43), une protéine de jonction communicante (*gap junction*) présente au niveau du trophoblaste villeux (Cronier *et al.*, 2002), est impliquée à la fois dans la communication, la fusion et la différenciation trophoblastique (Cronier *et al.*, 1994, Frendo *et al.*, 2003a). La glycoprotéine CD98 joue également un rôle positif dans la fusion trophoblastique (Kudo *et al.*, 2003a). Une étape importante de la fusion cellulaire est le remodelage du cytosquelette et la modification des membranes avec la dégradation des protéines

d’adhésion ; ces différentes réactions sont réalisées par des protéases spécifiques, les caspases (*cysteinyl aspartate-specific proteinases :* endoprotéases qui reconnaissent et clivent, des chaînes polypeptidiques au niveau d’un résidu aspartique de la partie carboxy-terminale. Elles jouent principalement un rôle dans l’inflammation, l’apoptose et la nécrose) (Fischer *et al.*, 2003). Dans le placenta, la caspase 8 joue un rôle majeur dans la différenciation (Black *et al.*, 2004, Huppertz *et al.*, 1999). Black *et al.* ont ainsi montré que la présence d’inhibiteurs de caspase 8 sur des explants de trophoblastes villeux de premier trimestre bloque la différenciation (Black *et al.*, 2004). L’implication des caspases 10 et 14 a également été suggérée (Huppertz *et al.*, 1999, White *et al.*, 2007).

Tableau 1.1 Principaux facteurs régulant la syncytialisation du trophoblaste villeux.

Acide nucléique			
AMPc	TT	Fusion+biochim.	(Keryer *et al.*, 1998)
Facteurs de croissance, hormones et cytokines			
Facteurs de croissance			
EGF	TT	Biochimique	(Morrish *et al.*, 1987)
CSF	TT	Biochimique	(Garcia-Lloret *et al.*, 1994)
GM-CSF	TT	Biochimique	(Garcia-Lloret *et al.*, 1994)
TGF-α	TT	Biochimique	(Yang *et al.*, 2003)
VEGF	TT, FT	Biochimique	(Crocker *et al.*, 2001)
PL74	TT	Biochimique	(H. Li *et al.*, 2005)
TGF-β	TT	Biochimique	(Morrish *et al.*, 1991)
Hormones			
hCG	TT	Biochimique	(Cronier *et al.*, 1994, Shi *et al.*, 1993)
Estradiol	TT	Fusion+biochim.	(Cronier *et al.*, 1999)
Dexaméthasone	TT	Fusion+biochim.	(Laurent Cronier *et al.*, 1998)
Cytokines			
LIF	TT	Biochimique	(Leduc *et al.*, 2012, Nachtigall *et al.*, 1996)
TNF-α	TT	Biochimique	(Leisser *et al.*, 2006)
Protéines kinases et facteurs de transcription			
Protéines kinases			
PKA	BeWo	Fusion	(Knerr *et al.*, 2005)
ERK 1/2	TT	Fusion+biochim.	(Daoud *et al.*, 2005, Vaillancourt *et al.*, 2009)
p38	TT	Fusion+biochim.	(Daoud et al., 2005; Vaillancourt et al., 2009)
Facteurs de transcription			
GCMa	BeWo	Fusion	(C. Yu *et al.*, 2002)
Mash-2	TT	Fusion	(Jiang *et al.*, 2000)

Protéines membranaires			
Transporteurs			
ASCT2	BeWo	Fusion	(Jansson, 2001, Kudo *et al.*, 2002)
CD98	BeWo	Fusion	(Kudo *et al.*, 2002, Kudo *et al.*, 2003a)
Autres			
Syncytine 1	TT, BeWo	Fusion	(Jean-Louis Frendo *et al.*, 2003b, Mi *et al.*, 2000)
Syncytine 2	TT, BeWo	Fusion	(Vargas *et al.*, 2009)
Galectine 3	BeWo	Fusion	(Dalton *et al.*, 2007)
Connexine 43	TT	Fusion	(Frendo *et al.*, 2003a)
Protéases			
Caspase 8	EV	Fusion	(Black *et al.*, 2004, Huppertz *et al.*, 1999)
Caspase 10	/	Fusion	(Huppertz *et al.*, 1999)
Caspase 14	/	Fusion	(White *et al.*, 2007)
ADAM12	/	Fusion	(Abe *et al.*, 1999)
Facteurs physico-chimiques			
Hypoxie	TT, BeWo	Fusion+biochim.	(Alsat *et al.*, 1996a, Kudo *et al.*, 2003b)
Calcium	TT, BeWo	Fusion	(Rote, 2005)
Structure membranaire			
Externalisation des PS	BeWo	Fusion	(Adler *et al.*, 1995)

En rouge les facteurs induisant la syncytialisation et en vert ceux l'inhibant. ADAM : *a disintegrin and metalloproteinase*, AMPc : adenosine monophosphate cyclique, ASCT2 : *anti-neutral amino acid transporter*, CSF-1 : *colony stimulating factor 1*, EGF : *epidermal growth factor*, ERK1/2 : *extracellular signal-regulated kinase 1/2,* EV : explants villeux, FT : trophoblastes de 1er trimestre, GCM : *glial cells missing*, GM-CSF : *granulocyte-macrophage colony-stimulating factor*, hCG : hormone gonadotrophine chorionique humaine, LIF : *leukemia inhibitory factor*, MASH-2 : *magnesium for aneurysmal subarachnoid haemorrhage 2*, MT : trophoblastes de 2^{e} trimestre, PKA : *protéine kinase A*, PS : phosphatidylsérine, TGF : *transforming growth factor*, TNFα : *tumor necrosis factor alpha*, TT : trophoblastes à terme, VEGF : *vascular endothelial growth factor*, / : n'a pas encore été découvert. Adapté d'après (Gauster *et al.*, 2009).

1.1.4 Cytotrophoblaste extravilleux (migration et invasion)

1.1.4.1 Généralités

Le cytotrophoblaste extravilleux (CTBev), cellule non polarisée, prolifère durant les trois premières semaines de grossesse, puis pénètre jusqu'au tiers supérieur du myomètre et envahit les artères spiralées maternelles (Fallet, 2010, Malassine, 2001). Cette migration/invasion permet à la fois l'implantation et le remodelage des artères spiralées utérines. Les CTBev migrent à contre-courant et remplacent progressivement les cellules endothéliales, ce qui transforme des artères spiralées utérines de haute résistance en des artères de basse résistance appelées artères utéro-placentaires (Huppertz *et al.*, 2009). Cette diminution de la résistance permet au sang maternel de pénétrer dans la chambre intervilleuse sans à-coups de pression, facilitant ainsi les échanges entre la mère et le fœtus (Sadler, 2012). Ce phénomène, permis grâce à l'invasion interstitielle et endovasculaire, est indispensable au bon développement fœtal et au bon déroulement de la grossesse (Kaufmann *et al.*, 2003). Cette invasion peut être qualifiée de pseudotumorale (Alsat *et al.*, 1999), car il s'agit une invasion de l'endomètre avec la sécrétion d'enzymes protéolytiques et « pseudo » car elle est parfaitement contrôlée et limitée à la fois dans le temps (elle n'a lieu qu'au 1er trimestre de la grossesse) et dans l'espace (seulement jusqu'au tiers supérieur du myomètre) (Halperin *et al.*, 2000).

1.1.4.2 Mécanismes impliqués dans la migration et l'invasion des cytotrophoblastes extravilleux

Entre la 10^{e} et la 12^{e} semaine de gestation, l'arrivée du sang dans la chambre intervilleuse provoque une augmentation de la pression d'oxygène. Il y a alors une modification de l'expression de gènes régulés par l'oxygène (Caniggia *et al.*, 2000). À ce moment, les CTBev se différencient et prennent un phénotype invasif. Lors de l'acquisition de leur caractère invasif, les CTBev passent de cellules de type épithéliales adhérentes à des cellules invasives (Peter Kaufmann *et al.*, 1997). Les CTBev n'expriment plus l'intégrine de surface α6β4, un récepteur des laminines permettant de

se fixer à la lame basale, mais expriment les intégrines α5β1 (un récepteur de la fibronectine) et α1β1 (un récepteur de la laminine et du collagène) (Alsat *et al.*, 1999, Damsky *et al.*, 1994, Peter Kaufmann *et al.*, 1997). Elles acquièrent également des marqueurs typiques des cellules endothéliales, tels que la molécule d'adhésion-1 plaquettes-cellules endothéliales (PECAM-1), la cadhérine de l'endothélium vasculaire et la molécule d'adhésion-1 des cellules vasculaires (VCAM-1) (Zhou *et al.*, 1997b). Les mécanismes moléculaires impliqués dans la migration et dans l'invasion des CTBev ne sont pas très bien connus à l'heure actuelle. De nombreux facteurs contrôlent la migration et l'invasion des CTBev *in vivo*, de manières autocrines et paracrines *(revues de littératures de* (Ji *et al.*, 2012) et (O'Tierney-Ginn *et al.*, 2014)) (Bischof *et al.*, 2000). Ces facteurs sont résumés dans le Tableau 1.2.

L'hypoxie induit l'expression du facteur *hypoxia inducible factor* 1 (HIF-1). Une étude sur des explants placentaires, *ex vivo*, a montré que ce facteur a un rôle très important (Caniggia *et al.*, 2000). Au 1^{er} trimestre (avant la 10^{e} semaine); les CTBev sont dans une atmosphère contenant peu d'oxygène, ce qui favorise leur prolifération et les maintient dans un phénotype immature et non invasif médié par le *transforming growth factor beta* 3 (TGFβ3) régulé par le facteur HIF-1 (Genbacev *et al.*, 1996). L'hypoxie a également un effet pro-apoptotique sur le CTBev. *In vitro,* en condition hypoxique, une augmentation des protéines pro-apoptotiques p53 (phosphoprotéine nucléaire contrôlant les réponses cellulaires aux dommages à l'ADN et les réponses aux stress par des arrêts du cycle cellulaire ou en induisant l'apoptose) et Bax, et une diminution de l'expression de la protéine anti-apoptotique Bcl-2, ont été décrites (Chen *et al.*, 2010, Humphrey *et al.*, 2008, May *et al.*, 1999). À une plus forte pression d'O_2, on observe une diminution de l'expression de l'HIF-1 et de TGFβ3, conduisant à un arrêt de la prolifération des CTBev. En parallèle, l'acquisition de marqueurs d'invasivité tels que MMP-9, ainsi qu'une diminution de l'expression de l'intégrine α5, ont été montrées (Caniggia *et al.*, 2000).

La hCG est également synthétisée par le CTBev sous une forme hyperglycosylée appelée hCG-H (Handschuh *et al.*, 2007b). Elle a la même structure polypeptidique que la hCG classique (deux sous-unité très glycosylée : la sous-unité α qui contient deux sites de N-glycosylation et une sous-unité β qui contient deux sites de N-glycosylation

et 4 sites de O-glycosylation) mais elle possède des chaînes N- et O-oligosaccharidique plus importantes. *In vitro,* la hCG-H d'origine extravilleuse stimule l'invasion trophoblastique de façon autocrine (Handschuh *et al.*, 2007a). Une étude *in vitro* a montré que la hCG-H diminue la sécrétion de TIMP-1 par les cellules endométriales, ce qui a pour conséquence de diminuer l'inhibition de MMP-2, et donc de faciliter l'invasion des CTBev (Tapia-Pizarro *et al.*, 2013). La leptine et son récepteur jouent également un rôle dans le processus invasif des CTBev, en modulant l'expression des MMP (MMP-2 et MMP-9) (Castellucci *et al.*, 2000).

Les cellules *natural killer* (NK) de la décidue maternelle sont impliquées dans l'invasion des CTBev directement, par des interactions cellule/cellule, et indirectement, en sécrétant des facteurs pro-invasifs tels que des facteurs de croissance (VEGF, *placental growth factor* (PLGF)), des facteurs chimioattractants (IL-8 et interferon-inducible protein (IP)-10) (Hanna et al., 2006). D'autre part, l'activine, produite notamment par l'endomètre et les cellules placentaires, joue un rôle important dans la formation de colonnes de CTBev, en favorisant le pouvoir migratoire et invasif des CTBev (Caniggia *et al.*, 1997, Gundacker *et al.*, 2016, Hughes *et al.*, 2015, Ji *et al.*, 2012). D'autres facteurs de croissance, dont l'*epidermal growth factor* (EGF) et le *vascular endothelial growth factor* (VEGF), régulent l'invasion trophoblastique en activant différentes voies de signalisation, notamment la voie PI3K/Akt/mTOR (Pollheimer *et al.*, 2005). Les interleukines IL-1, IL-8 et IL-10, présentes en grande concentration au niveau de l'interface fœto-maternelle (Roth *et al.*, 1999), régulent aussi l'invasion trophoblastique (Challis *et al.*, 2009, Pollheimer *et al.*, 2012). En outre, l'IL-1β stimule la sécrétion de métalloprotéase matricielle 9 (MMP-9) (Librach *et al.*, 1994). D'autre part, Roth *et al.* ont montré que l'IL-10 diminue l'expression de la MMP-9, diminuant par conséquent l'invasion des cytotrophoblastes (Roth *et al.*, 1999). Le facteur de transcription *signal transducers and activators of transcription 3* (STAT3) joue un rôle important dans l'acquisition du caractère invasif des CTBev (Leduc *et al.*, 2012).

Tableau 1.2 Principaux facteurs modulant la prolifération, la migration ou l'invasion du cytotrophoblaste extravilleux.

Facteurs de croissance, hormones et cytokines	
Facteurs de croissance	
IGF-2	(Hamilton *et al.*, 1998, J. Zhou *et al.*, 1992)
Activine	(Caniggia *et al.*, 1997, Y. Li *et al.*, 2015)
EGF	(Pollheimer *et al.*, 2005, Y. Xie *et al.*, 2015)
TGF-β	(Caniggia *et al.*, 1999, Lash *et al.*, 2005)
VEGF	(Pollheimer *et al.*, 2005)
Hormones	
hCG-H	(Islami *et al.*, 2001, Saleh *et al.*, 2007, Tapia-Pizarro *et al.*, 2013)
Leptine	(Castellucci *et al.*, 2000)
Cytokines	
TNF-α	(Bauer *et al.*, 2004)
Interleukines (IL1, IL8, IL10, IL11, IL16)	(Challis *et al.*, 2009, Y. Huang *et al.*, 2006, Jovanovic *et al.*, 2010, Librach *et al.*, 1994, Paiva *et al.*, 2009, Roth *et al.*, 1999)
Protéases, inhibiteurs de protéases et facteurs de transcription	
Protéases	
MMP-9	(Cohen *et al.*, 2006a)
MMP-2	(Cohen *et al.*, 2006a)
Collagénase IV	(Librach *et al.*, 1991)
uPA	(Huber *et al.*, 2006)
DPP4	(Fujiwara *et al.*, 2005, Sato *et al.*, 2002)
PAPP-A	(Handschuh *et al.*, 2006)
Inhibiteurs de protéases	
PAI-1/2	(Huber *et al.*, 2006) (Bauer *et al.*, 2004)
TIMP-1	(Huppertz *et al.*, 1998b)

Facteurs de transcription	
HIF-1	(Caniggia *et al.*, 2000)
STAT3	(Leduc *et al.*, 2012, Poehlmann *et al.*, 2005)
Autres	
Pression en oxygène	(Huppertz *et al.*, 2009)
Décidue	(Hanna *et al.*, 2006)

Les facteurs induisant la migration, l'invasion ou la prolifération sont en rouge, ceux l'inhibant sont en vert. DPP4 : dipeptidyl peptidase-4, EGF : *epidermal growth factor,* hCG : hormone gonadotrophine chorionique humaine, IGF : *insulin-like growth factor,* IL : interleukine, MMP : métalloprotéase matricielle, PAI-1 : *plasminogen activator inhibitor-1,* PAPP-A : *pregnancy-associated plasma protein-A,* TGFβ : *transforming growth factor beta,* TIMP-1 *: tissue inhibitor of metalloproteinase-1,* TNFα : *tumor necrosis factor alpha,* STAT3 *: signal transducers and activators of transcription 3,* uPA : *urokinase plasminogen activator,* VEGF : *vascular endothelial growth factor*. Adapté de (Fournier *et al.*, 2007).

La *dipeptidyl peptidase IV* (DPPIV) est une peptidase membranaire dont l'expression est associée au phénotype non-invasif des CTBev (Sato *et al.*, 2002). En parallèle de leur capacité à synthétiser des protéases, les CTBev sécrètent des inhibiteurs de ces protéases, comme l'inhibiteur de l'activateur du plasminogène 1/2 (PAI 1/2) et le *tissue inhibitor of metalloproteinase-1* (TIMP). Ce mécanisme d'autorégulation permet de limiter l'invasion des CTBev à une certaine profondeur (Hofmann *et al.*, 1994, Huppertz *et al.*, 1998b). Les CTBev interstitiels sécrètent aussi des protéases afin de faciliter leur pénétration dans la matrice extracellulaire de la décidue. Ils synthétisent notamment de l'*urokinase plasminogen activator* (uPA), la *pregnancy-associated plasma protein-A* (PAPP-A) ainsi que les métalloprotéases matricielles : MMP-2 (ou gélatinase A) et MMP-9 (ou gélatinase B) (Cohen *et al.*, 2006a, Handschuh *et al.*, 2006) (voir section 1.1.4.3). Enfin, le *tumor necrosis factor alpha* (TNF-α) inhibe aussi la migration et l'invasion de le CTBev en agissant sur la voie des *plasminogen activator inhibitor-1* (Bauer *et al.*, 2004).

1.1.4.3 Métalloprotéinases matricielles

Les MMP forment une famille constituée de 28 enzymes capables de dégrader les composants de la matrice extracellulaire et la membrane basale (Hendrick *et al.*, 2003b). Elles peuvent être sécrétées, comme le MMP-1 ou le MMP 18, ou fixées à la membrane cellulaire, comme les MMP-14 et MMP-25. Leurs rôles dans les phénomènes de cicatrisation, de pathologie cardiovasculaire, d'invasion métastatique et d'embryogenèse ne sont plus à démontrer (Billon *et al.*, 2000, Ketelhuth *et al.*, 2011, Shi *et al.*, 2007, Torzilli *et al.*, 2012, Wang *et al.*, 2011). Les MMP, contenant un atome de zinc dans leur site catalytique, sont synthétisées sous une forme inactive appelée zymogène (pro-enzyme). Elles sont majoritairement activées au niveau du compartiment extracellulaire et à la surface cellulaire. Cette activation se fait grâce à une réaction de protéolyse qui clive leur pro-domaine et permet de libérer le site actif (Figure1.4). Certaines MMP ont la capacité de s'auto-activer ou d'activer d'autres MMP (Dong *et al.*, 2002). Elles peuvent également être activées par des cytokines ou des protéinases comme l'*urokinase plasminogen activator* (uPA).

Les MMPs sont regroupées en six sous-familles, selon leurs substrats matriciels et leur structure : les collagénases, les gélatinases, les stromélysines, les matrilysines, les MMP membranaires et la sous-famille des autres MMP (Dong *et al.*, 2002). Huit MMP sont synthétisées par les cellules déciduales ou les CTBev (Tableau 1.3) (Cohen *et al.*, 2006a, Ferretti *et al.*, 2007, Gellersen *et al.*, 2010). Les gélatinases (MMP-2 et MMP-9) sont les MMPs qui ont été les plus étudiées pour leur implication dans l'invasion trophoblastique (Isaka *et al.*, 2003). S'il a été démontré qu'elles sont toutes deux indispensables à l'invasion trophoblastique, des études ont montré que la MMP-9 joue un rôle plus important que la MMP-2 dans ce phénomène (Polette *et al.*, 1994, Shimonovitz *et al.*, 1994, P. Xu *et al.*, 2000). Le taux de sécrétion des MMPs varie en fonction du stade de la grossesse. La MMP-2 est sécrétée dès le début de la grossesse, puis sa sécrétion diminue de la 6^{e} à la 11^{e} semaine, tandis qu'aucune sécrétion de MMP-9 n'est observée jusqu'à la 6^{e} semaine, puis augmente jusqu'à la 11^{e} semaine (Staun-Ram *et al.*, 2004, P. Xu *et al.*, 2000). Les MMP-14 et MMP-15 sont également exprimées dans le placenta humain durant le 1er trimestre de la grossesse. Outre leur activité protéolytique, elles activent la MMP-2 (Bjorn *et al.*, 2000, Hurskainen

et al., 1998). D'autres MMPs, telles que les MMP-26, MMP-1, MMP-3 et MMP-7 jouent un rôle dans l'invasion des CTBev. Elles agissent soit directement, en remodelant la matrice extracellulaire (MEC), soit indirectement, en activant d'autres protéases (Tableau 1.3) (Cohen *et al.*, 2006a, W. Qiu *et al.*, 2005, Vettraino *et al.*, 1996).

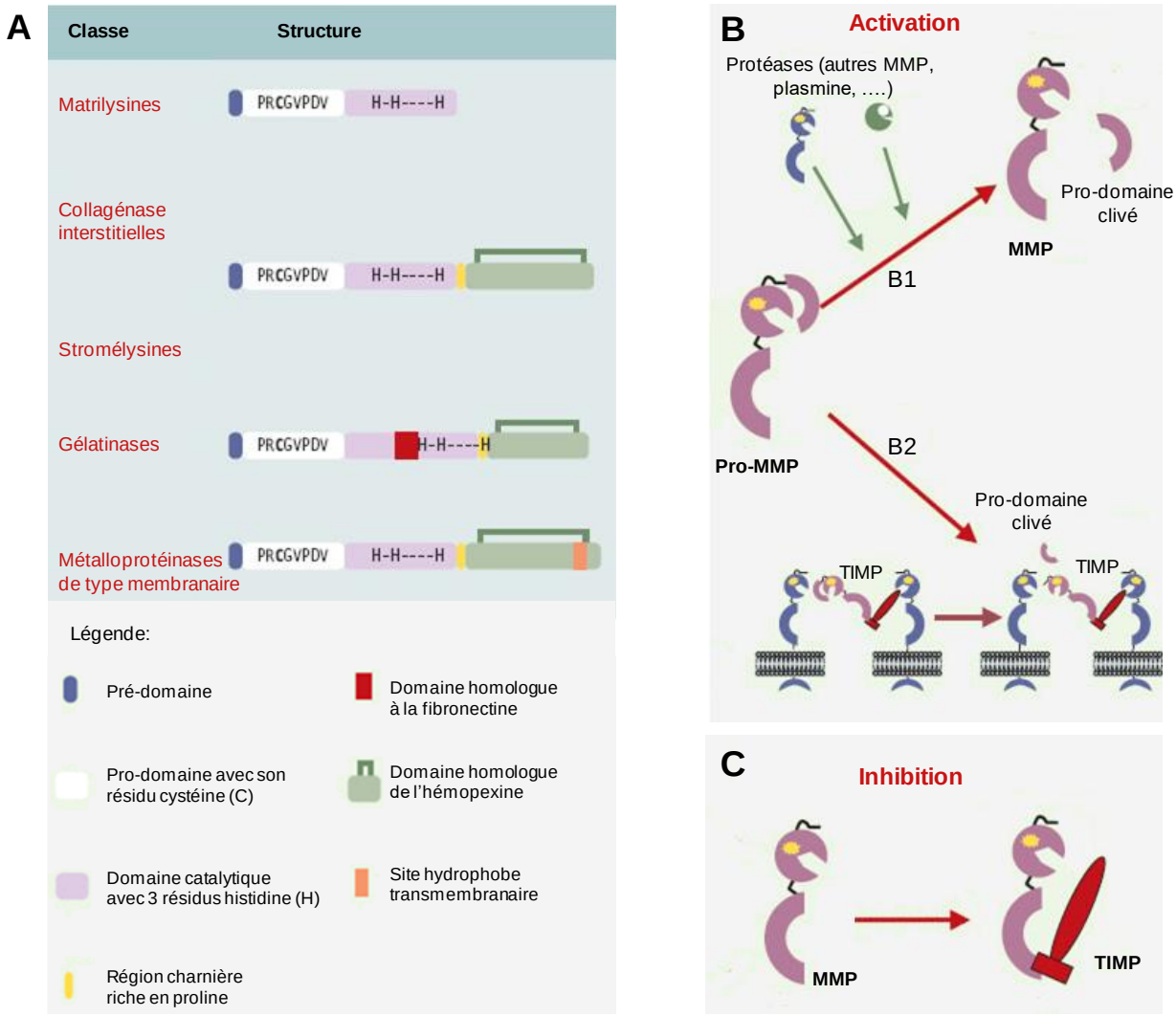

Figure 1.4 **Structures et mécanismes de régulation de l'activité des métalloprotéases matricielles (MMP).** (A) Structures des MMP selon leur classe. (B) Mécanismes d'activation des MMP. Sécrétées sous forme de pro-enzymes, elles sont activées soit (B1) dans le milieu extracellulaire par clivage protéolytique par une autre MMP, ou par la plasmine soit (B2) à la surface cellulaire comme c'est le cas pour la pro-MMP2 (violet), qui est activée par la MT1-MMP (bleu), à laquelle elle se lie après avoir formé un complexe avec le *tissue inhibitor of metalloproteinase* (TIMP)-2 (rouge). (C) Mécanismes d'inhibition des MMP par la formation d'un complexe avec le TIMP. Modifié de (Pidoux *et al.*, 2004).

Tableau 1.3 Différentes métalloprotéinases matricielles présentes dans le trophoblaste.

MMP	Autres noms	Substrats	Expression par le trophoblaste selon le stade de la grossesse
Collagénase			
MMP-1	collagénase interstitielle, collagénase fibroblastique	Col I, II, III, VII et X, gélatine, entactine, aggrécane, protéine de jonction	Durant toute la gestation
Gélatinase			
MMP-2	Gélatinase A de 72 kDa	Col I, IV, V, VII, X et XI, gélatines, fibronectines, laminines, aggrécane, élastines, entactines, vitronectine	Début de la grossesse. Diminue de la 6^{e} à la 11^{e} semaine
MMP-9	Gélatinase B de 92 kDa	Col IV, V, et XIV, gélatines, agrécane, élastines, entactines, vitronectine.	Début de la grossesse. Augmente de la 7^{e} à la 11^{e} semaine
Stromélysine			
MMP-3	Stormélysine-1, transine-1	Col III, IV, IX et X, gélatines, aggrécane, fobronectine, laminine, élactine, vitronectine, caséine, ténascine-C	Durant toute la gestation
MMP-7	Matrilysine, PUMP-1	Col IV, aggrécane, IGFBP-1, fibronectine, laminine, gélatine, élastine, entactine, vitronectine, caséine, ténascine-C	Durant toute la gestation
MMP-26	Matrilysine-2, endométase	Gélatine, β-caséine, fibronectine	Début de la grossesse. Augmente de la 7^{e} à la 11^{e} semaine
MMP membranaire			
MMP-14	MT1-MMP, MMP-XI	Col I, II, III, fibronectine, laminine-1, vitronectine, dermatane, protéoglycane de type dermatan sulfate, active pro-MMP-2 et pro MMP-13	Présent au 1er trimestre
MMP-15	MT2-MMP	Active pro-MMP-2	Présent au 1er trimestre

Col : collagénase ; MMP : métalloprotéase matricielle, PUMP-1 : *putative metalloproteiase-1*. Adapté de (Cohen *et al.*, 2006a) et (Dong et al., 2002).

Tout comme leur activation, l'inhibition des MMPs est complexe et étroitement régulée (Figure1.4). Les *tissue inhibitors of metalloproteinases* (TIMP), au nombre de quatre (TIMP-1, TIMP-2, TIMP-3, TIMP-4), sont des inhibiteurs spécifiques et réversibles de l'activité des MMPs (Tableau 1.4) (Baker *et al.*, 2002). Ils forment un complexe non covalent avec la forme active de ces enzymes. Ils se lient au niveau de leur site catalytique, en mimant leur substrat (Billon *et al.*, 2000). Les TIMPs sont produits par les cellules déciduales maternelles, qui contrôlent ainsi l'invasion des cellules trophoblastiques (Gellersen *et al.*, 2010). Outre leurs activités anti-protéasiques, les TIMPs jouent un rôle dans la croissance cellulaire et l'apoptose (Chambers *et al.*, 1997). Enfin, les MMPs peuvent aussi être inactivées par des chélateurs de métaux (ex. acide egtazique, acide ellagique) (Hung *et al.*, 2006).

Tableau 1.4 Différents *tissue inhibitors of metalloproteinases* (TIMP).

TIMP	Autres noms	MMP substrats
TIMP-1	*MMP inhibitor 1, erythroid potentiating activity, fibroblast collagenase inhibitor,* inhibiteur de collagénase	MMP-1, -2, -3, -7, -9
TIMP-2	*MMP inhibitor 2*, CSC-21K	MMP-1, -2, -3, -7, -9, -14, -15
TIMP-3	*MMP inhibitor 3*, protéine MIG-5	MMP-1, -2, -3, -7, -9, -14, -15
TIMP-4	*MMP inhibitor 4*	MMP-1, -2, -3, -7, -9

Seuls les MMPs présents au niveau du trophoblaste sont cités. Modifié de (Dong *et al.*, 2002).

En résumé, la prolifération, la migration et l'invasion des trophoblastes durant la grossesse sont contrôlées à la fois par la pression en oxygène au niveau utérin et par des facteurs maternels (déciduaux et sanguins), fœtaux et trophoblastiques. Bien que tous les mécanismes moléculaires impliqués ne soient pas parfaitement élucidés, tous les scientifiques s'entendent à dire qu'un défaut d'implantation trophoblastique peut engendrer des pathologies de grossesse.

1.1.5 Anomalies placentaires associées à une altération du trophoblaste villeux et extravilleux

Un dysfonctionnement placentaire est impliqué dans 5 à 10 % des grossesses pathologiques (Chen *et al.*, 2012). Diverses complications obstétricales, telles que des fausses couches, des restrictions de croissance ou des accouchements prématurés, ont comme origine physiopathologique une altération de l'homéostasie du trophoblaste villeux et/ou extravilleux (Evain-Brion, 2001, John *et al.*, 2012). Par exemple, un défaut d'invasion des CTBev est impliqué dans une pathologie majeure de la grossesse : la prééclampsie. Cette pathologie, qui atteint de 2 à 3 % des femmes enceintes, est associée à une hypertension artérielle et à une protéinurie maternelle (Fournie, 2012, Fournier *et al.*, 2008). Bien que la physiopathologie de cette maladie de la grossesse ne soit pas encore totalement élucidée, de nombreux facteurs intervenant dans l'invasion trophoblastique semblent impliqués, tels que le VEGF, la hCG et la caspase 8 (Ackerman *et al.*, 2012, Ji *et al.*, 2012). Une des hypothèses est qu'un défaut d'invasion des CTBev provoquerait un remodelage incomplet des artères spiralées maternelles, qui aurait pour conséquences des fluctuations dans l'apport d'oxygène au niveau de la chambre intervilleuse, provoquant un stress oxydant. Ce dernier affecterait alors l'homéostasie des trophoblastes villeux, en provoquant une augmentation de l'apoptose du STB (Adibi *et al.*, 2010, Hung *et al.*, 2006, Redman, 2011). Un défaut d'invasion du CTBev a également été décrit comme pouvant être impliqué dans la restriction de croissance intra-utérine (RCIU). En outre, une modification du profil des intégrines de surface, de la cadhérine et des immunoglobulines (Ig, glycoprotéines majoritairement membranaires ayant un rôle dans la reconnaissance, la liaison et l'adhésion des cellules) a été observée, avec notamment une augmentation de l'expression de l'intégrine α5β3, et une absence de α1β1 (Zhou *et al.*, 1993, Zhou *et al.*, 1997a). Par ailleurs, une altération de l'homéostasie du trophoblaste villeux, le plus souvent au niveau de l'apoptose du STB, est impliquée dans certaines pathologies de la grossesse telles que la RCIU, la prééclampsie, les môles (dégénérescence kystique des villosités choriales, ayant l'aspect d'un amas de petites vésicules) hydatiformes complètes ou partielles, ou encore dans certains carcinomes trophoblastiques (Kohli *et al.*, 2017, Philippe *et al.*, 1998, Scifres *et al.*, 2009).

En résumé, le placenta est un organe complexe, indispensable au bon déroulement de la grossesse, et dont le fonctionnement fait intervenir de très nombreux facteurs. Un défaut d'invasion peut entrainer un mauvais remodelage des artères utérines maternelles, et donc un apport sanguin insuffisant au niveau de la chambre intervilleuse, tandis qu'un défaut de formation du STB peut engendrer un mauvais transfert de nutriments et d'oxygène, et altérer la production des hormones essentielles au développement du fœtus. Toute cette homéostasie risque d'être perturbée par des xénobiotiques (comme des antidépresseurs), ou par des pathologies maternelles, telles que la dépression (voir section 1.2.3.3).

1.2 Dépression, antidépresseurs et grossesse

1.2.1 Dépression

1.2.1.1 Dépression et symptômes

La cause et les mécanismes d'action impliqués dans la dépression demeurent controversés à l'heure actuelle. Au niveau chimique, elle se caractérise par une diminution des taux de différents neuromédiateurs, les principaux étant la sérotonine, la noradrénaline, la dopamine, et l'acide gamma-aminobutyrique (GABA) (Peacock *et al.*, 2016). Cependant, l'hypothèse sérotoninergique, qui se base sur le fait que la dépression serait due à une diminution des taux de sérotonine au niveau central, reste prédominante. Les inhibiteurs sélectifs de la recapture de la sérotonine (ISRS) sont d'ailleurs des antidépresseurs largement prescrits, y compris pour des femmes enceintes, ce qui rend prioritaire l'étude de leur effet sur le placenta.

1.2.1.2 Hypothèse sérotoninergique

La sérotonine ou 5-hydroxytryptamine (5-HT) est produite dans de nombreux tissus, dont le système nerveux central (SNC), les ostéoclastes, ou encore les cellules gastro-intestinales (Jonnakuty *et al.*, 2008). Au niveau périphérique, elle est stockée à plus de 90 % au niveau des plaquettes sanguines (Odile, 2012). Notre laboratoire a récemment montré que les primocultures de trophoblastes villeux de placentas humains synthétisent la sérotonine (Laurent *et al.*, 2017). Cette monoamine est synthétisée à partir d'un acide aminé essentiel : le L-tryptophane. Cette synthèse nécessite deux étapes enzymatiques, une hydroxylation et une décarboxylation (Tyce, 1990), qui sont résumées dans la figure 1.5.

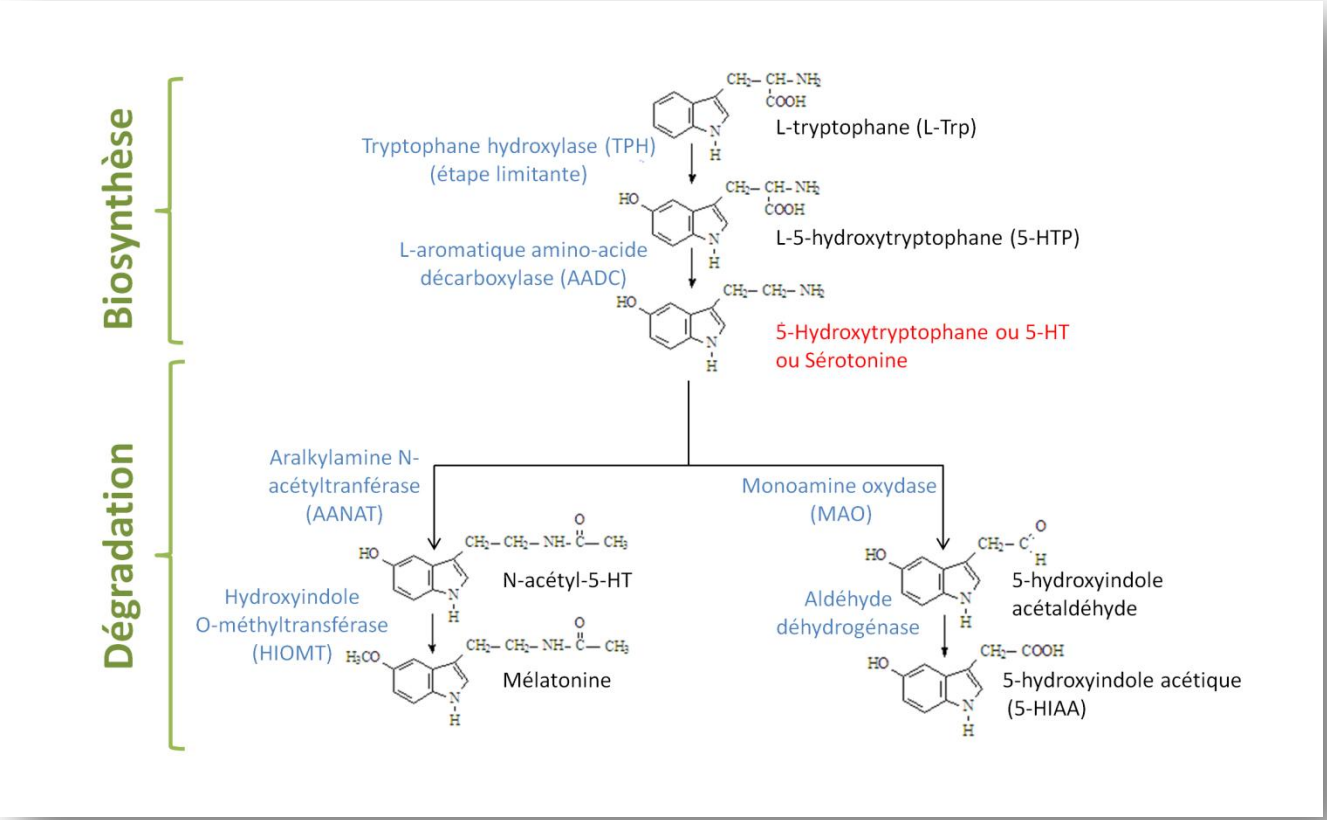

Figure 1.5 **Étapes de la biosynthèse et de la dégradation de la sérotonine.** Modifié à partir de (Pharmacorama, 2017). L'acide aminé essentiel L-tryptophane (L-Trp) est hydroxylé en L-5-hydroxytryptophane (5-HTP) par les enzymes tryptophanes hydroxylases 1 ou 2 (TPH1 ou TPH2). Le 5-HTP est converti en sérotonine (5-HT) par la L-aromatique amino-acide décarboxylase (AADC). La 5-HT est transformée en mélatonine suite à une acétylation par l'aralkylamine N-acétyltransférase (AANAT) puis à une méthylation par l'hydroxyindol O-méthyltransférase (HIOMT). La 5-HT est dégradée sous l'action successive de la monoamine oxydase (MAO) et de l'aldéhyde déhydrogénase pour former la 5-hydroxyindole acétique (5-HIAA).

La sérotonine est considérée à la fois comme un neuromédiateur, une hormone et un facteur de croissance. Dans le système nerveux central (SNC), elle est impliquée dans le cycle veille/sommeil, la prise alimentaire, et dans le contrôle de l'humeur (Jacobs *et al.*, 1992, Jonnakuty *et al.*, 2008). On sait qu'un dysfonctionnement du système sérotoninergique est impliqué dans les troubles anxieux, le comportement suicidaire et la dépression (Daszuta *et al.*, 2005, Handley, 1995, Mann, 1999). Par ailleurs, elle joue un rôle dans certaines complications obstétricales, dont la prééclampsie (complication

majeure de la grossesse associée à une hypertension artérielle et une protéinurie maternelle) (Bolte *et al.*, 2001, Sabolovic Rudman *et al.*, 2015).

Au sein des neurones sérotoninergiques du SNC, la sérotonine est stockée dans des vésicules synaptiques avant d'être libérée par exocytose, au niveau de la fente synaptique, suite à une dépolarisation membranaire. La sérotonine se fixe alors sur les récepteurs de la sérotonine (R-5HT) et y joue son rôle de neurotransmetteur, ou sinon elle est recapturée par les neurones pré-synaptiques, via les transporteurs de la sérotonine (SERT ou 5-HTT), pour ensuite être dégradée par la monoamine oxydase (MAO), ou de nouveau endocytée dans des vésicules de sécrétion (Figure 1.6).

Tel qu'évoqué plus haut, la dépression est associée à une diminution des taux de sérotonine au niveau du système nerveux central (SNC). Les taux de sérotonine périphérique sont également modifiés dans cette pathologie alors que la sérotonine ne traverse pas la barrière hémato-encéphalique (BHE) (Cleare, 1997, Mann, 1999, Maurer-Spurej *et al.*, 2007). Par ailleurs, l'expression et l'activité d'autres composants de système sérotonine sont altérées dans les cas de dépression sévère. On note une diminution de l'expression du transporteur de la sérotonine (SERT), une augmentation du nombre de récepteurs 5-HT2 au niveau des plaquettes sanguines (Du *et al.*, 2000, Hrdina *et al.*, 1995, Muller-Oerlinghausen *et al.*, 2004, Pandey *et al.*, 1995). Une augmentation de l'expression des SERT au niveau placentaire a aussi été décrite dans les cas de dépression (Ponder *et al.*, 2011).

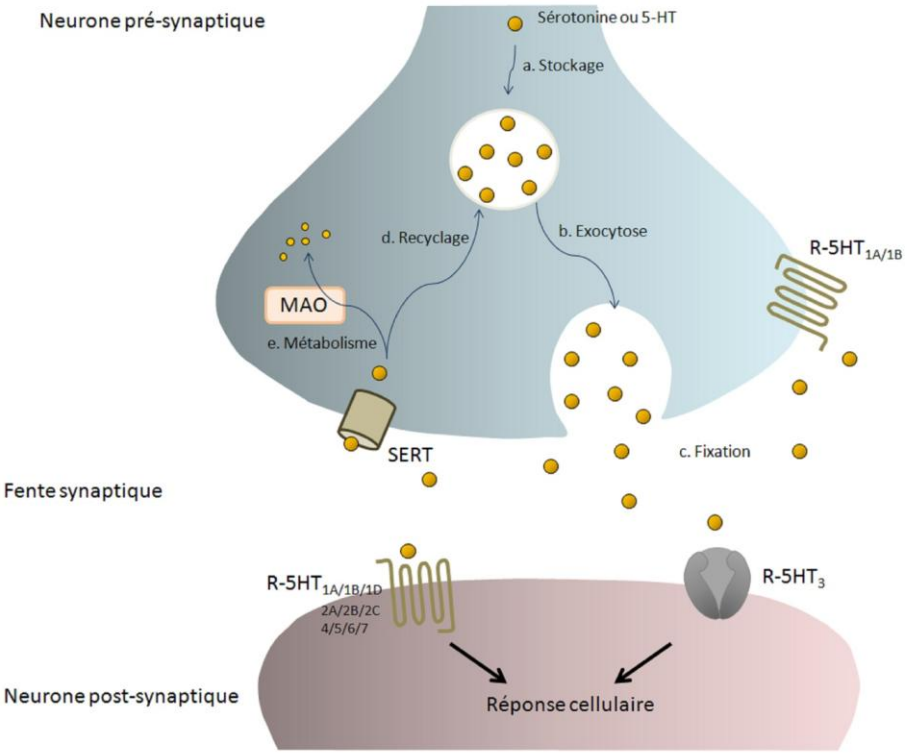

Figure 1.6 **Neurotransmission sérotoninergique.** A : La sérotonine (5-HT) est stockée dans des vésicules. B : Suite à un potentiel d'action, elle est exocytée dans la fente synaptique. C : La sérotonine peut se fixer sur ses récepteurs (R-5HT) post-synaptiques, être recapturée par ses transporteurs (SERT) pré-synaptiques, ou activer ses récepteurs pré-synaptiques. D : La sérotonine est de nouveau endocytée dans des vésicules : *vesicular monoamine transporter* (VMAT), ou sinon E : elle est dégradée par la monoamine oxydase (MAO).

Si une diminution de la synthèse de sérotonine a longtemps été une explication à la diminution de sérotonine dans le SNC (dû à une baisse des concentrations du 5HIAA dans le liquide céphalorachidien de patients déprimés ainsi qu'à une diminution de tryptophane plasmatique chez ses mêmes patients) (Bach *et al.*, 2014, Lidberg *et al.*, 2000, Traskman *et al.*, 1981), cette théorie est cependant remise en cause, car elle est considérée comme étant trop simpliste par rapport aux multiples facteurs impliqués dans cette pathologie (génétique, épigénétique, hormonaux) (Hamon, 2010).

1.2.2 Traitements

Différentes stratégies médicamenteuses ont été mises en place pour le traitement de la dépression, leur but étant d'augmenter le taux des neuromédiateurs sérotoninergiques, dopaminergiques et noradrénergiques au niveau de la fente synaptique (Tableau 1.5) (Costentin, 2009, Lôo *et al.*, 2004, Niederhoffer *et al.*, 2016). D'autres médicaments, n'appartenant pas à la famille des antidépresseurs tels que les thymorégulateurs comme le lithium (anticonvulsivant, psychotrope), les neuroleptiques, et les psychotropes, sont parfois utilisés pour le traitement de la dépression, seuls ou en association avec des antidépresseurs. De plus, des recherches sont encore en cours pour trouver de nouvelles cibles thérapeutiques, par exemple une action antagoniste sur les récepteurs N-methyl-D-aspartate (NMDA) du glutamate, ou encore en ciblant les récepteurs opioïdes Kappa et mu (Machado-Vieira *et al.*, 2017).

Quatre antidépresseurs testés dans le cadre de cette thèse sont de la classe des ISRS, qui bloquent le SERT (fluoxétine, paroxétine, citalopram et sertraline), alors que le cinquième est un inhibiteur de la recapture de la sérotonine et de la noradrénaline (ISRN), qui bloque les SERT et les transporteurs de la noradrénaline (NET) (venlafaxine). Ces cinq ISRS font partie des antidépresseurs les plus prescrits actuellement, aussi bien dans la population générale que chez les femmes enceintes (Alwan *et al.*, 2011, Andrade *et al.*, 2008, Ilyas *et al.*, 2012, Karkare *et al.*, 2011, Mars *et al.*, 2017). Notons que la venlafaxine a une affinité 30 fois plus importante pour les SERT que pour les NET (Montgomery, 2008). Ainsi, afin de faciliter la lecture, nous écrirons dans la suite du manuscrit que la venlafaxine est un ISRS.

Tableau 1.5 Principales classes d'antidépresseurs et leurs mécanismes d'action.

Classes d'antidépresseurs	Mécanismes d'action
Imipraminiques ou tricyclique (TCA)	Inhibent transporteurs membranaires de la recapture pré-synaptique de la noradrénaline et/ou de la sérotonine (autres effets pharmacologiques : anticholinergique périphérique et central M1, antihistaminique H_1, bloqueur adrénergique α, bloqueur 5-HT_2 post-synaptique)
Inhibiteurs de la monoamine oxydase (IMAO)	Bloquent les enzymes MAO dégradant des bioamines cérébrales (MAO-A et/ou MAO-B) La MAO-A dégrade préférentiellement la noradrénaline et la sérotonine, tandis que la MAO-B dégrade la dopamine.
Inhibiteurs sélectifs de la recapture de la sérotonine (ISRS)	Inhibent la recapture de la sérotonine par blocage sélectif du SERT (autres effets pharmacologiques : voir tableau 1.6)
Inhibiteurs de recapture de la sérotonine et de la noradrénaline (IRSN)	Inhibitent la recapture de la sérotonine et de la noradrénaline par blocage sélectif du SERT et du NET (autres effets pharmacologiques : voir tableau 1.6)
Autres antidépresseurs	- Antidépresseurs tétracycliques : bloquent notamment les récepteurs α-2 présynaptiques - Antidépresseurs mélatoninergiques : agonistes des récepteurs de la mélatonine MT1 et MT2 , antagonistes du récepteur $5HT_{2c}$ - Millepertuis (phytomédicament) : inhibiteur de la recapture de la sérotonine, de la noradrénaline et de la dopamine, bloque MAO, agit sur la sécrétion de la mélatonine - Vortioxétine : inhibiteur du recapture de 5-HT et également agoniste aux récepteurs 5-HT_{1A}, agoniste partiel aux récepteurs 5-HT_{1B} et antagoniste aux récepteurs 5-HT_3, 5-HT_{1D} et 5-HT_7.

Créé à partir de (Buxeraud *et al.*, 2017, Niederhoffer *et al.*, 2016).

1.2.2.1. Antidépresseurs de type Inhibiteurs sélectifs de recapture de la sérotonine (ISRS)

Les ISRS, bien qu'ayant des propriétés communes, ont des structures chimiques différentes. Ils sont dérivés de la phénylphtalane, de la phénoxypropylamine ou de la naphtylamine (Lôo *et al.*, 2004) (Figure 1.7).

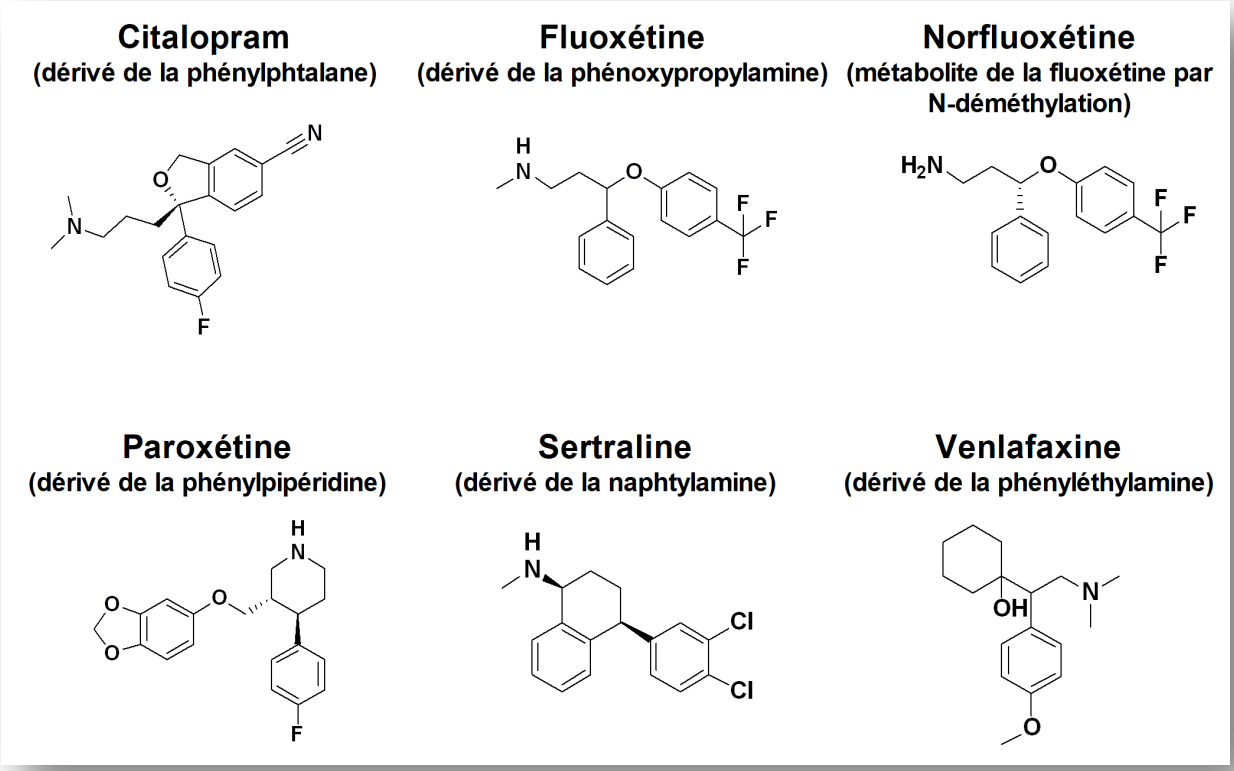

Figure 1.7 **Structure des différents inhibiteurs sélectifs de recapture de la sérotonine étudiés dans cette thèse.**

La découverte des ISRS a séduit la communauté scientifique à cause de sa sélectivité d'action, de son importante affinité pour le SERT, et du peu d'effets indésirables comparé aux autres classes d'antidépresseurs. Cependant, ces antidépresseurs ne sont pas totalement spécifiques au SERT, et peuvent se fixer avec des affinités variables à d'autres transporteurs et récepteurs (Tableau 1.6).

Tableau 1.6 Affinité des ISRS pour les neurotransporteurs et neurorécepteurs.

	Inhibition des transporteurs			Stimulation des récepteurs				
	DAT	NET	SERT	R-α-1A	R-D_2	R-H_1	R-5HT_2	R-MA_1
Citalopram	+	++	+++++	++	+	+++	++	+++
Fluoxétine	++	+++	+++++	++	+	++	+++	++
Norfluoxétine	++	+++	+++++	++	+	+	++	++
Paroxétine	++	+++	+++++	+	N.D.	+	+	+++
Sertraline	+++	+++	+++++	+++	+	+	+	+++
Venlafaxine	+	++++	+++++	++	N.D.	++	++	++

DAT : transporteur de la dopamine, NET : transporteur de la noradrénaline, SERT : transporteur de la sérotonine, R-α-1A : récepteur alpha-1 adrénergique, R-D2 : récepteur dopaminergique D2, R-H1 : récepteur histaminique H1, R-5HT_2 : récepteur sérotoninergique de type 2, R-MA_1 : récepteur muscarinique de type 1, N.D. : non déterminé. +++++ à + signifie de la plus grande à la plus petite affinité pour le transporteur ou le récepteur. Tableau créé à partir de données issues de (Bolden-Watson *et al.*, 1993, Cusack *et al.*, 1994, Hyttel, 1993, Preskorn *et al.*, 2009).

Les ISRS subissent un important effet de premier passage hépatique (environ 50 %), et sont fortement liés aux protéines plasmatiques (≥ 50 %, principalement à l'albumine et à l'alpha 1-glycoprotéine), excepté la venlafaxine (25-29 %). Leurs demi-vies plasmatiques varient de 5 h à plusieurs jours. La métabolisation est essentiellement hépatique ; différents cytochromes peuvent être impliqués (Tableau 1.7), produisant différents métabolites qui peuvent être pharmacologiquement actifs (exemple : norfluoxétine et didéméthylcitalopram), ou peu ou pas actifs (exemple : desméthyl-sertraline et les métabolites de la paroxétine) (Niederhoffer *et al.*, 2016). Les ISRS sont éliminés principalement par les reins, excepté le citalopram et la sertraline, qui sont excrétés principalement par voie fécale (Hiemke *et al.*, 2000).

Tableau 1.7 Demi-vie, liaison protéique et enzymes de métabolisation des ISRS.

Classe thérapeutique	ISRS	ISRS	ISRS	ISRS	ISRN
Principe actif (nom commercial)	Citalopram (Celexa®)	Fluoxétine (Prozac®)	Paroxétine (Paxil®)	Sertraline (Zoloft®)	Venlafaxine (Effexor®)
Liaison protéines plasmatiques	50 %	95 %	95 %	98 %	25-29%
$t_{1/2}$	33 h	4-6 j (norfluoxetine : 7-15j)	24 h	26 h	5 h
Métabolisé par	CYP2C19, CYP2D6, CYP3A4	CYP2D6, CYP2C9, CYP2C19, CYP3A4	CYP2D6, CYP3A4	CYP2B6 CYP2C9, CYP2C19, CYP2D6, CYP3A4	CYP2D6, CYP3A4

$t_{1/2}$: temps de demi-vie, CYP : cytochrome P450. Modifié de (Jin et al., 2016, Shea et al., 2012, Wille et al., 2008).

Les ISRS activent ou inhibent (directement ou indirectement) de nombreux gènes : 738 connus pour la fluoxétine, 176 pour la sertraline, 76 pour la venlafaxine et 69 pour le citalopram et pour la paroxétine (CTD, 2017a, CTD, 2017b, CTD, 2017c, CTD, 2017d, CTD, 2017e) (Tableau 1.8). Il faut cependant garder à l'esprit que si plus d'interactions ont été retrouvées avec fluoxétine, ce n'est pas forcément que cet antidépresseur est plus réactif, mais plutôt que c'est historiquement la molécule la plus étudiée. De nombreux gènes, dont l'expression est modulée par les antidépresseurs, sont impliqués dans la différenciation des CTBv comme l'EGF (paroxétine et fluoxétine), VEGF (paroxétine et fluoxétine), et connexine-43 (fluoxétine) ou encore dans la migration et l'invasion du CTBev : *TNF-α* (fluoxétine, paroxétine et sertraline), *MMP-9* (fluoxétine), *STAT3* (paroxétine, sertraline) (Allaman *et al.*, 2011, Branco-de-Almeida *et al.*, 2012, Chen *et al.*, 2014b, Fatemi *et al.*, 2008, B. Li *et al.*, 2009, Roumestan *et al.*, 2007, Taler *et al.*, 2007).

Tableau 1.8 Principaux gènes avec lesquels interagissent les ISRS (fluoxétine, citalopram, paroxétine, sertraline et venlafaxine).

Antidépresseur	Toxicogénomique (interaction génique)	Références
Fluoxétine	***R 5-HT2B***	(B. Li *et al.*, 2009, B. Li *et al.*, 2008)
	CYP2D6	(A. *et al.*, 2004, Scordo *et al.*, 2005)
	BDNF	(Allaman *et al.*, 2011, Molteni *et al.*, 2006)
	MAPK1	(B. Li *et al.*, 2008, Z. Wang *et al.*, 2013b)
	SLC6A4	(Neumaier *et al.*, 1996, Shishkina *et al.*, 2012)
	MAPK3	(Li et al., 2008) (B. Li *et al.*, 2008, Z. Wang *et al.*, 2013b)
	FOS	(Miyata *et al.*, 2005, Salchner *et al.*, 2006)
	R 5-HT1A	(Le Poul *et al.*, 2000, Song *et al.*, 2015)
	PLA2G4A	(B. Li *et al.*, 2009, Rao *et al.*, 2006)
	R 5-HT2C	(Englander *et al.*, 2005, Tyler *et al.*, 2014)
Citalopram	***SLC6A4***	(Henry *et al.*, 2006, Z. Xie *et al.*, 2007)
	PRL	(Michopoulos *et al.*, 2011, Smith *et al.*, 2004)
	TAAR1	(Z. Xie *et al.*, 2007)
	CYP2C19	(Rochat *et al.*, 1997, B. N. Yu *et al.*, 2003)
	CYP3A4	(Dorell *et al.*, 2005, Rochat *et al.*, 1997)
Paroxétine	***CYP2D6***	(Lam *et al.*, 2002, Lim *et al.*, 2005)
	R 5-HT2C	(B. Li *et al.*, 2009)
	SLC6A4	(Benmansour *et al.*, 1999, Henry *et al.*, 2006)
	EGFR	(B. Li *et al.*, 2009)
	IRS1	(Levkovitz *et al.*, 2007)
	KCNJ6	(Kobayashi *et al.*, 2006)
Sertraline	***SLC6A4***	(Benmansour *et al.*, 2002, Zhao *et al.*, 2009)
	CYP2C19	(Obach *et al.*, 2005, J. H. Wang *et al.*, 2001)

	CYP3A4	(Kumar *et al.*, 2009, Obach *et al.*, 2005)
	DDIT3	(Chen *et al.*, 2014a)
	MAP4K4	(Chen *et al.*, 2014a)
	MAPK1	(Chen *et al.*, 2014b, Levkovitz *et al.*, 2007)
	TPH2	(Kim *et al.*, 2002)
	CASP3	(Chen *et al.*, 2014a, Chen *et al.*, 2014b)
	EIF2AK3	(Chen *et al.*, 2014a)
	HSPA8	(Chen *et al.*, 2014a, Yamada *et al.*, 1999)
	R 5-HT2A	(Sell *et al.*, 2008)
	R 5-HT2C	(Sell *et al.*, 2008)
	KCNJ3	(Kobayashi *et al.*, 2011)
	MAPK3	(Chen *et al.*, 2014b, Levkovitz *et al.*, 2007)
	TNF	(Chen *et al.*, 2014b, Taler *et al.*, 2007)
Venlafaxine	***CYP2D6***	(Kingback *et al.*, 2012, Shams *et al.*, 2006)
	ARC	(Pei *et al.*, 2004, Pei *et al.*, 2003)
	CYP3A4	(Kingback *et al.*, 2012)

ARC : *actin-related protein*, BDNF : *brain-derived neurotrophic factor*, CASP : caspase, CYP : cytochrome, DDIT : *DNA damage inducible transcript,* EGFR : *epidermal growth factor receptor*, EIF2AK3 : *eukaryotic translation initiation factor 2 alpha kinase 3,* FOS : *FBJ murine osteosarcoma*, HSPA : *heat shock protein family A*, IRS : *insulin receptor substrate*, KCNJ : *potassium inwardly-rectifying channel, subfamily J*, MAP4K4 : *mitogen-actived protein kinase kinase kinase kinase 4,* MAPK : *mitogen-activated protein kinase*, PLA2G4A : *phospholipase A2, group IVA*, PRL : prolactine, R 5-HT : récepteur sérotoninergique, SLC6A4 : *solute carrier family 6 member 4*, TAAR : *trace amine associated receptor*, TNF : *tumor necrosis factor,* TPH : tryptophane hydroxylase. Minimum de 3 interactions retrouvées sauf pour la fluoxétine : 10 principaux gènes avec lesquels elle interagit. Créé d'après les données de (CTD, 2017b).

1.2.3 Dépression et grossesse

1.2.3.1 Épidémiologie et facteurs de risque de la dépression chez les femmes enceintes

Avec environ 2,4 millions de personnes atteintes de troubles de l'humeur en 2014 au Canada, la dépression est un véritable enjeu de santé publique (Statistique.Canada, 2017a). Elle atteint les femmes avec une incidence beaucoup plus forte que les hommes (revue de littérature de Perry et *al.* (Perry *et al.*, 2017)). En 2014, au Canada, on dénombrait 1,5 million de femmes diagnostiquées pour 890 000 hommes (Statistique.Canada, 2017a). L'incidence de la dépression chez la femme varie en fonction de l'âge, avec une plus forte fréquence au cours de la période fertile (Figure 1.8)**.** Les hormones sexuelles joueraient un rôle important dans la différence de prévalence de dépression entre les femmes et les hommes (Shors *et al.*, 2003)**.** En effet, la dépression atteint à des taux semblables les femmes et les hommes avant la puberté alors que les femmes sont deux fois plus atteintes que les hommes entre la puberté et la ménopause. Cette différence s'estompe ensuite chez les personnes âgées (Forlani *et al.*, 2014, Freeman *et al.*, 2004, Vigod *et al.*, 2009). Les femmes sont particulièrement susceptibles d'être atteintes de dépression dans des périodes où les taux d'estrogènes varient (post-partum, transition de ménopause) (Bennett *et al.*, 2004, Freeman, 2010, Paskova *et al.*, 2014). Par ailleurs, des facteurs psychosociaux liés au genre peuvent aussi expliquer cette différence de prévalence. En effet, les femmes déclarent plus facilement leur détresse et vont plus facilement consulter que les hommes (Association_canadienne_pour_la_santé_mentale, 2018, Gouvernement_du_Canada, 2006, OMS, 2013). En présentant les mêmes symptômes, un diagnostic de dépression serait plus facilement posé pour une femme que pour un homme (OMS, 2013).

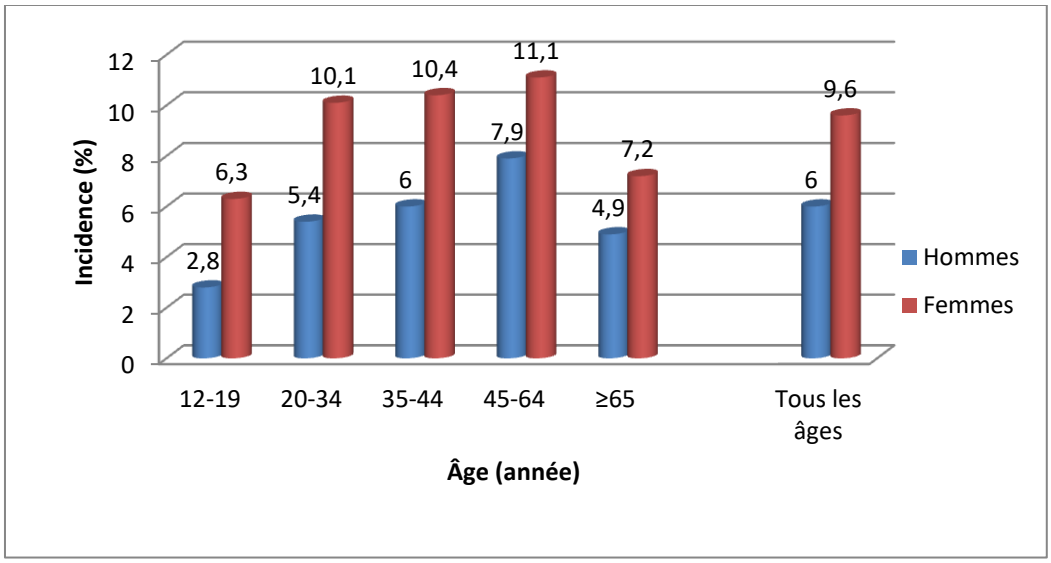

Figure 1.8 **Incidence de l'âge sur les troubles de l'humeur en fonction du sexe, au Canada, en 2014.** D'après les données de Statistique Canada (Statistique.Canada, 2017b).

De 10 à 20 % des femmes seront dépressives durant leur grossesse (Bennett *et al.*, 2004, Gavin *et al.*, 2005, Gentile, 2015). La dépression de grossesse est associée à différents facteurs de risques, qui se classifient en quatre grandes catégories : sociaux, psychologiques, liés à la santé durant la grossesse, et spécifiques à la grossesse (Tableau 1.9). Biaggi *et al.* ont proposé un classement des facteurs de risques de dépression de grossesse (Biaggi *et al.*, 2016). Il s'agit, par ordre décroissant, de : l'absence de partenaire, du manque de soutien de l'entourage, d'antécédent d'abus ou de violence domestique, d'antécédent de pathologies psychiatriques, de grossesse non désirée, et d'évènement stressant.

Tableau 1.9 Facteurs de risques de développer une dépression de grossesse

	Références
Facteurs sociaux	
Âge maternel extrême	(Bodecs *et al.*, 2013, Raisanen *et al.*, 2014, Rubertsson *et al.*, 2014)
Célibataire	(Brittain *et al.*, 2015, Jeong *et al.*, 2013)
Relation avec le partenaire (manque de soutien du partenaire, conflit, violence)	(Fonseca-Machado Mde *et al.*, 2015, Groves *et al.*, 2012, Mahenge *et al.*, 2013)
Manque de soutien du réseau social	(Agostini *et al.*, 2015, Jeong *et al.*, 2013)
Faible niveau d'éducation/chômage	(Bodecs *et al.*, 2013, Lydsdottir *et al.*, 2014)
Facteurs psychologiques	
Stress/évènement dramatique	(Husain *et al.*, 2012, St-Pierre *et al.*, 2016)
Antécédent de mauvais traitement/ d'abus	(Robertson-Blackmore *et al.*, 2013, Seng *et al.*, 2014)
Antécédent de troubles psychiatriques	(Bayrampour *et al.*, 2015, Shakeel *et al.*, 2015)
Facteurs liés à la santé durant la grossesse	
Consommation tabac, alcool, drogues	(Marcus *et al.*, 2003, Smedberg *et al.*, 2015)
Facteurs spécifiques à la grossesse	
Grossesse non désirée	(Bunevicius *et al.*, 2009, Fellenzer *et al.*, 2014)
Précédent de complication de grossesse/fausse couche	(Gong *et al.*, 2013, Waqas *et al.*, 2015)

Adapté de (Biaggi *et al.*, 2016, Ulrich *et al.*, 2016)

1.2.3.2 Épidémiologie de la prise d'inhibiteurs de recapture de sérotonine (ISRS) durant la grossesse

Si, en première ligne, le traitement de la dépression passe par un accompagnement non médicamenteux (accompagnement psychologique individuel ou collectif, yoga) (Field *et al.*, 2013a, Field *et al.*, 2013b, Spinelli *et al.*, 2016), il s'avère souvent insuffisant, et doit alors être associé à une prescription d'antidépresseurs. L'incidence de la prescription d'antidépresseurs chez les femmes enceintes est en nette progression, et a triplé aux États-Unis entre 1998 et 2005 (Alwan *et al.*, 2011). Les ISRS, incluant la venlafaxine, sont les antidépresseurs les plus prescrits aux femmes enceintes, suivis par le bupropion et les tricycliques (environ 0,5 %) (Alwan *et al.*, 2011, Andrade *et al.*, 2008). Selon les sources, on estime que de 2 à 10 % des femmes enceintes prennent des ISRS durant leur grossesse (Alwan *et al.*, 2011, Charlton *et al.*, 2015, Cooper *et al.*, 2007). Alwan *et al.* ont classé le type d'ISRS pris par les femmes enceintes entre 1998 et 2005 aux États-Unis: sertraline (1,6 %), fluoxétine (1,1 %), paroxétine (0,8 %), citalopram (0,2 %), et venlafaxine (0,2 %) (Alwan *et al.*, 2011). La prescription de paroxétine est néanmoins en nette diminution depuis 2003 ; elle a été divisée par 4 entre 2003 et 2007 (Huybrechts *et al.*, 2013). Ce phénomène s'explique par la prise en compte, par les autorités de santé publique, d'effets indésirables majeurs de ce médicament, tels que le suicide et des malformations congénitales (Berard *et al.*, 2016, Cole *et al.*, 2007, Huybrechts *et al.*, 2013).

1.2.3.3 Recommandation et polémique sur le bien fondé du traitement de la dépression de grossesse par des ISRS

Les autorités de santé et les cliniciens font face à un dilemme : le traitement thérapeutique ou non de la dépression de grossesse. Une dépression durant la grossesse peut avoir un effet négatif sur la santé de la mère, mais aussi sur celle du fœtus (Christian *et al.*, 2009, de Paz *et al.*, 2011, Gentile *et al.*, 2017, Ponder *et al.*, 2011). Ce danger peut être d'origine directe, par l'intermédiaire d'un milieu hormonal altéré ou d'un mauvais développement placentaire, ou indirecte, par l'intermédiaire des comportements maternels, tels que l'abus de substances (alcool, drogues), une

mauvaise alimentation, voire même le suicide. Les femmes enceintes dépressives non traitées ont un risque plus élevé de complications obstétricales associant un défaut de développement et de fonctionnement placentaire, telles que la prééclampsie, la restriction de croissance intra-utérine et la prématurité (Tableau 1.10). La dépression maternelle pose donc de nombreux risques pour la mère et le fœtus, et l'absence de traitement peut avoir un impact négatif sur le développement du fœtus.

Malgré l'utilisation importante d'ISRS par les femmes enceintes ; il n'existe pas à ce jour de consensus médical sur la prescription de cette classe d'antidépresseurs. Si son utilisation est acceptée au vu des bénéfices qu'elle peut avoir pour la mère et l'enfant, elle reste controversée pour les différentes autorités de santé, notamment à cause des effets indésirables que ces médicaments peuvent engendrer (CRAT, 2017, FDA, 2011). Chez les nouveau-nés de mère ayant pris un ISRS en fin de grossesse, on a rapporté de l'irritabilité, des tremblements, de l'hypotonie, des pleurs persistants, de la difficulté de succion, ou des troubles du sommeil, symptômes d'un syndrome de sevrage sérotoninergique (Levinson-Castiel *et al.*, 2006, Vidal, 2017b). Plusieurs études ont rapporté, suite à une exposition aux ISRS, un risque accru de faible poids de naissance, d'admissions accrues en soins intensifs, ou encore de toxicité cardiaque et pulmonaire (Tableau 1.10). D'autres effets de ces antidépresseurs sont plus controversés (prématurité, prééclampsie, tératogénicité, trouble du spectre de l'autisme), et si certaines études ont identifié ces effets, d'autres, par contre, n'ont pas retrouvé de corrélation. Ceci s'explique notamment par le fait qu'il est difficile de séparer l'effet de la dépression de grossesse de l'effet des antidépresseurs en tant que tels (Millard *et al.*, 2017).

Tableau 1.10 Conséquences maternelles, obstétricales et sur l'enfant d'une dépression de grossesse traitée ou non par inhibiteur sélectif de recapture de la sérotonine (ISRS).

Dépression de grossesse non traitée		Dépression de grossesse traitée par ISRS	
Conséquences maternelles			
Conduite à risque (tabac, alcool, drogues)	(Alhusen *et al.*, 2016, Olivier *et al.*, 2014)	*Note : cette liste n'est pas exhaustive ; seuls les effets indésirables les plus fréquents sont référencés ici.*	
Malnutrition, obésité, anémie, diabète	(Alhusen *et al.*, 2016, Bansil *et al.*, 2010, Olivier *et al.*, 2014)	Troubles psychiatriques : agitation, insomnie, sédation, somnolence, rêves anormaux	(Vidal, 2017a, Vidal, 2017c, Vidal, 2017d, Vidal, 2017e)
Suicide	(Filippi *et al.*, 2016, Oates, 2003)	Troubles gastro-intestinaux : nausée, vomissement, diarrhée, constipation, sécheresse buccale	
		Troubles du système nerveux : vertige, tremblement, céphalée, difficultés de concentration	
		Bâillement excessif	
		Troubles de vision : flou ou trouble	
		Dysfonction sexuelle : baisse de libido	
		Hypersudation	
		Troubles généraux (asthénie, prise de poids, anorexie)	
Conséquences obstétricales			
Hypertension / prééclampsie	(Bansil *et al.*, 2010, Kurki *et al.*, 2000)	Hypertension / prééclampsie (controversé)	(Avalos *et al.*, 2015, Uguz, 2017)
Prématurité	(Grote *et al.*, 2010, Sahingoz *et al.*, 2014)	Prématurité (controversé)	(Eke *et al.*, 2016, Hendrick *et al.*, 2003a, H. Huang *et al.*, 2014, Sahingoz *et al.*, 2014)

Accouchement par césarienne accru	(Malm *et al.*, 2015)		
Restriction de croissance intra-utérine	(Bansil *et al.*, 2010, Grote *et al.*, 2010)		
Conséquences sur l'enfant			
Morbidité infantile	(Bansil *et al.*, 2010)	Syndrome de sevrage néonatal	(Levinson-Castiel *et al.*, 2006)
Faible poids à la naissance	(Grote *et al.*, 2010, Sahingoz *et al.*, 2014)	Faible poids à la naissance	(Hendrick *et al.*, 2003a, H. Huang *et al.*, 2014, Oberlander *et al.*, 2006)
		Hypertension artérielle pulmonaire persistante du nouveau-né (controversé)	(Alwan, Bandoli, & Chambers, 2016; Andrade et al., 2009; Berard et al., 2017)
Troubles psychologiques et comportementaux	(Davalos *et al.*, 2012, Hammen *et al.*, 2003)	Faibles scores d'Apgar	(Jensen *et al.*, 2013, Malm *et al.*, 2015)
		Toxicité cardiaque/cardiovasculaire (surtout la paroxétine)	(Berard *et al.*, 2014, Berard *et al.*, 2016, Nembhard *et al.*, 2017, Ornoy *et al.*, 2017)
Retard voir faible développement cognitif et retard psychomoteur	(Deave *et al.*, 2008, Nulman *et al.*, 2012)	Tératogénicité (controversée)	(Jordan *et al.*, 2016, Reefhuis *et al.*, 2015)
		Admission en soin intensive accrue	(Malm *et al.*, 2015, Norby *et al.*, 2016)
Risque accru dépression, anxiété, trouble du déficit de l'attention avec ou sans hyperactivité, trouble de spectre de l'autisme.	(Kaplan *et al.*, 2017, Lahti *et al.*, 2017, Rodriguez *et al.*, 2005)	Trouble de spectre de l'autisme.	(Boukhris *et al.*, 2016, Kaplan *et al.*, 2017)

1.2.4 Dépression de grossesse et placenta

1.2.4.1 Pharmacocinétique maternelle et placentaire des ISRS

Chez les humains, tous les ISRS traversent le placenta et sont retrouvés au niveau du fœtus. La comparaison des taux de ces antidépresseurs, dosés à la fois dans le sérum maternel et dans le sang de cordon (Figure 1.9), permet d'estimer la concentration placentaire.

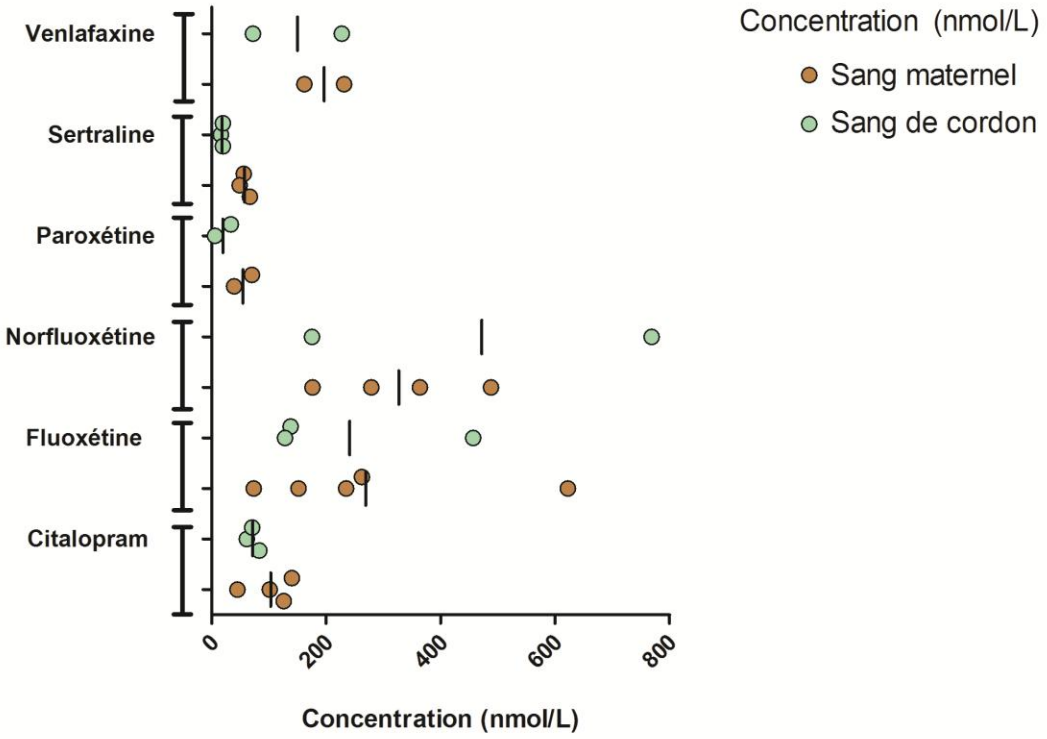

Figure 1.9 **Concentration d'inhibiteurs sélectifs de recapture de la sérotonine (ISRS) mesurée dans le sang maternel et dans le sang de cordon.** Réalisé d'après les données de (Heikkinen *et al.*, 2002, Heikkinen *et al.*, 2003, Hendrick *et al.*, 2003b, Rampono *et al.*, 2009, D. Sit *et al.*, 2011). La barre représente la moyenne.

Les mécanismes régulant le transfert placentaire des ISRS sont complexes et mal compris. Le placenta constitue une barrière lipidique avec une perméabilité sélective. Les ISRS, de par leurs petites tailles et leurs propriétés non ioniques et lipophiles,

traversent le placenta par diffusion passive (Evseenko *et al.*, 2006). Durant l'avancée de la grossesse, la diffusion de cette classe d'antidépresseurs est facilitée car, d'une part, la surface de contact est augmentée (les microvillosités placentaires sont plus nombreuses), et d'autre part, la distance entre le sang fœtal et le sang maternel est moindre (les microvillosités étant plus minces (Ewing *et al.*, 2015, Syme *et al.*, 2004). Les ISRS (excepté la fluoxétine) traversent également le placenta par transport actif grâce à la protéine membranaire P-glycoprotéine (P-gp), qui appartient à la superfamille des transporteurs d'efflux *ATP binding cassette* (ABC) (Wang *et al.*, 2007). Il est intéressant de noter que l'expression des P-gp diminue au cours de la grossesse, ce qui peut influencer le transfert des ISRS (DeVane *et al.*, 2006, Syme *et al.*, 2004).

Les ISRS peuvent aussi être métabolisés au niveau placentaire. En effet, une large variété de cytochromes P450 (CYP) est exprimée par les cellules trophoblastiques notamment CYP1A1, CYP1A2, CYP1B1, CYP2D6, CYP2E1, CYP2F1, CYP3A4, etc. (Hakkola *et al.*, 1996a, Hakkola *et al.*, 1996b, Pasanen, 1999). Parmi ces CYP, tel qu'évoqué à la section 1.2.2.1, le CYP3A4 est impliqué dans le métabolisme du citalopram, de la fluoxétine, de la paroxétine, de la sertraline et de la venlafaxine, tandis que le CYP2B6 métabolise la sertraline (Tableau 1.7). Cependant, le niveau d'expression des CYP placentaires est inférieur à ceux du foie, et leurs activités diminuent au cours de la grossesse (Prouillac *et al.*, 2010, Syme *et al.*, 2004). D'autre part, une modification de la pharmacocinétique (absorption, distribution, métabolisation et élimination) de la mère au cours de la grossesse influence la concentration placentaire des ISRS (Anderson, 2005, Loebstein *et al.*, 1997). En effet, l'expression des CYP hépatiques maternels varie (globalement augmente) au cours de la grossesse, ce qui diminue le taux d'ISRS biodisponibles (Sit *et al.*, 2008, Ververs *et al.*, 2009). Ces études suggèrent que les métabolites des ISRS auxquels est exposé le placenta ont principalement été métabolisés par la mère. De plus, seuls les ISRS qui sont sous forme libre, c'est-à-dire non liés aux protéines plasmatiques telles que l'albumine ou l'alpha-1 glycoprotéine acide (AGP), peuvent traverser le placenta. Or, ces protéines diminuent au cours de la grossesse, augmentant ainsi la fraction libre d'ISRS dans le sang maternel (Hutson *et al.*, 2011, Ram *et al.*, 2015). Cela laisse suggérer qu'au terme de la grossesse, une quantité plus importante d'ISRS sous forme

libre est donc biodisponible pour le placenta et le fœtus, par rapport au 1er trimestre de grossesse.

1.2.4.2 Système sérotoninergique placentaire

Les cellules placentaires possèdent un système sérotoninergique fonctionnel (Figure 1.10). En 1989, Balkovetz et al. ont été les premiers à démontrer, *ex vivo*, la présence du SERT au niveau du tissu placentaire humain (Balkovetz et al., 1989). Depuis, ces résultats ont été confirmés par la mise en évidence d'ARNm de SERT dans les trophoblastes villeux humains à terme (Bottalico et al., 2004, Prasad et al., 1996), ainsi que de son activité (Cool et al., 1990). Notre équipe a également montré, par immunohistochimie réalisée sur des tissus placentaires humains normaux à terme, la présence des protéines du SERT au niveau des trophoblastes villeux (CTBv et STB), ainsi que des capillaires fœtaux (Viau *et al.*, 2009). Nous avons également mis en évidence à partir des mêmes tissus, l'expression d'ARNm et de protéines de SERT, à la fois dans les CTBv, mais aussi dans le STB (Viau *et al.*, 2009). De plus, le SERT est exprimé au niveau du STB, autant du côté maternel (membrane apicale) que fœtal (membrane basale) à la fois chez la souris (Hadden *et al.*, 2017) et chez l'humain (Viau, 2008). Les récepteurs de la sérotonine de type $5HT_{2A,\ 2B,\ 2C}$ (Huang *et al.*, 1998, Sonier *et al.*, 2005, Vaillancourt *et al.*, 1994, Viau *et al.*, 2009), et la monoamine oxydase (MAO, enzyme dégradant les monoamines) (Auda *et al.*, 1998, Billett, 2004, Blakeley *et al.*, 2013), sont également présents au niveau des cellules placentaires humaines. Autant les ARNm, que les protéines du récepteur $5HT_{2A}$, ont été mis en évidence dans les CTBv et les STB issus de placentas humains normaux à terme (Viau *et al.*, 2009).

À la fois chez la souris (Bonnin *et al.*, 2011a) et chez l'humain (Laurent *et al.*, 2017), le placenta est capable de synthétiser *de novo* la sérotonine. En outre, les cellules trophoblastiques humaines (CTBev, CTBv et STB), de 1er et de 3e trimestre de grossesse, expriment les tryptophanes hydroxylases (TPH-1 et TPH-2), suggérant un rôle de la sérotonine placentaire dans le développement du fœtus (Laurent *et al.*, 2017). Dans ce sens, Bonnin *et al.* ont montré que la sérotonine placentaire est la source de sérotonine impliquée pour le développement du prosencéphale du fœtus, à 14,5 jours embryonnaires chez la souris (Bonnin *et al.*, 2011a, Bonnin *et al.*, 2011b).

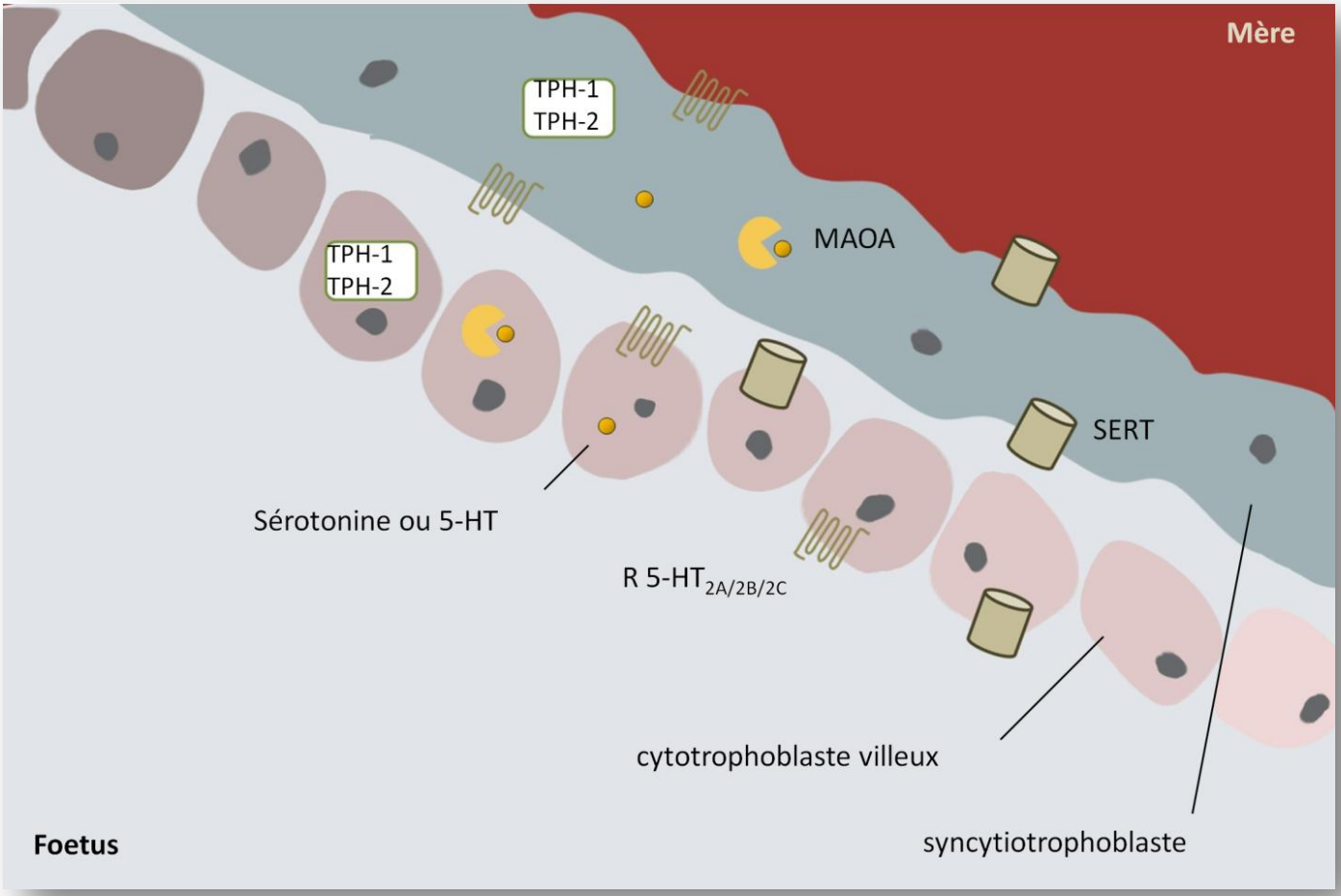

Figure 1.10 **Système sérotoninergique dans le placenta humain.** MAOA : monoamine oxydase A, SERT : transporteur de la sérotonine, TPH : tryptophane hydroxylase, R 5-HT : récepteur de la sérotonine.

1.3 **Modèles d'études**

Le placenta humain a une structure et un développement unique parmi les mammifères ce qui fait que le modèle animal n'est pas un bon modèle d'étude. Par contre, les lignées cellulaires (BeWo : modèle de trophoblastes villeux ; JEG-3 et HIPEC : modèles de trophoblastes extravilleux) et les primocultures de trophoblastes humains sont des modèles de choix. La lignée cellulaire de choriocarcinome de placenta humain BeWo représente un modèle pertinent du trophoblaste villeux pour l'étude de la syncytialisation. En effet, elle sécrète des hormones (hCG, hPL) et est capable de fusionner (Alsat *et al.*, 1999), comme les primocultures. Ces cellules se différencient en syncytium (fusionnent et sécrètent la hCG) lorsqu'elles sont stimulées par la forskoline, qui est un activateur direct de l'adénylate cyclase (AC) (Orendi et al., 2010). Chez les cellules BeWo comme pour les primocultures de CTBv, on observe une diminution de l'expression des protéines E-cadhérine et syncytine lors de la différenciation (Kudo et al., 2002). Les lignées cellulaires JEG-3 et HIPEC (*human invasive, proliferative extravillous cytotrophoblast*) sont de bons modèles du trophoblaste extravilleux, de par leurs capacités de migration et d'invasion. La lignée HIPEC est dérivée de primocultures humaines de trophoblastes extravilleux, et a été immortalisée par transfection de gènes de virus Simien 40 (Pavan et al., 2003), tandis que les cellules JEG-3 sont une lignée de choriocarcinomes placentaires humains (Hertz, 1959). Ces deux lignées possèdent un phénotype extravilleux : elles prolifèrent, migrent, envahissent, et sécrètent des MMP. (Kohler et al., 1971, Pavan et al., 2003). De plus, ces trois lignées cellulaires (BeWo, JEG-3 et HIPEC) possèdent un système sérotoninergique. Notre équipe et d'autres ont montré que les cellules BeWo et JEG-3 expriment les récepteurs 5-$HT_{2A,\ 2B\ et\ 2C}$ et le SERT (Hudon Thibeault *et al.*, 2016, Keating *et al.*, 2006, Morikawa *et al.*, 1998, Sonier *et al.*, 2005, Viau *et al.*, 2009). La présence des TPH-1 et -2 a été démontrée dans les 3 lignées cellulaires (Laurent et al., 2017). Ces lignées représentent donc de bons modèles pour étudier l'effet des ISRS sur les trophoblastes humains.

D’autre part, les primocultures de trophoblastes villeux sont un bon modèle d’étude. En effet, un placenta permet d’obtenir un grand nombre de cellules : environ 300 millions de cellules (Clabault *et al.*, 2018, Sagrillo-Fagundes *et al.*, 2016). La technique d’isolement et de purification des trophoblastes villeux, initialement mise au point par Kliman *et al.* (Kliman et al., 1986), a été modifiée et améliorée (entre autres) par notre laboratoire et fait l’objet de deux publications (chapitre 2 et annexe 2). Ce modèle *in vitro* unique est largement utilisé dans la littérature pour l’étude de la différenciation à la fois morphologique (fusion) et biochimique (sécrétion de hCG) du trophoblaste villeux (Daoud et al., 2005, Racca et al., 2015, Steinberg et al., 2016). Les primocultures de CTBv fusionnent spontanément, en présence de sérum fœtal bovin (FBS) ou de facteurs de croissance, pour former un syncytium (syncytiotrophoblaste) après quatre jours de mise en culture (Kliman et al., 1986).

1.4 **Hypothèse et objectifs de recherche**

Peu d'études se sont intéressées aux effets des antidépresseurs ISRS sur le développement du placenta, qui est pourtant un organe clef dans le bon déroulement de la grossesse et du développement fœtal. Les ISRS étant couramment utilisés durant la grossesse, une meilleure compréhension de leurs effets sur le développement du trophoblaste villeux et extravilleux est donc primordiale. Dans différents types cellulaires, la sérotonine induit la différenciation cellulaire via l'activation des récepteurs 5-HT_2 (Etienne et al., 2004, Guillet-Deniau et al., 1997, Gustafsson et al., 2006, Koch et al., 2002, Ni et al., 1997, Palvimaki et al., 1996). Cependant, la littérature demeure controversée (pas d'effet, inhibition ou activation) quant à l'effet des ISRS sur la différenciation cellulaire et l'apoptose selon le type cellulaire, et les concentrations (Guillet-Deniau *et al.*, 1997, Gustafsson *et al.*, 2006, Hadden *et al.*, 2017, Hodge *et al.*, 2013, X. Hu *et al.*, 2012, Schaz *et al.*, 2011). Les concentrations utilisées (0,03 à 10 µM) dans la présente étude correspondent aux concentrations retrouvées dans le sang maternel et dans le sang de cordon (sang fœtal). À ces doses, nous pensons que ces antidépresseurs, comme démontré pour les ostéoclastes (Gustafsson et al., 2006), n'ont pas d'effet sur la syncytialisation (différenciation) ni sur l'apoptose du trophoblaste villeux.

La sérotonine régule, via son récepteur 5-HT_{2A}, l'invasion et la prolifération des cellules du muscle squelettique, des cellules musculaires lisses vasculaires, ainsi que des cellules mésangiales du glomérule (Banes et al., 2005, Guillet-Deniau et al., 1997, Nebigil et al., 1995). Notre laboratoire a récemment mis en évidence que la sérotonine régule l'invasion, la migration et la prolifération des trophoblastes extravilleux, via ce même récepteur, et l'activation des voies de signalisation ERK1/2 et JAK2/STAT3 (Oufkir *et al.*, 2010, Oufkir *et al.*, 2011). Non seulement les ISRS inhibent la recapture de la sérotonine en bloquant le SERT, mais ils ont également des effets antagonistes sur le récepteur de la 5-HT_{2A} (Dempsey et al., 2005, Pitt et al., 1994). Nous suggérons donc que les ISRS altèrent le développement des CTBev, soit directement en

interagissant avec le récepteur 5-HT_{2A}, soit indirectement en se fixant sur le SERT, diminuant ainsi les taux de sérotonine.

Hypothèse 1 : Nous proposons que les ISRS, aux concentrations utilisées chez les femmes enceintes, n'ont pas d'effet sur la syncytialisation (différenciation fonctionnelle, fusion et apoptose) du trophoblaste villeux.

Hypothèse 2 : Nous proposons que les ISRS altèrent le processus d'implantation en inhibant l'invasion, la migration et la prolifération du trophoblaste extravilleux.

L'objectif général de ce projet de recherche est donc de déterminer les effets de cinq des ISRS les plus prescrits chez les femmes enceintes sur le développement trophoblastique. **Les objectifs spécifiques** sont de déterminer l'effet des antidépresseurs de type ISRS (fluoxétine (et son métabolite la norfluoxétine), sertraline, paroxétine, citalopram et venlafaxine ; 0,03 à 10 µM) sur :

1- La syncytialisation du trophoblaste villeux, soit i) la prolifération et le cycle cellulaire des cellules BeWo (modèle du trophoblaste villeux), ii) la différenciation fonctionnelle (sécrétion de hCG) ; iii) la différenciation morphologique (fusion) ; et iv) l'apoptose dans les cellules BeWo et dans les primocultures de trophoblastes villeux de placentas de grossesses normales à terme ;
2- La prolifération, le cycle cellulaire, l'invasion et la migration des trophoblastes extravilleux, en utilisant les lignées JEG-3 et HIPEC.

2 CHAPITRE 2 : ISOLATION AND PURIFICATION OF VILLOUS CYTOTROPHOBLAST CELLS FROM TERM HUMAN PLACENTA

2.1 Présentation de l'article

Titre en français: Isolement et purification des cellules de cytotrophoblastes villeux issues de placentas humains à terme

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2.2 **Résumé de l’article en français**

Le placenta est un élément clef durant la grossesse à la fois pour le fœtus et pour la mère, ce qui justifie l’importance des études placentaires. Les cultures primaires de cytotrophoblastes villeux issus de placentas humains à terme représentent un des meilleurs modèles pour les études placentaires. Dans ce chapitre, nous présentons de manière détaillée : 1) l'isolement des cytotrophoblastes villeux, comprenant la préparation tissulaire, les digestions, le gradient de Percoll, et 2) l'immunopurification des cellules et leur mise en plaque.

2.3 **Contribution de l’étudiante**

L’étudiante a rédigé la partie portant sur la préparation tissulaire, les digestions, le gradient de Percoll et la congélation des cellules. Elle a relu et corrigé la seconde partie du chapitre et a contribué aux discussions et corrections nécessaires à la publication de la version finale du chapitre.

2.4 **Article**

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Chapter 17

Isolation and Purification of Villous Cytotrophoblast Cells from Term Human Placenta

Hélène Clabault, Laetitia Laurent, J. Thomas Sanderson, and Cathy Vaillancourt

Abstract

The placenta is a key element during pregnancy for the health of the fetus and the mother, which justifies why placental studies are so important. One of the best models for placental studies is the primary cell culture of cytotrophoblast cells from human term placentas. In this chapter, we will detail firstly the isolation of cytotrophoblast cells, with tissue preparation, digestion, Percoll gradient, and cell freezing, and secondly the cell immunopurification and seeding.

Key words Immunopurification, Percoll gradient, Syncytiotrophoblast, Placenta, Primary cell culture

1 Introduction

There are several models to study the development and functioning of the placenta. Each model, which will be described quickly here, allows studying part of the mechanisms involved. Nevertheless, since the placenta is a very complex organ, none of the below-mentioned models can exactly reflect all the functions of the human placental tissue in vivo. First of all, placental tissues from human or animal models can be fast frozen and then used to evaluate the level of expression of different targets under physiological conditions or also following some treatment or diseases. Unfortunately, this model cannot allow studying the behavior of the cytotrophoblast cells, which is essential for the understanding of the placental functions. Villous explants containing the whole villous structure and cell types are often used to study placental physiology. This model has the advantages of closely matching human placental physiology but is limited for intensive biochemistry and molecular

Hélène Clabault and Laetitia Laurent contributed equally to this work.

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biology studies. There are also a few trophoblastic cell lines, all derivate from choriocarcinoma, which are commercially available such as BeWo, Jeg-3, and JAR (the latter are derivate from the former), and are commonly used to study human placental functions [1–3]. An inconvenient of these cell lines is the necessity of chemical induction for achieving a differentiation. BeWo cells, which are the most used model, can biochemically and morphologically differentiate following an induction (*e.g.*, forskolin stimulation), which make them good differentiation and fusion models. They have also the ability to form confluent monolayer on permeable support, which allows studying the transplacental transport of drugs [4]. Caution should be used, however, as these lines unlikely reflect in vivo trophoblast function. A recent study showed a very weak correlation between gene expression in human cytotrophoblasts and BeWo cells [5].

Finally, the other way to study the cytotrophoblastic function is to establish a primary cell culture from placenta. The advantage of using placenta is that it represents a large reservoir of materials for isolation of trophoblast cells compared to biopsies, the usual way to obtain human live tissue. Moreover, primary cells are able to syncytialize spontaneously and, as such, allow studying human trophoblast cell fusion, syncytiotrophoblast formation, and regeneration. Taking this into account, we believe that human primary trophoblast culture remains a suitable and robust model for studying placenta.

Concretely, for this manipulation, it is important to consider that it is a relatively long experiment, with significant handling costs. Indeed, it takes approximately 8 h of continuous manipulation after obtaining the placenta and then 3 h for trophoblasts purification. We estimate that the isolation of cytotrophoblast cells from a term human placenta costs about 400 USD (for double preparation) of consumable products such as chemicals and small disposable materials. The most expensive consumables are DNase type IV (130 USD), trypsin (100 USD), and DMEM high glucose (40 USD). For the thawing and the immunomagnetic purification, we estimate 130 USD. The isolation technique requires little work space, ideally a double bench. The necessary equipment is fairly basic (centrifuge, sterile hood). The immunopurification, however, requires an autoMACS® magnetic separator.

In this chapter, we describe the procedure to isolate and purify the cytotrophoblast cells from human term placenta. The procedures, adapted from Kliman et al., are based on enzymatic dissociation of villous placental tissue, followed by gradient centrifugation and immunomagnetic bead purification [6]. All steps are summarized in Table 1; a video is also available on JoVE [7].

Table 1
Summary of isolation, purification, and purity analysis of villous cytotrophoblasts (vCTB) by step

Steps	Proceedings	Succinct descriptions
Isolation		
1	Transportation	– Quick transportation of placenta (≤1 h)
2	Tissue preparation	– Remove fetal and maternal membranes – Mince 30–35 g of tissue after having remove blood vessels
3	Digestion	– Four digestions for 30 min at 37 °C in shaking water bath with HBSS + $CaCl_2$ + $MgSO_4$ + digestive enzymes: trypsin I and DNAse I type IV ± P/S
4	Centrifugation	– Keep supernatant from digestions 2 to 4, put it on FCS – Centrifugation (1250 × *g*, 15 min at RT) – Pellets rinsing with DMEM-P/S
5	Percoll	– Deposit cells on Percoll gradients – Centrifugation without brake (507 × *g*, 30 min, at RT)
6	Centrifugation	– Keep vCTB which are between 1.048 and 1.060 densities – Wash cells in DMEM-P/S – Centrifugation (1250 × *g*, 10 min, at RT)
7	Count	– Pellet resuspension in FBS – Count and cell viability determination
8	Cryofreezing	– Cryofreezing in FBS and 10% DMSO
Purification		
9	Thawing	– Quick thawing frozen cells – Resuspension with autoMACS® running buffer + P/S
10	Count	– Cells count with trypan blue
11	Labeling	– Incubation 30 min with running buffer + anti-HLA-ABC Ab – Wash
12	Immunopurification	– Cells resuspension with magnet microbeads MACS® – vCTB immunopurification
13	Count	– Cells count with trypan blue
14	Seeding	– vCTB seeding after resuspension in culture media (DMEM + HEPES + FBS + P/S)
15	Changing medium	– 4 h after incubation at 37 °C, wash (×2) with medium culture

HBSS Hank's balanced salt solution, *$CaCl_2$* calcium chloride, *$MgSO_4$* magnesium sulfate, *DNAse* deoxyribonuclease, *P/S* penicillin/streptomycin, *FCS* fetal calf serum, *RT* room temperature, *DMEM* Dulbecco's modified eagle medium, *FBS* fetal bovine serum, *DMSO* dimethyl sulfoxide, *Ab* antibody, *HEPES* 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid
Adapted from [10] according to the protocol used in our laboratory [7]

2 Materials

2.1 Equipment

The quantities for a double preparation are given in brackets. Note that all surgical equipment should be sterile.

1. autoMACS® columns.
2. autoMACS® magnetic separator.
3. Büchner funnel.
4. Cell strainers 100 μm nylon.
5. Fine-toothed forceps [2].
6. Gauze sponge.
7. 50 mL corex tube [2].
8. Metzenbaum [2] and pointed scissors [2].
9. Peristaltic pump.
10. Pyrex flat (21 × 30 cm).
11. 250 mL sterile trypsinizing flasks [2].
12. Watch glass [2].

2.2 Chemical Products

1. Anti-human HLA ABC purified clone W6/32.
2. Anti-mouse IgG microbeads.
3. autoMACS® running buffer.
4. autoMACS® rinsing solution.
5. 100 mM calcium chloride (store at 4 °C).
6. Culture medium: Dulbecco's modified eagle medium (DMEM) high glucose with 25 mM HEPES, 10% of fetal bovine serum (FBS) (store at 4 °C).
7. Deoxyribonuclease (DNase) type IV.
8. Fetal calf serum (FCS).
9. 10× Hank's buffered salt solution (HBSS) without calcium and magnesium.
10. 800 mM magnesium sulfate (store at 4 °C).
11. Modified HBSS: 1 vial (9.25 g) 1× HBSS without calcium and magnesium, 25 mL 1 M HEPES, pH = 7.4 (store at 4 °C).
12. Penicillin/streptomycin (P/S; 10,000 units/mL penicillin G, 100 mg/mL streptomycin sulfate).
13. Percoll.
14. Saline (NaCl) solution (10 L; 0.9%).
15. Trypsin type I.

3 Methods

3.1 Cell Isolation

3.1.1 Eve or the Day of the Manipulation

1. Weigh the different quantities of trypsin (Table 2), twice for a double preparation, in a plastic weight boat. Label, cover with paraffin, and store them at 4 °C until use.
2. Reconstitute DNase by dissolving 1 vial (100 mg) in 1 mL of modified HBSS. Keep at 4 °C until use.
3. Prepare a 90% Percoll solution: mixing 36 mL of Percoll with 4 mL of 10× HBSS. Prepare Percoll solutions in disposable culture tubes, as written in Table 3 (*see* **Note 1**). Keep at 4 °C until use.
4. Add 4 mL of P/S in 200 mL of DMEM. Keep at 4 °C until use.

3.1.2 Isolation of Trophoblast Cells

Be aware that during the whole process, the placenta and its pieces must be kept in saline buffer.

1. In a water bath at 37 °C, warm two aliquots of 1 mL P/S, eight bottles of modified HBSS for digestions (digestion 1, 2 × 150 mL; digestion 2, 2 × 100 mL; digestion 3, 2 × 75 mL; and digestion 4, 2 × 75 mL), 200 mL of DMEM-P/S, and 50 mL of FCS. For FCS, as soon as thawed, put it at room temperature for acquiring the right density.
2. Following the delivery of the baby, bring the placenta to the laboratory as quickly as possible (≤1 h) in a cold saline buffer (*see* **Note 2**).
3. Dispose of the placental blood in a liquid trash. Weigh the placenta in a pre-weighed flat plastic pot.

Table 2
Quantities for digestion solutions

	Digestion 1	Digestion 2	Digestion 3	Digestion 4
Modified HBSS (mL)	150	100	75	75
$MgSO_4$ (µL)	150	100	75	75
$CaCl_2$ (µL)	150	100	75	75
Trypsine (U)	1,824,000	1,200,000	960,000	960,000
DNase IV (µL) (0.1 mg/µL)	300	200	150	150
P/S (mL)	1	/	/	/

For a double digestion, all solutions have to be prepared twice
HBSS Hank's buffered salt solution, *$MgSO_4$* magnesium sulfate, *$CaCl_2$* calcium chloride, *DNase* deoxyribonuclease, *P/S* penicillin and streptomycin

Table 3
Quantities for Percoll solution

Concentration (%)	70	65	60	55	50	45	40	35	30	25	20	15	10	5
90% Percoll (mL)	2.33	2.17	2.00	1.83	1.67	1.50	1.33	1.17	1.00	0.83	0.67	0.50	0.33	0.17
Modified HBSS (mL)	0.67	0.83	1.00	1.17	1.33	1.50	1.67	1.83	2.00	2.17	2.33	2.50	2.67	2.83

4. Cover the placenta with saline solution.
5. Take note of the following: umbilical cord length, implantation (central or not), placental length, width and form (oval, discoid), membranes color, cotyledons (entire or not), and pathologies.
6. Cut the umbilical cord, by doing a 1 cm diameter circle in the placental cord base. Keep it in formalin if required by the histopathology hospital department.
7. Remove the amnion (thin membrane covering the fetus) and the outer 2 cm of the placenta (this part is too thin and does not contain many trophoblasts).
8. Cut the entire placenta in 5 × 5 cm pieces. Rinse several times (~four times) in saline buffer to remove blood cells. After this step, the liquid should be limpid.
9. Remove fetal (amnion and chorion) and maternal membranes. In a watch glass, mince tissue to remove blood vessels and calcifications using forceps and the back of a Metzenbaum scissors. When a piece of placenta is completely minced, put it in a Büchner funnel under a beaker (*see* **Note 3**). Shed the saline buffer, rinse again, and place it in a weighing boat on ice. Repeat until having 60–70 g of minced tissue for a double preparation (30–35 g for a single preparation).
10. Equally divide the 60–70 g of minced placental tissue in the two trypsinization flasks.
11. Prepare the first digestion solutions with heated modified HBSS, $MgSO_4$, $CaCl_2$, trypsine, DNase and P/S, according to Table 2. Transfer these solutions in the trypsinization flasks.
12. Vigorously mix, before putting the flasks in a shaking water bath for 30 min (50 cycles/min). Mix flasks every 5 min to provide a homogenous digestion (*see* **Note 4**).
13. At the end of the first digestion, remove the flasks from the water bath and put them on a bench at a 45° angle. Decant for about 1 min.
14. With the help of a 10 mL sterile pipette, discard 80 mL of liquid, avoiding any tissue.
15. Prepare the second digestion solution as described in Table 2, put the solution in trypsinization flasks, mix, and incubate 30 min in a water bath, mixing every 5 min (as in **step 12**).
16. At the end of the second digestion, remove the flasks from the water bath and put them on a bench at a 45° angle. Decant for about 1 min. With the help of a 10 mL sterile pipette, withdraw 80 mL of liquid and put it gently on a cell strainer (100 μm nylon) placed on top of a 50 mL tube.

17. Aliquot 13.5 mL of the second digestion collected liquid in 15 mL tubes. With a glass Pasteur pipette, very gently and slowly, add 1.5 mL of FCS at the bottom of tubes. The two liquids must not mix. Centrifuge all tubes at 1250 × *g* without brake at room temperature during 20 min.
18. After the centrifugation, pellets with four layers should be formed from top to bottom: digestion solution, FCS with DNase and trypsin, trophoblast cells, and blood cells. With a vacuum pump, aspirate supernatants, taking care to avoid withdrawing the trophoblasts layer. It is very important to remove the whitish precipitate at the FCS interface, by aspirating along the tube's wall (Fig. 1).
19. Resuspend each pellet with 1 mL of warm DMEM-P/S (flick each tube four or five times). Collect all resuspended cells in two tubes with a Pasteur pipette. Let the tubes stand by at room temperature.
20. Begin layering the Percoll gradient. Take two 50 mL corex tubes, and fix the outflow tubing of the peristaltic pump on each tube. The liquid should flow along the tube wall without forming droplets.

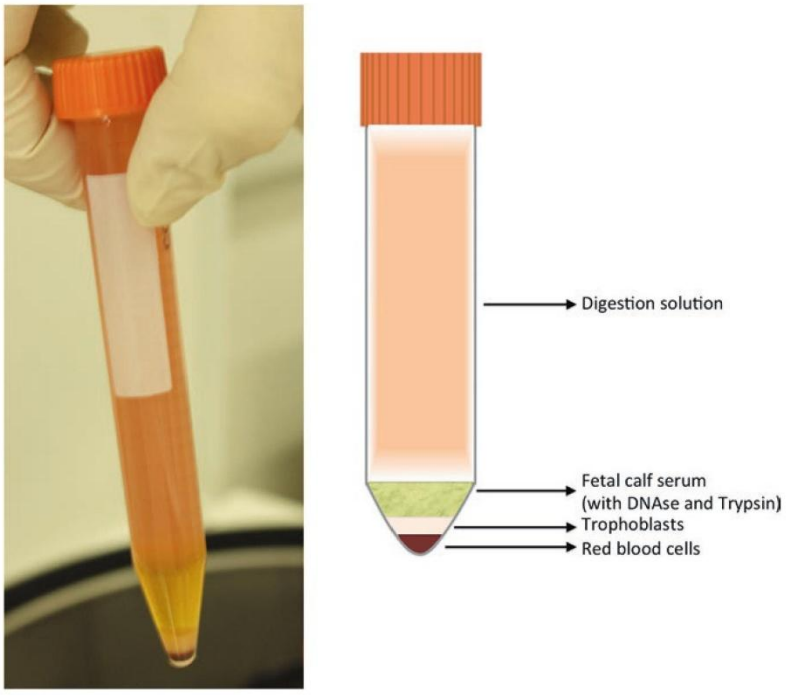

Fig. 1 Digestion tube. After the centrifugation, pellets with four layers should be formed from top to bottom: digestion solution, FCS with DNase and trypsin, trophoblast cells, and blood cells

21. Take the different Percoll solutions previously prepared (as described in Table 3). Layer each Percoll solution, in a decreasing fashion (70–5% concentrations), with a slow peristaltic pump (1 mL/min) (*see* **Note 5**). Vortex each Percoll solution before layering it. Keep at room temperature.
22. At the end of the third and fourth digestions, perform again **steps 16–19**. For the last digestion, collect as much supernatant as possible.
23. Once all digestions are completed, there will be eight tubes with trophoblasts. Fill them to 15 mL with warm DMEM--P/S. Centrifuge at 1250 × *g* with brake, during 10 min at room temperature. Remove the supernatant with the vacuum pump, taking care to avoid aspirating the white pellets.
24. Resuspend each pellet with 1 mL of warm DMEM-P/S by flicking all tubes four or five times each. Collect all liquids in two 15 mL tubes (*see* **Note 6**). It is very important to rinse tubes well, to avoid losing cells. Fill with warm DMEM-P/S, to have two tubes of 8 mL.
25. With a Pasteur pipette, very gently and slowly, take support on the wall of the tube and apply cell suspensions in Percoll gradients. Centrifuge without brake at 507 × *g* during 30 min at room temperature. It is important that the centrifuge is perfectly balanced. Handle the tubes very gently to avoid mixing the gradients.
26. At the end of the centrifugation, move the tubes near the vacuum pump. Place a lamp behind to visualize the bands well. Trophoblasts cells are located between 40% and 50% Percoll concentrations (*see* **Note 7**). With the vacuum pump, take support on the tube wall and remove the top layer up to the trophoblast cells (>50% Percoll concentration). With a Pasteur pipette, take the cells of interest and put them in a 50 mL tube (*see* **Note 8**).
27. Fill the tube to 50 mL with warm DMEM-P/S. Centrifuge with brake at 1250 × *g* during 10 min at room temperature (*see* **Note 9**).

From this step on, work under sterile conditions.

28. Resuspend the pellet with 20 mL of FBS. Keep on ice.
29. Count the cells and determine their viability using trypan blue dye.
30. Add 2.22 mL of sterile DMSO. Mix gently by flicking. Aliquot 1.5 mL of cell suspension into cryogenic vials. Put in freezing container and store at −80 °C for 12–24 h, before putting the vials in a liquid nitrogen tank.

3.2 Cell Purification

3.2.1 Preparation Steps

1. Prepare the autoMACS® by installing the autoMACS® rinsing solution, the autoMACS® running buffer, and a new autoMACS® column in the negative port. Solutions need to be at room temperature for the purification of cells.
2. Perform the "clean program" of the autoMACS®.
3. Clean up the ports, then place three 50 mL tubes identified negative 1, positive 1, and positive 2 under respective ports.
4. Prepare a sterile aliquot of cold running buffer: 50 mL is necessary for 50 million cells. Add 1 mL of P/S in 50 mL of running buffer. Keep solutions on ice.
5. Add 1% of P/S in the culture medium and keep it at 37 °C.

3.2.2 Cell Purification Steps

1. If starting from a frozen stock of villous mononuclear cells, thaw cells quickly in a 37 °C water bath. Transfer the cells into a 50 mL tube and resuspend gently in about 20 mL cold running buffer containing P/S.
2. Centrifuge at 450 × *g* for 5 min at 4 °C.
3. Remove gently the supernatant, then resuspend in 20 mL cold running buffer. Keeping the cells on ice, count the cells and determine their viability using trypan blue dye.
4. Centrifuge again at 450 × *g* for 5 min at 4 °C. Carefully remove the supernatant and then resuspend in 1 mL cold running buffer. Add 10 µL of mouse anti-HLA-ABC.
5. The cell suspension will be viscous; mix well by flicking the tube several times and incubate for 30 min at 4 °C. Mix gently the cells every 5 min.
6. After the incubation, add 6 mL of cold running buffer and centrifuge at 450 × *g* for 5 min at 4 °C.
7. Remove gently and discard the supernatant, resuspend in 6 mL of cold running buffer, and repeat the centrifugation at 450 × *g* for 5 min at 4 °C.
8. After discarding the supernatant, resuspend cells in 900 µL of cold running buffer. Label cells by adding 100 µL anti-mouse IgG microbeads. Incubate for 15 min at 4 °C. Mix gently the cells every 5 min.
9. Following the incubation, wash cells by adding 6 mL of cold running buffer and centrifuge at 450 × *g* for 5 min at 4 °C.
10. Discard the supernatant, then resuspend in 5 mL of cold running buffer.
11. Place the cell suspension under the uptake port and perform the separation program on the autoMACS.
12. Keep the negative fraction, which contains villous trophoblasts, and add 20 mL of cold running buffer. Centrifuge the purified cells at 450 × *g* for 5 min at 4 °C.

13. Remove gently the supernatant, then resuspend in 20 mL of warm culture medium. Keeping the cells at room temperature, count the cells and determine their viability using trypan blue dye (*see* **Note 10**).
14. Seed cells in the warm culture medium:
    (a) 96 wells plate: 150,000 cells/well
    (b) 24 wells plate: 1.6 million cells/well
    (c) 6 wells plate or 35-mm petri dish: 4.5 million cells/well.
15. Incubate at 37 °C and 5% CO_2 for at least 4–6 h, which allows cells to attach to the surface of the plate and then rinse twice with pre-warmed culture medium to remove nonattached cells. Refresh the medium every 24 h.

4 Notes

1. Two layers of Percoll solution can be used. Put 20 mL of 60% Percoll at the bottom of the tube and then, with the peristaltic pump, add 20 mL of 25% Percoll. After centrifugation, trophoblast cells will be at the interface between both layers of the Percoll.
2. Transport buffer (~500 mL/placenta) can be used instead of saline. This buffer is composed of DMEM medium (26.74 g) completed with HEPES (11.916 g), sodium bicarbonate (7.4 g), penicillin (4 mg), gentamicin (100 mg), and amphotericin (10 mg). Dissolve all powders in 2 L of milliQ water, adjust pH at 7, and filter (0.2 μm).
3. Instead of a Büchner funnel to rinse minced placenta with saline solution, it is possible to use gauze sponge or sieve with an appropriate pore size.
4. For a uniform heat transfer, the bath water level should exceed the liquid level in the trypsinization flask.
5. If there is no peristaltic pump in your laboratory, you can flow the Percoll very gently with Pasteur pipettes.
6. It is possible to discard the fourth digestion if the white pellet is very small.
7. Given that trophoblasts cells localization is approximate in the gradient Percoll, it can vary in each experiment.
8. It is possible that it will be difficult to take only trophoblast cells because blood cells are often too close by. If there are blood cells mixed with your trophoblasts, keep going with the experiment. Blood cells will be eliminated during the immunopurification and centrifugation steps realized to wash the cells after freezing. If blood cells are still present even after all these

steps, it is nevertheless possible to seed them and then wash cells carefully several times with warm culture medium at each change of medium.

9. At this point, it is possible not to freeze the trophoblast cells and directly perform the purification. For that, replace the medium by cold running buffer (under sterile conditions), then continue to Subheading 3.2.2 below.
10. To verify cell purity using flow cytometry, keep one million cells (2 × 500,000 cells in two microtubes). Briefly, wash the cells twice with HBSS and then block nonspecific antigen sites with HBSS containing FBS during 30 min at room temperature. Wash once and then stain the positive tube cells with anti-cytokeratin-7 antibody (1.25 μg/million cells) or add HBSS-BSA in the negative tube. After 45 min of incubation at room temperature, wash the cells and proceed with the flow cytometry analysis [8, 9]. A cell preparation is considered to be pure when there is a minimum of 96% of positive cells staining with the anti-cytokeratin-7 antibody.

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References

1. Pattillo RA, Gey GO (1968) The establishment of a cell line of human hormone-synthesizing trophoblastic cells in vitro. Cancer Res 28:1231–1236
2. Kohler PO, Bridson WE (1971) Isolation of hormone-producing clonal lines of human choriocarcinoma. J Clin Endocrinol Metab 32:683–687
3. Hochberg A, Rachmilewitz J, Eldar-Geva T, Salant T, Schneider T, de Groot N (1992) Differentiation of choriocarcinoma cell line (JAr). Cancer Res 52:3713–3717
4. Prouillac C, Lecoeur S (2010) The role of the placenta in fetal exposure to xenobiotics: importance of membrane transporters and human models for transfer studies. Drug Metab Dispos 38:1623–1635
5. Bilban M, Tauber S, Haslinger P, Pollheimer J, Saleh L, Pehamberger H, Wagner O, Knofler M (2010) Trophoblast invasion: assessment of cellular models using gene expression signatures. Placenta 31:989–996
6. Kliman HJ, Nestler JE, Sermasi E, Sanger JM, Strauss JF III (1986) Purification, characterization, and in vitro differentiation of cytotrophoblasts from human term placentae. Endocrinology 118:1567–1582
7. Sagrillo-Fagundes L, Clabault H, Laurent L, Hudon-Thibeault AA, Salustiano EM, Fortier M, Bienvenue-Pariseault J, Wong Yen P, Sanderson JT, Vaillancourt C (2016) Human primary trophoblast cell culture model to study the protective effects of melatonin against hypoxia/reoxygenation-induced disruption. J Vis Exp (113). https://doi.org/10.3791/54228

3 CHAPITRE 3 : AN ELECTRICAL IMPEDANCE-BASED ASSAY TO EXAMINE FUNCTIONS OF VARIOUS PLACENTAL CELL TYPES *IN VITRO*

3.1 Présentation de l'article

Titre en français: Expérience basée sur l'impédance électrique pour examiner, *in vitro*, les fonctions de diverses cellules placentaires

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3.2 **Résumé de l'article en français**

In vitro, les analyses fonctionnelles des cellules sont abondamment utilisées afin d'étudier les mécanismes moléculaires impliqués dans la prééclampsie. Les fonctions cellulaires de base étudiées comprennent l'adhésion, l'apoptose, la prolifération, la migration, et l'invasion. Actuellement, la plupart des chercheurs utilisent des expériences à des temps donnés, ce qui permet de déterminer la fonction de la cellule seulement à un temps précis, avec la nécessité de répéter l'expérience avec différentes durées. Ici, nous décrivons un appareil basé sur la mesure de l'impédance électrique qui permet la surveillance des cellules en temps réel, et ainsi d'analyser le comportement des cellules à une multitude de temps durant toute la durée d'une expérience.

3.3 **Contribution de l'étudiante**

L'étudiante a contribué à la rédaction du chapitre de façon égale avec Tejasvy Chollangi et Andrée-Anne Hudon Thibeault, a réalisé les tableaux et figures, et a participé aux discussions et corrections nécessaires à la publication de la version finale du chapitre.

3.4 **Article**

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Chapter 20

An Electrical Impedance-Based Assay to Examine Functions of Various Placental Cell Types In Vitro

Tejasvy Chollangi, Hélène Clabault, Andrée-Anne Hudon Thibeault, Hannah E.J. Yong, Shagun Narula, Ellen Menkhorst, J. Thomas Sanderson, Cathy Vaillancourt, and Padma Murthi

Abstract

In vitro functional analyses of cells are widely used to investigate the molecular mechanisms involved in preeclampsia. Common cellular functions studied include adhesion, apoptosis, proliferation, migration, and invasion. At present, most researchers will use endpoint experimental assays that only allow the determination of cell function at a single time point, with the need to repeat the experiment for an alternate time point. Here, we describe an electrical impedance-based tool that allows real-time monitoring of cells, which enables the efficient assessment of multiple time points over the duration of a single experiment.

Key words Functional assays, Adhesion, Proliferation, Apoptosis, Migration, Invasion, xCELLigence® RTCA systems

1 Introduction

A large variety of cell types are involved in placentation. Understanding how these cells function is imperative to further the current knowledge of placental disorders such as preeclampsia. For example, much research performed has focused on the migration and invasion of extravillous trophoblast cells into the decidua. Another example would be determining apoptosis of endothelial cells in the context of endothelial dysfunction. Current assays that are commonly used, such as migration scratch assays and cell viability assays, are tedious and require multiple experiments to measure different time points. In this chapter, we describe the use of the xCELLigence® real-time cell analysis (RTCA) systems (ACEA

Cathy Vaillancourt and Padma Murthi share joint senior authorship.
Tejasvy Chollangi, Hélène Clabault, Andrée-Anne Hudon Thibeault, and Hannah E.J. Yong, contributed equally to this study.

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Biosciences Inc., San Diego, CA), which offer the opportunity to continuously monitor cellular behavior and explore multiple cell functions in real time [1]. The xCELLigence® RTCA systems can be used to study basic cell behaviors, such as adhesion, apoptosis, migration, proliferation, and invasion, as well as be customized to determine specialized cell functions. These systems enable cell activity and kinesis to be measured and quantified as changes in the impedance of an electric current (Fig. 1). Gold microelectrodes embedded in the base of the wells of a cell culture plate transmit the current at specific intervals. The presence of adherent cells impedes the flow of electrons when current is applied. This impedance in the conduction of electricity is measured as a quantifiable signal referred to as the cell index (Fig. 1a). The cell index is an arbitrary unit of measurement defined as $\frac{R(t)-R(t_0)}{15}$. $R(t_0)$ represents the background impedance in the well with only medium, while $R(t)$ reflects the impedance at any given time point when the cells are present [2, 3]. The cell index accounts for background impedance through the use of an initial "blanking" step, which enables the system to correct for the impedance caused by medium alone. The cell index rises as cells spread across the electrodes (Fig. 1b). Eventually, when the cells reach confluence in the well, the cell index will plateau for a period of time. Any subsequent decline will reflect the cell death or detachment. Therefore, the real-time monitoring of cell activity allows users to select the optimal time points for other endpoint experiments.

There are seven different xCELLigence® RTCA models, with different plate compatibilities. For placental studies, the most popular models are the xCELLigence® dual plate (DP), single plate (SP), and multiple plate (MP) (Table 1). Customized plates allow various functions to be tested. The E-plate can contain anywhere from 8 to 384 wells depending on the xCELLigence® RTCA model with the most popular plates having 16 or 96 wells. E-plates can be used to measure events such as cell adhesion, proliferation, and apoptosis (Table 1). E-Plates are similar to regular cell culture plates except that the base of each well contains electrodes. The alternative cell invasion/migration (CIM)-plate, which is only available in 16-well format, is used to determine cell migration or invasion in wells that are pre-coated with a gel matrix. The CIM-plate utilizes a modified Boyden chamber, and each well consists of an upper and lower chamber separated by a microporous membrane (8 μm pore). The microelectrodes are present at the underside of the upper chamber to detect electrical impedance change caused by migratory and invasive cells that have passed through the membrane. E-Plate inserts, which are to be used for co-culture assays, are also available can be added on top of a 16- or 96-well E-plate. There is no impedance monitoring in the insert. E-plate

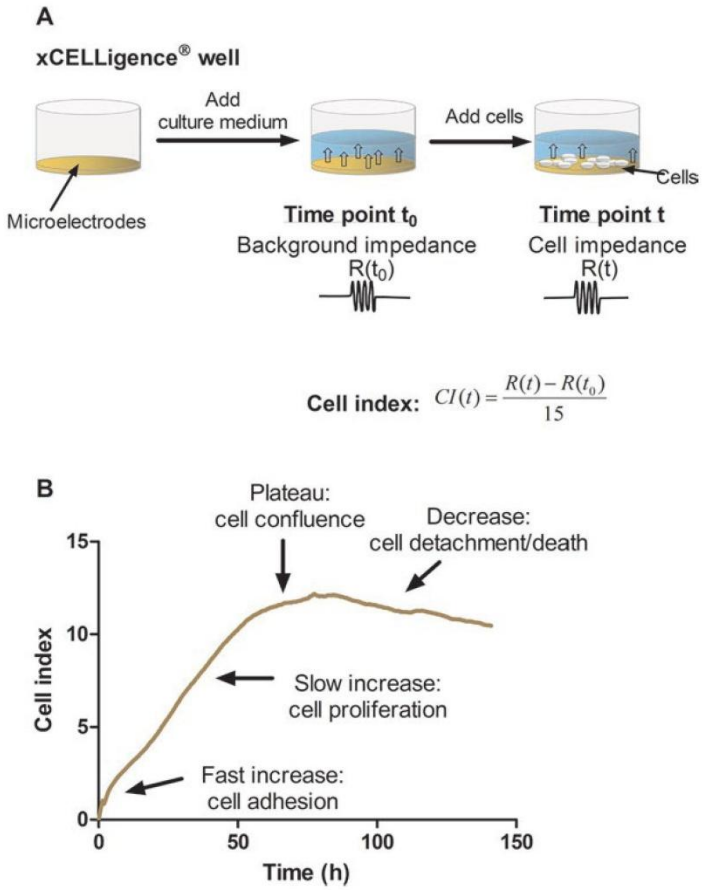

Fig. 1 Basic principles of the xCELLigence® RTCA systems. (**a**) Impedance and cell index measurements. The xCELLigence® RTCA systems utilize differences in electrical impedance caused by cell attachment to provide a measurement of cell function expressed as a cell index. The background impedance ($R(t_0)$) is determined at the start of the experiment, with its cell index always rendered to a value of 0. Any subsequent measurements of impedance caused by cell attachment are then reflected in positive cell index values ($R(t)$). (**b**) A typical real-time impedance curve

inserts are rather used to evaluate the influence of another cell type on the cells in the E-plate wells.

The use of xCELLigence® RTCA instruments can be challenging when setting up experiments with non-adherent or non-proliferative cells. Cell impedance monitoring is also sensitive to any change in the atmospheric conditions, and experiments can be affected by humidity and temperature changes in the incubator due to door opening, for instance. Consumables can also be expensive (*see* **Note 1**). Nevertheless, xCELLigence® RTCA systems have

Table 1
Description of the most commonly used xCELLigence instruments to study placental cell lines

Instrument	Assays – Cell characterization – Proliferation and cytotoxicity – Adhesion – Receptor signaling – Cell interaction: co-culture – Hypoxia studies – Phenotypic screening (mode of action of drugs)	– Invasion – Migration	Format	Compatible plate
RTCA DP	Yes	Yes	3 × 16 wells	– CIM-plate 16 – E-plate 16 (±View and ±PET) – E-plate insert
RTCA SP	Yes	No	1 × 96 wells	– E-plate 96 (±View and ±PET) – E-plate insert
RTCA MP	Yes	No	3 × 96 wells	

RTCA real-time cell analyzers, *DP* dual plate, *SP* single plate, *MP* multiple plates, *CIM* cell invasion/migration, *View* wells have a clear inspection window to visualize cells (no electrode in this window), *PET* bottomed plates, polyethylene terephthalate is an alternative to more expensive glass-bottomed plates. Offered only for E-plate 16 and 96 VIEW

numerous advantages over conventional end-point-based cell assays. The benefits of xCELLigence® RTCA systems include considerable time savings and the ability to continuously measure cellular events without the use of invasive cell labels. Furthermore, xCELLigence® RTCA systems have the capacity to adapt a running protocol in real-time response to the collected data. It is also possible to harvest the conditioned medium and the cells after xCELLigence monitoring to perform other analyses such as the staining of cell markers or measurement of cell secretions, such as human chorionic gonadotropin (hCG), human placental lactogen (hPL), or steroid hormones.

2 Material

In the present chapter, we will focus on three xCELLigence® models: DP, SP, and MP.

2.1 Cell Passage and Counting

1. Cells of interest.
2. Phosphate-buffered saline (PBS); pH 7.4.

3. Trypsin or other cell dissociation buffers.
4. Cell culture medium.
5. Treatment (optional).
6. Manual or automated cytometer.
7. Trypan blue (optional).

2.2 The xCELLigence Assays

1. The xCELLigence® RTCA instrument: dual plate (DP, 16 wells × 3 cradles), single plate (SP, 96 wells × 1 cradle), or multiple plate (MP, 96 wells × 6 cradles) (ACEA Biosciences).
2. E-Plates 16 or 96 for adhesion, proliferation, and apoptosis assays (ACEA Biosciences).
3. CIM-plates 16 for migration and invasion assays (ACEA Biosciences).
4. Extracellular matrix (e.g., Matrigel™) for invasion assays.

3 Methods

Handle all materials in a sterile hood. The xCELLigence instrument® RTCA should be placed in an incubator and maintained at standard humidified conditions (37 °C/5% CO_2). Ensure that the instrument is properly connected to the computer and functional before starting the experiment (*see* **Note 2**). Optimal time points and cell densities must be determined for each cell type to be used. The following steps are a guide only; the xCELLigence user manual should be consulted before use.

3.1 Adhesion/ Proliferation Assay

1. Add 100 µL of medium to each well (*see* **Notes 3–5**). Leave E-plate to equilibrate for 30 min at room temperature.
2. During plate equilibration, trypsinize cells and resuspend in culture medium to obtain the appropriate dilution of cell suspension (100 µL per well will be necessary). The ideal cell density has to be optimized for each cell type studied (Fig. 2 and Table 2).
3. Once the plate is equilibrated, do a background measurement in the xCELLigence instrument (*see* Subheading 3.3).
4. Add 100 µL of cell suspension and allow cells to settle on the base of the well for 30 min at room temperature (*see* **Note 6**).
5. Place plate into the instrument cradle and begin the experiment (*see* **Notes 7–9**). If performing a co-culture experiment, *see* Subheading 3.2 in Chapter 23 for a detailed protocol.

3.2 Migration/ Invasion Assay

1. When performing an invasion assay, pre-coat the upper chamber with Matrigel™ or another desired gel matrix (*see* **Note 10**).

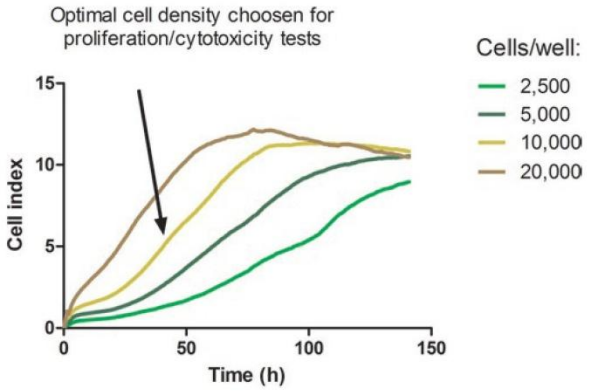

Fig. 2 The xCELLigence® RTCA system can be used to produce typical cell titration curves to determine optimal cell densities and time points for other experiments (an example is given for the proliferation of BeWo cells, 200 μL of medium by well)

Table 2
Optimal cell densities for placental cell lines using an xCELLigence® RTCA system

Cell line	Plate type	Cell concentration (cells/well)	References
Proliferation tests			
BeWo	E-plate 16	1×10^4	[5]
JEG-3	E-plate 96	2.5×10^3 and 5×10^3	[6] (Clabault data not published)
HIPEC	E-plate 96	2.5×10^3	(Clabault data not published)
HTR8/SVneo	E-plate 16	4×10^3–4×10^4	[3, 7]
SGHPL-4	E-plate 16	1.25×10^3–4×10^4	[3]
BeWo/H295R co-culture	E-plate 16 and E-plate insert	H295R (well): 2×10^4 BeWo (insert): 1×10^4	[5]
Migration/invasion tests			
HTR8/SVneo migration	CIM-plate 16	4×10^4–2×10^5	[3, 7–9]
HTR8/SVneo invasion	CIM-plate 16	4×10^4	[8]

2. Add 165 μL of culture medium to the bottom chamber of each well. Attach the upper chamber and add a further 50 μL of culture medium to the upper chamber of each well (*see* **Note 11**). Leave CIM-plate to equilibrate for 1 h in the xCELLigence instrument.
3. During plate equilibration, trypsinize cells and resuspend in culture medium to obtain the appropriate cell density. The ideal number of cells has to be optimized for each cell type studied (Table 2).

4. Once the plate is equilibrated, do a background measurement of medium alone in the xCELLigence instrument (*see* Subheading 3.3).
5. After the background check, add 100 μL of cell suspension and allow cells to settle on the base of the upper chamber of each well for 30 min at room temperature. This is a critical step as convection currents from the warm medium (37 °C) may affect cell attachment and introduce variations into the experimental results.
6. Place plate into the instrument cradle and begin the experiment.

3.3 Using the xCELLigence Software

1. Create a new experiment.
2. Enter the relevant information such as treatment conditions, cell types, cell density, and number of replicates in the "Layout" tab.
3. In the "Schedule" tab, add at least two steps. The first step is the background measurement step and the default settings should not be modified. The second step is the start of the experiment and can be split into sub-steps if desired to, for example, require readings to be taken every 5 min in the first hour (12 sweeps during 1 h) and once an hour for the next 48 h (48 sweeps during 48 h). Experimental protocols can be set up and saved in the computer software beforehand.
4. After placing the plate into the instrument cradle, check the "Message" tab to ensure that all connections are good. The message should say "Plate scanned. Connections ok." Usually at this step, pressing the "Play" button will prompt the software to ask if you wish to save the experiment. Click "Yes" and the background measurement will be recorded and accounted for. When completed, remove the plate and proceed to add the cells.
5. After cells have been added to the wells and allowed to settle, place the plate back into the cradle to begin the experiment. Before pressing the "Play" button, ensure that there are no connection errors in the "Message" tab as above. When the plate has to be removed from the instrument cradle (e.g., for treatments), press the "Pause" button.
6. When the experiment is finished (either at the end of the last step or after abortion of the experiment), problematic curves may be repaired using the curve adjustment tool (shift or line fit) under the "Plot" tab in the xCELLigence software.
7. Data can be normalized relative to a given time point, usually at the beginning of the experiment after the cells have settled down. Normalization corrects for well-to-well variation in initial cell densities that are not due to experimental conditions/treatments. It is also possible to express cell index as the

difference between an experimental condition and a baseline at each given time point, which is called delta cell index.

8. Analyze data using the data analysis tool.

4 Notes

1. Plates may potentially be reused to minimize costs [4]. It is up to the end user to decide if they wish to do so. However, we personally would not recommend it as there is a substantial risk of contamination once the plate is placed in the incubator and exposed to a non-sterile environment. Additionally, the process of removing the cells and spent medium may damage the sensitive electrodes within the wells, which would affect subsequent experimental results.
2. The instrument must be regularly maintained as per manufacturer's instructions. Routinely check for any corrosion of cradle pins as this will likely affect experimental results. Ensure that control plates (supplied with the instrument) are run to identify any problems if the instrument has not been used for a significant period of time.
3. It is possible to add PBS in the gaps between the wells to minimize evaporation from the wells.
4. The incubator housing the xCELLigence instrument should have sufficient water in the water tray to ensure high humidity, as the electrodes are highly sensitive to fluctuations in surrounding humidity. Care should be taken to recognize a potential "edge effect" in the plate, as wells on the periphery of the plate are not exposed to the same humidity level as the wells within, which may affect cell behavior depending on the cell type studied.
5. If not all the wells are used in an experiment, the unused wells can be kept sterile with a sealing adhesive film (e.g., PCR sealing film).
6. Ensure that no air bubbles are present after pipetting the culture medium into the wells. The air bubbles can affect the electrical impedance detected in the well and give an inaccurate background measurement. Be extra careful when pipetting medium containing fetal calf serum, which can produce a lot of bubbles. One method to avoid air bubbles is to perform reverse pipetting, which will allow the target volume to be released without introducing any air.
7. If you need to treat the cells, pause the cell impedance monitoring step, remove the plate from the station and add the test compounds to the culture medium or replace the culture

medium in the wells with pretreated culture medium. Another possibility is to plate cells directly in pretreated culture medium at the beginning of the experiment, but be aware that treatments may affect cell adhesion.

8. If at any time point of the experiment, the culture medium needs to be removed to either renew the medium or to add pretreated culture medium to the wells, avoid touching the bottom of the wells with the pipet tip as it could detach cells and introduce additional variability to the experiment. This can be done by leaving a small amount of culture medium (10 μL) in each well. Fresh culture medium should be added carefully, ideally by pipetting down slowly against the inside wall of the well.
9. Using E-Plate View, it is possible to visually inspect the cells (e.g., morphology, proliferative state), while pausing the experiment.
10. When coating the wells with Matrigel™ for the invasion assays, ensure that ice-cold medium is used to dilute the Matrigel™. It is also imperative that there are no air bubbles in the Matrigel™ coating, as these will interfere with cell movement through the matrix. From our experience, the best technique to ensure this is by reverse pipetting. The coated plate can then be incubated for 30 min at 37 °C or left in the cell culture cabinet overnight at room temperature to set. If using the incubator, do not leave the gel to set for more than 2 h as the coating may dry out.
11. There should be a clear meniscus on top of the wells in the lower chamber. This comes into direct contact with the electrodes. A "click" sound should be heard when attaching the upper and lower chambers together.

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References

1. ACEA Biosciences Inc (2016) xCELLigence RTCA systems. ACEA Biosciences Inc, San Diego, CA
2. Ke N, Wang X, Xu X, Abassi YA (2011) The xCELLigence system for real-time and label-free monitoring of cell viability. Methods Mol Biol 740:33–43
3. Keogh RJ (2010) New technology for investigating trophoblast function. Placenta 31: 347–350
4. Stefanowicz-Hajduk J, Adamska A, Bartoszewski R, Ochocka JR (2016) Reuse of E-plate cell sensor arrays in the xCELLigence real-time cell analyzer. BioTechniques 61:117–122
5. Thibeault AA, Deroy K, Vaillancourt C, Sanderson JT (2014) A unique co-culture model for fundamental and applied studies of human fetoplacental steroidogenesis and interference by environmental chemicals. Environ Health Perspect 122:371–377
6. Kaitu'u-Lino TJ, Pattison S, Ye L, Tuohey L, Sluka P, MacDiarmid J, Brahmbhatt H, Johns T, Horne AW, Brown J, Tong S (2013) Targeted nanoparticle delivery of doxorubicin into placental tissues to treat ectopic pregnancies. Endocrinology 154:911–919
7. Peng W, Chen Y, Luo X, Shan N, Lan X, Olson D, Zhang H, Ding YB, Qi HB (2016) DNA methylation-associated repression of MEST/PEG1 expression contributes to the invasion of extravillous trophoblast cells. Placenta 46:92–101
8. Chau SE, Murthi P, Wong MH, Whitley GS, Brennecke SP, Keogh RJ (2013) Control of extravillous trophoblast function by the eotaxins CCL11, CCL24 and CCL26. Hum Reprod 28:1497–1507
9. Nystad M, Sitras V, Larsen M, Acharya G (2014) Placental expression of aminopeptidase-Q (laeverin) and its role in the pathophysiology of preeclampsia. Am J Obstet Gynecol 211(686): e681–e631

4 CHAPITRE 4 : EFFECTS OF SELECTIVE SEROTONIN-REUPTAKE INHIBITORS (SSRIs) ON HUMAN VILLOUS TROPHOBLASTS SYNCYTIALIZATION

4.1 Présentation de l'article

Titre en français: Effets des antidépresseurs de la classe des inhibiteurs sélectifs de la recapture de la sérotonine (ISRS) sur la syncytialisation des trophoblastes villeux humains.

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Le manuscrit a été soumis à « Toxicology and Applied Pharmacology » (TAAP-D-17-01218), et accepté le 14 avril 2018.

4.2 **Résumé de l'article en français**

Les inhibiteurs sélectifs de la recapture de la sérotonine (ISRS) sont la classe d'antidépresseurs la plus prescrite durant la grossesse. Le placenta humain est un organe unique permettant la croissance et le développement du fœtus. Ainsi, le but de cette étude est d'analyser l'effet des ISRS sur les cellules trophoblastiques villeuses, en utilisant la lignée cellulaire BeWo et des cellules primaires humaines de trophoblastes villeux. La fluoxétine et son métabolite la norfluoxétine, la sertraline et la venlafaxine n'ont pas affecté la prolifération, la viabilité des cellules BeWo, ni le pourcentage de cellules primaires trophoblastiques positives au M30 (marqueur apoptotique). Aucun de ces ISRS n'a affecté la fusion de cellules BeWo, stimulées ou non avec de la forskoline, alors que la sertraline et la venlafaxine ont augmenté la fusion de cellules trophoblastiques primaires. La sertraline et la venlafaxine ont inhibé la différenciation fonctionnelle (sécrétion d'hormone gonadotrophine chorionique humaine : hCG) des cellules BeWo, mais elles n'ont pas eu d'effet sur la sécrétion d'hCG dans les cellules primaires. La norfluoxetine augmente l'expression génique de *chorionic gonadotropin beta (CGB)* et diminue celle de *gap junction protein alpha 1 (GJA-1)* (biomarqueurs de syncytialisation) des cellules BeWo, alors qu'au niveau des cellules primaires, aucun des ISRS testés n'a effecté l'expression de ces gènes. Cette étude démontre que certains ISRS peuvent affecter la syncytialisation de cellules trophoblastiques villeuses de manière structure- et concentration-dépendante, et suggère que certains ISRS pourraient compromettre l'homéostasie placentaire. De plus, cet article met l'accent sur l'importance de l'utilisation de cellules primaires au lieu de lignées cellulaires, pour évaluer l'effet des médicaments sur les fonctions des trophoblastes villeux.

4.3 **Contribution de l’étudiante**

L’étudiante a participé à l’élaboration de l’étude, réalisé toutes les expériences et analysé les résultats. Elle a par ailleurs rédigé l’article et participé au choix du journal de publication.

4.4 **Article**

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Effects of selective serotonin-reuptake inhibitors (SSRIs) on human villous trophoblasts syncytialization

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ABSTRACT

Selective serotonin-reuptake inhibitors (SSRIs) are the most commonly prescribed antidepressants during pregnancy. The human placenta is a highly specialized organ supporting normal growth and development of the fetus. Therefore, this study aims to analyze the effects of SSRIs on villous cytotrophoblasts cells, using BeWo cells and human placental trophoblast cells in primary culture. The SSRIs fluoxetine and its metabolite norfluoxetine, sertraline and venlafaxine did not affect BeWo cell proliferation and viability, nor the percentage of M30-positive (apoptotic) primary trophoblast cells. None of the SSRIs affected basal or forskolin-stimulated BeWo cell fusion, whereas sertraline and venlafaxine increased the fusion of primary villous trophoblasts. Sertraline and venlafaxine also modified human chorionic gonadotropin beta (β-hCG) secretion by BeWo cells, whereas none of the SSRIs affected β-hCG secretion in primary trophoblasts. Norfluoxetine increased *CGB* (chorionic gonadotropin beta) and *GJA1* (gap junction protein alpha 1) levels of gene expression (biomarkers of syncytialization) in BeWo cells, whereas in primary trophoblasts none of the SSRIs tested affected the expression of these genes. This study shows that SSRIs affect villous trophoblast syncytialization in a structure- and concentration-dependent manner and suggests that certain SSRIs may compromise placental health. In addition, it highlights the importance of using primary trophoblast cells instead of "trophoblast -like" cell lines to assess the effects of medications on human villous trophoblast function.

1. Introduction

Depression affects an estimated 350 million people worldwide (Kumar and Gupta, 2014) and is more common in women than men (Perry et al., 2017). Although pregnancy is often associated with increased well-being, it does not protect against this disease. According to the literature, between 10% and 20% of women suffer from depression during pregnancy (Bennett et al., 2004b; Gavin et al., 2005; Gentile, 2015). In addition to psychotherapy, various classes of antidepressants are prescribed to pregnant women, including selective serotonin-reuptake inhibitors (SSRIs), selective serotonin- and norepinephrine-reuptake inhibitors (SNRIs), tricyclic antidepressants, and more rarely, monoamine oxidase inhibitors (MAOIs) (Gentile, 2014; Ram and Gandotra, 2015). Up to 5% of pregnant woman are treated with SSRIs or SNRIs (Andrade et al., 2008; Alwan et al., 2011; Charlton et al., 2015). Fluoxetine, sertraline and venlafaxine are among the most

Abbreviations: SSRIs, selective serotonin-reuptake inhibitors; hCG, human chorionic gonadotropin; vCTBs, villous cytotrophoblast cells; STB, cytotrophoblasts; *CGB*, chorionic gonadotropin beta, ; *CSH*, chorionic somatomammotropin hormone; *ERVFRD-1*, endogenous retrovirus group FRD member 1; *ERVW-1*, endogenous retrovirus group W member 1; *GJA1*, gap junction protein alpha 1; hPL, human placental lactogen.

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prescribed antidepressants in the general population including pregnant women (Andrade et al., 2008; Alwan et al., 2011).

Although classified as an SNRI, in the present study venlafaxine will be considered as an SSRI because its inhibition potency for serotonin reuptake is 30-fold greater than that for norepinephrine - (Montgomery, 2008; Laurent et al., 2016). Both classes of antidepressants increase extracellular serotonin levels in the synaptic cleft of serotonergic neurons by blocking the serotonin transporter (SERT). Health care providers need to balance the risks and benefits of antidepressant treatment with those of untreated depression. In fact, untreated depression is a serious medical condition associated with poor prenatal behaviors, such as poor diet, low attendance at prenatal checkups and increased substance abuse, and with conditions such as preterm delivery, low birth weight, increased risk of postnatal depression, and maternal and fetal mortality (Bennett et al., 2004a; Grote et al., 2010; Filippi et al., 2016). On the other hand, SSRI treatment has been associated with secondary effects, including adverse outcomes of pregnancy, such as premature delivery (Huang et al., 2014; Eke et al., 2016), perinatal complications (respiratory distress, feeding difficulties, cardiac malformations) (Byatt et al., 2013; Laurent et al., 2016), low birth weight (Ramos et al., 2010; Huang et al., 2014), neurobehavioral sequelae (Weikum et al., 2012), and autism spectrum disorder (Boukhris et al., 2016). The scientific community is divided on whether a correlation between SSRI intake and an increased incidence of fetal malformations exist (Chambers et al., 1996; Kulin et al., 1998; Alwan et al., 2007; Wemakor et al., 2015; Laurent et al., 2016).

The placenta plays a crucial role in successful pregnancy by providing fundamental trophic, immunological and endocrine support, as well as an essential exchange of nutrients, oxygen and wastes between mother and fetus (Malassine, 2001; Desforges and Sibley, 2010; Fournier, 2016). Many of these key functions are performed by the placental trophoblast cells. The villous cytotrophoblasts (vCTBs) in the floating villi fuses into a large plurinucleated syncytiotrophoblast (STB) in a process called morphological differentiation. The STB is in direct contact with maternal blood and forms a physical and immunological barrier between the fetus and the maternal blood. The STB also allows transplacental exchanges (Albrecht and Pepe, 1990; Pepe and Albrecht, 1995) and the process of syncytialization is also associated with a biochemical (or functional) differentiation, characterized by an increased production of estrogens, human chorionic gonadotropin (hCG; *CBG* gene) and human placental lactogen (hPL; *CSH* gene) (Kliman et al., 1986; Morrish et al., 1987; Alsat et al., 1999; Vaillancourt et al., 2009).

Various mechanisms and factors are involved in the stimulation or inhibition of vCTB differentiation, including growth factors, hormones, kinases, and membrane proteins (Gauster and Huppertz, 2008; Vargas et al., 2009; Ji et al., 2012). Membrane proteins such as syncytin-1 (*ERVW-1* gene), syncytin-2 (*ERVFRD-1* gene) and connexin-43 (*GJA1* gene), as well as hCG and hPL are syncytialization biomarkers. Syncytin-1 and -2 are fusogenic protein encoded by human endogenous retroviral (*HERVs*) genes, which promote trophoblast cell fusion. In primary trophoblast cells, syncytin-1 is significantly up-regulated after stimulation with AMPc (Frendo et al., 2003b). The gap junction connexin-3 is involved in the trophoblast fusion and biochemical differentiation (Frendo et al., 2003a).

Placental cells have a functional serotonergic (5-HT) system. Our laboratory and others have shown that in both mouse and human trophoblast cells tryptophan hydroxylases (TPHs) (the rate-limiting enzymes in 5-HT synthesis), 5-HT_{2A} receptors, monoamine oxidase (MAO) and SERT are present and that trophoblasts are able to produce serotonin *de novo* (Balkovetz et al., 1989; Viau et al., 2009; Bonnin et al., 2011; Laurent et al., 2017). Serotonin has a key role in pregnancy and is involved in the regulation of embryogenesis, placentation and placental function (Sonier et al., 2005; Oufkir et al., 2010; Cikos et al., 2011; Hadden et al., 2017). It is also involved in certain obstetric disorders, such as gestational hypertension (De Ocampo et al., 2016), preeclampsia (Bolte et al., 2001; Sabolovic Rudman et al., 2015) and gestational diabetes (Viau et al., 2009; Kwak et al., 2012).

It is known that xenobiotics such as phthalates or valproic acid can affect villous trophoblasts homeostasis (Adibi et al., 2010; Kwiecinska et al., 2011). Our laboratory recently demonstrated that fluoxetine and norfluoxetine disrupt placental estrogen synthesis (Hudon Thibeault et al., 2016). Despite these findings and the high prescription rate of SSRIs for pregnant women together with the critical role that the villous trophoblast plays in successful pregnancy and healthy fetal development, the effects of SSRIs on vCTB syncytialization have never been studied. Therefore, the aim of this study was to determine whether therapeutically relevant concentrations of SSRIs affect villous trophoblast syncytialization, using BeWo human trophoblast-like choriocarcinoma cells and human trophoblast cells in primary culture.

2. Material and methods

2.1. Cell culture

BeWo cells (CCL-98 clone) were obtained from the American Type Culture Collection (ATCC; Rockville, MD) and maintained in Dulbecco's modified Eagle's medium (DMEM)/F-12 without phenol red, supplemented with 10% fetal bovine serum (FBS, Hyclone, Tempe, AZ) and 0.6 g/L of sodium bicarbonate (Sigma-Aldrich, Oakville, ON, Canada). Cells were cultured in a humidified atmosphere of 5% carbon dioxide (CO_2) at 37 °C. Cells were passaged when they reached about 90% confluence, using 0.5% trypLE (cell dissociation buffer) (Thermo Fisher Scientific, Waltham, MA).

The study was performed according to the Declaration of Helsinki. All placentas were obtained following approval from the local ethics committee (*Comité de Protection des Personnes 2015-mai-13909*) and with the written consent of the women. Placental tissues from healthy mothers with uncomplicated pregnancies at term (37–39 weeks of amenorrhea) were obtained immediately after normal caesarean section at the Cochin Port-Royal, Antony and Montsouris maternity units (Paris, France). The accepted caesarean section reasons were breech delivery or transverse presentation, covering placenta, multi-cicatricial uterus, narrow pelvis. There was no available additional information on participant demographics, or previous SSRI or SNRI use. Villous cytotrophoblast cells (vCTBs) from term placentas were isolated as described previously (Kliman et al., 1986; Alsat et al., 1993; Sagrillo-Fagundes et al., 2016; Clabault et al., 2018), using sequential trypsin-DNase digestions and further purification on a Percoll gradient. The vCTBs were suspended in DMEM – high glucose (Sigma-Aldrich), supplemented with 10% FBS and penicillin– streptomycin. They were then plated and acclimatized for 18 h after isolation before starting treatments. More than 95% of the cells were positive for cytokeratin-7 and negative for vimentin, confirming their trophoblastic nature.

2.2. Cell treatments

SSRIs were purchased from Sigma-Aldrich (Fig. S1). Norfluoxetine (a metabolite of fluoxetine) and venlafaxine were dissolved in H_2O miliQ; fluoxetine and sertraline in DMSO (Sigma-Aldrich). BeWo and primary trophoblast cells were treated with increasing concentrations of SSRIs (0 to 10 μM) in culture medium. The SSRI concentrations used corresponded to concentrations found in maternal (15–130 nM) and cord blood (65–250 nM) during treatment for depression (Hendrick et al., 2003; Sit et al., 2011). Control cells were exposed to a final concentration of 0.1% DMSO. To induce biochemical and morphological differentiation, BeWo cells were stimulated with 20 μM forskolin (Sigma-

Aldrich). Cell culture medium with or without SSRI treatment was changed every 24 h.

2.3. Cell proliferation assay

BeWo cell proliferation was monitored in real-time by measuring cell impedance using an xCELLigence™ RTCA SP instrument (ACEA Biosciences, San Diego, CA) as described recently (Chollangi et al., 2018). Electronic 96-well microtiter plates (E-Plate® 96) were filled with 100 µL of complete culture medium, and after 30 min, background impedance was measured. Next, BeWo cells (1×10^5 cells/mL, 100 µL/well) were added to the E-Plates. After 24 h, cells were treated with various concentrations of the SSRIs. Cells exposed to 0.1% DMSO vehicle were used as controls. Real-time proliferation was determined quantitatively by measuring changes in impedance every 10 min over a period of 72 h, and data were analyzed using RTCA Data Analysis Software v1.0 (ACEA Biosciences). Cell indices were based on the slope of the normalized linear part of the proliferation curves.

Proliferation and cell cycle experiments were not performed with primary trophoblast cells because these cells do not proliferate *in vitro* (Aplin, 1991; Yui et al., 1994; Garcia-Lloret et al., 1996).

2.4. Cell cycle assay

BeWo cells (4×10^4 cells/mL) were added to 24-well plates (CellBind; Corning LifeSciences, Lowell, MA) in culture medium and allowed to acclimatize for 24 h. The medium was then refreshed, and various concentrations of the SSRIs were added. After 24 h, cells were collected, washed and fixed with ice-cold 70% ethanol (v/v), and stored at 4 °C overnight. Cells were then centrifuged (310 x g, 10 min) and the cell pellets suspended in phosphate-buffered saline (PBS) containing 50 µg/mL propidium iodide (Sigma-Aldrich), 100 µg/mL RNase A (Sigma-Aldrich) and incubated for 15 min in the dark at room temperature. Cell cycle was then analyzed using a FACSCalibur flow cytometer (Becton Dickinson, Franklin Lakes, NJ). The percentage of the cell population in each cell cycle phase was determined with Cell Quest Pro Software (BD Biosciences, San Jose, CA). To evaluate the cell cycle, 3000 events per sample were recorded.

2.5. Cell fusion assay

BeWo cells (5×10^4 cells/well) were added to 24-well CellBind® plates in complete culture medium. After 24 h, the medium was refreshed and various concentrations of the SSRIs were added. After another 48 h, cell culture medium was removed and cells were washed with PBS. The cells were then fixed in methanol for 8 min at −20 °C, rinsed with PBS and incubated for 1 h at room temperature with 2% (v/v) FBS in PBS to eliminate non-specific antigen binding. To determine cell fusion, BeWo cells were incubated for 1 h at room temperature with mouse anti-desmoplakin antibody (#Ab16434, Abcam, Toronto, ON, Canada) diluted 1:300 (v/v) in PBS supplemented with 0.5% bovine serum albumin (BSA). The cells were then washed with PBS and incubated for 1 h in the dark at room temperature with donkey anti-mouse IgG Alexa Fluor 488 (#A11029, Thermo Fisher Scientific) diluted 1:1000 in PBS supplemented with 0.2% BSA. Nuclei were stained in the dark for 30 min at room temperature using 50 µg/µL propidium iodide in PBS.

Primary villous cytotrophoblast cells (2×10^5 cells/well) were added to 24-well CellBind® plates in complete culture medium (as described in Section 2.1.). After 18 h, the medium was refreshed and various concentrations of SSRIs were added for 48 h or 72 h. The protocol was similar with a few modifications. The cells were incubated in blocking buffer (PBS-3% BSA) for 2 h, and then incubated overnight at 4 °C with the primary antibodies anti-desmoplakin (1:300 dilution; Sigma-Aldrich) and M30 CytoDEATH (specific for epithelial apoptotic cells (Jordan and Butchko, 2002) (1:100 dilution; Roche, Indianapolis, IN). The next day, the cells were washed and incubated with the secondary antibodies AlexaFluor488-labeled donkey anti-mouse and AlexaFluor555-labeled donkey anti-rabbit (1:500 dilution; Thermo Fisher Scientific). Nuclei were stained with DAPI (Sigma-Aldrich) in PBS for 30 min at room temperature in the dark. After staining, cells were washed using 0.5 mL/well PBS and stored at 4 °C until microscopic assessment using a Nikon A1R confocal microscope (Nikon Canada, Mississauga, ON). At least four fields were randomly selected for analysis. A syncytium was defined as a cell containing three or more nuclei in the same cytoplasm without desmoplakin-stained membranes in between. The results were expressed as the percentage of nuclei contained in multinuclear syncytia as described by Pidoux et al. (2010). Two replicate wells per treatment were randomly selected for analysis to determine a final cellular fusion index.

2.6. hCG secretion

To determine biochemical differentiation, BeWo cells (5×10^4 cells/well in 24-well plates) and primary cells (1×10^6 cells/well in CellBind 6-well plates) were treated for 24 h (BeWo) or 72 h (primary cells). Supernatants were collected, centrifuged at 2000*g* at 4 °C for 10 min to remove debris, and stored at −20 °C until further analysis. Secretion of hCG by BeWo cells was determined by enzyme-linked immunosorbent assay (ELISA) using a kit from DRG Diagnostics (#EIA-1911, Marburg, Germany) following the enclosed instructions. Protein concentrations were quantified using a bicinchoninic acid (BCA) protein assay (Pierce Biotechnology, Rockford, CA) and used for normalization. The amount of hCG secreted was expressed initially in mUI/mL/mg protein and then as a percentage (mean ± SEM) of DMSO control or forskolin control. The amount of hCG secreted by primary cells was measured using a COBAS 8000 analyzer (Roche Diagnostics, Rotkreuz, Switzerland) and expressed as a percentage (mean ± SEM) of DMSO control at 48 h.

2.7. mRNA expression levels analysis

Cells were cultured under the same conditions as for the hCG secretion assays. Total RNA was isolated using an AllPrep DNA/RNA/Protein Mini kit (Qiagen, Toronto, ON, Canada) according to manufacturer instructions. RNA purity and quantity were determined using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA). An Experion automated electrophoresis system (Bio-Rad, Mississauga, Ontario, Canada) was used to measure RNA integrity (RNA quality indicator > 9). Synthesis of cDNA was performed using an iScript cDNA synthesis kit (Bio-Rad). cDNA samples were pre-amplified using SSoAdvanced PreAmpl Supermix (Bio-Rad). Sequences of the primer pairs used for amplification are shown in Table 1. Their selectivity was established using the Primer-Blast program (www.ncbi.nlm. nih.gov/tools/primer-blast/). Samples were then amplified by PCR with SSoAdvanced Universal SYBR Green Supermix using a CFX-96 Real-Time PCR Detection System (Bio-Rad). Target gene amplification responses were normalized using *peptidylprolyl isomerase A* (*PPIA*), *topoisomerase 1* (*TOP-1*) and *succinate dehydrogenase complex subunit A* (*SDHA*) as reference genes, which were selected using GeNorm software (BioGazelle, Zwijnaarde, Belgium) (Lanoix et al., 2012). PCR products were verified by electrophoresis on a 2% agarose gel containing 0.007% ethidium bromide and made visible under UV light.

Table 1
Oligonucleotide primer sequences used for gene amplification.

Gene	Accession number	Primer sequence (5′-3′)	
		Sense	Antisense
CGB	NM_001319065.1 (Frendo et al., 2003a)	GCTACTGCCCCACCATGACC	ATGGACTCGAAGCGCACATC
CSH	NM_001317.5	GCATGACTCCCAGACCTCCTT	TGCGGAGCAGCTCTAGATTGG
ERVW-1	NM_001130925.1 (Wang et al., 2013)	GCCATTCAAACAACGATAGG	TGGAACAACTTCAGCACAGA
ERVFRD-1	NM_207582.2 (Toufaily et al., 2013)	GCAGCTCGTTTTGTGACCAG	CGGTAGGCTGCTAGTGAAGG
GJA1	NM_000165.4	AGCCACTAGCCATTGTGGAC	CCCATACACCCCCAGTGAAC
PPIA	NM_021130 (Lanoix et al., 2012)	GTTTGCAGACAAGGTCCCA	ACCCGTATGCTTTAGGATG
SDHA	NM_004168 (Lanoix et al., 2012)	TACAAGGTGCGGATTGATG	CGATCACGGGTCTATATTCAA
TOP-1	NM_003286.2	GGCGAGTGAATCTAAGG	CTTAAAGGGTACAGCGAATG

CGB: chorionic gonadotropin beta; *CSH*: chorionic somatomammotropin hormone; *ERVFRD-1*: endogenous retrovirus group FRD member 1; *ERVW-1*: endogenous retrovirus group W member 1; *GJA1*: gap jounction protein alpha 1; *PPIA*: peptidylprolyl isomerase A; *TOP-1*: topoisomerase 1; *SDHA*: succinate dehydrogenase complex subunit A.

2.8. Statistical analysis

Experiments with BeWo cells were performed using three to five different cell passages, whereas for primary cell cultures, three to five different placentas were used. Statistical analyses were performed using IBM SPSS Statistics 21.0 software (Chicago, IL) and figures were made using GraphPad Prism v5.0 (GraphPad Software, San Diego, CA). First, the assumption of normality was verified by Shapiro-Wilks test. Then, the homogeneity of variance was determined by Levene test or manually if the Shapiro-Wilks test was not significant ($p < 0.05$). After determining the validity of these two assumptions (normality and homogeneity of variance), one of the following statistical analysis tests was used as specified in the figure legends: one-way ANOVA with a Dunnett posteriori test, Welch's test, a Kruskal-Wallis test, Mood's median test (for differences between vehicle control and SSRI treatments), a Student *t*-test or a Mann-Whitney *U* test (for differences between DMSO and forskolin treatments). A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. SSRIs do not affect on BeWo cell proliferation

None of the four tested SSRIs (fluoxetine, norfluoxetine, sertraline and venlafaxine) had a statistically significant effect on BeWo cell proliferation at the concentrations tested (0.03 to 10 μM; Fig. 1).

3.2. SSRIs increase the percentage of BeWo cells in G0-G1 phase and decrease the percentage of BeWo cells in G2-M phase

Flow cytometry showed statistically significant changes in the BeWo cell cycle after a 24 h exposure to the SSRIs (Fig. 2). Compared to DMSO, a relative increase of 8.9%, 13.28%, 6.66% and 13.7% of cells in G0-G1 was observed respectively after a treatment with 1 and 10 μM of fluoxetine ($P = 0.045$ and $P = 0.007$, respectively), and 1 and 10 μM of sertraline ($P = 0.025$ and $P = 0.006$, respectively) (Fig. 2). In parallel, the relative percentage of BeWo cells in the G2-M phase decreases concentration-dependently, which was statistically significant at 10 μM of fluoxetine (−14.7%, $P = 0.013$), at 0.1, 1, and 10 μM of norfluoxetine (−10.9%, −12.72% and − 13.8%; $P = 0.03$, $P = 0.019$ and $P = 0.017$, respectively) and at 1 and 10 μM of sertraline (−16.5% and − 18.9%, respectively; $P = 0.017$ and $P = 0.002$, respectively) compared to DMSO control (Fig. 2). None of the SSRIs affected the percentage of BeWo cells in phase S or sub G0.

3.3. SSRIs do not alter BeWo cells fusion

BeWo cells in culture aggregated and fused (syncytialization) when stimulated with 20 μM forskolin, a direct inducer of adenylate cyclase which increases intracellular cyclic adenosine monophosphate (cAMP) levels, to form a multinucleated syncytiotrophoblast (Fig. 3). The induction of fusion was apparent when comparing forskolin-treated cells with those exposed to 0.1% DMSO only (Fig. 3A). In BeWo cells treated for 48 h with increasing concentrations of fluoxetine or norfluoxetine, we did not observe any effect on BeWo syncytialization, compared to cells exposed to vehicle control (Fig. 3B and D). Conversely, the same SSRI treatments, in the presence of 20 μM forskolin, did not affect BeWo syncytialization (Fig. 3C and D). The same observations were made with the other tested SSRIs (data not shown).

3.4. Sertraline and venlafaxine alters hCG secretion in BeWo cells

A 48 h exposure to fluoxetine or norfluoxetine (0.1–10 μM) did not significantly alter hCG release from BeWo cells, either in the absence or presence of 20 μM forskolin, compared to controls (Fig. 4). In cells treated with sertraline, whether stimulated or not with forskolin, we observed a significant decrease of hCG secretion. In the absence of forskolin, sertraline at 0.1, 1 and 3 μM, decreased hCG release by 17.13% ($P = 0.009$), 9.75% ($P = 0.04$) and 12.75% ($P = 0.029$), respectively, compared to DMSO control; in the presence of forskolin, sertraline at 0.1, 1 and 10 μM, decreased hCG release by 7.59% ($P = 0.033$), 18.88% ($P = 0.013$) and 63.71% ($P = 0.013$), respectively, compared to forskolin control. In BeWo cells stimulated with forskolin, hCG release was increased by venlafaxine by 27.75% ($P = 0.033$) and 34.84% ($P = 0.002$) compared to forskolin control at 1 and 3 μM, respectively (Fig. 4).

3.5. In BeWo cells, norfluoxetine and sertraline affect mRNA levels of biomarkers of syncytialization

In BeWo cells treated with fluoxetine, norfluoxetine, sertraline and venlafaxine, 20 μM forskolin significantly increased *CGB* (β-hCG) and *ERVRFRD-1* (syncytin-2) and decreased *CSH* (hPL) mRNA expression compared to vehicle control (0.1% DMSO); *ERVW-1* (syncytin-1) and *GJA1* (connexin 43) expression was not statistically significantly affected (Fig. 5).

None of the tested SSRIs affected *CSH* or *ERVRFRD-1* mRNA levels (Fig. 5). Norfluoxetine treatment increased *CGB* mRNA levels in BeWo cells 1.82-fold at 0.3 μM ($P = 0.019$), 3.88-fold at 1 μM ($P = 0.0099$) and 3.10-fold at 3 μM ($P = 0.02$), compared to DMSO control. In forskolin-stimulated BeWo cells, sertraline increased *ERVW-1* mRNA levels 2.28-fold at 0.3 μM ($P = 0.025$), 2.24-fold at 1 μM ($P = 0.028$)

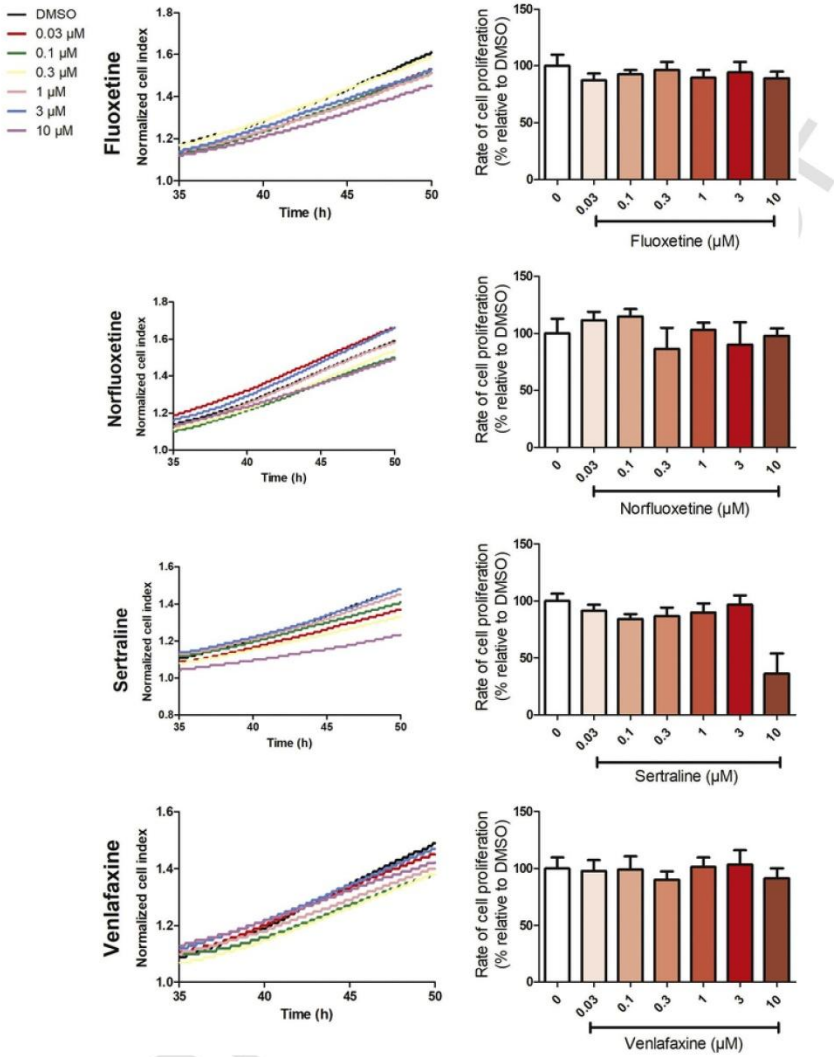

Fig. 1. Effects of fluoxetine, norfluoxetine, sertraline and venlafaxine on real-time proliferation of BeWo cells. Relative cell proliferation rates are presented as mean ± SEM. No statistically significant differences were detected between SSRIs treatments and vehicle control (0.1% DMSO) (one-way ANOVA or Kruskal-Wallis test, $P > 0.05$). Each experiment was performed five times; per experiment each treatment was tested in triplicate.

and 2.25-fold at 3 µM ($P = 0.028$) compared to forskolin control (Fig. 5); a similar increasing tendency was seen in unstimulated cells, but was not statistically significant. In BeWo cells treated with 3 µM norfluoxetine, *GJA1* mRNA levels increased significantly, 3.79-fold ($P = 0.034$) of DMSO control.

3.6. Sertraline induces fusion in primary cell cultures

Mononucleated vCTBs aggregated and fused spontaneously between 48 and 72 h in culture, which was made visible by desmoplakin immunostaining (Fig. 6A) and quantified by fusion index (Fig. 6B). Fluoxetine, norfluoxetine and venlafaxine did not alter syncytial formation (except at a 48 h exposure to 0.3 µM venlafaxine, $P = 0.016$). Sertraline stimulated vCTB fusion after 48 h (0.03 µM: $P = 0.006$; 0.3 µM: $P = 0.041$; 1 µM: $P = 0.029$) as well as 72 h (0.03 µM: $P = 0.012$; 0.3 µM: $P = 0.022$) of exposure (Fig. 6B).

There was a small but statistically significant ($P = 0.021$) increase from about 5.6 to 8.0% in the percentage of M30-positive trophoblast cells (a measure of caspase-dependent apoptosis) between 48 and 72 h of primary culture in the presence of 0.1% DMSO vehicle control (Fig. 6C), which is in agreement with the literature. For example, Daoud et al. reported 11.3% of apoptotic cells after 24 h, and 10.2% after 72 h (Daoud et al., 2006; Singh et al., 2012). None of the tested SSRIs altered the percentage of apoptotic trophoblast cells at either 48 h or 72 h of exposure.

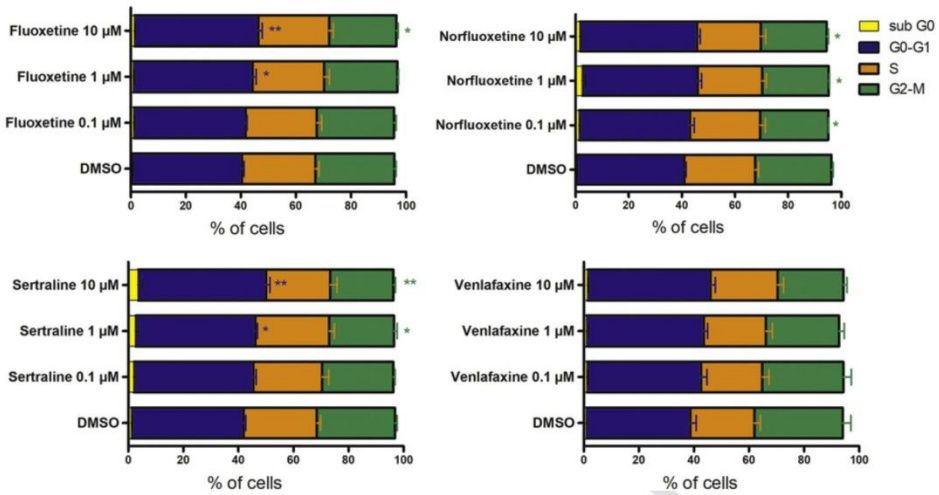

Fig. 2. Effects of fluoxetine, norfluoxetine, sertraline and venlafaxine on cell cycle of BeWo cells. After 24 h in culture, cells (1×10^5 cells/well) were treated for 24 h with the SSRIs, then stained using propidium iodide and analyzed by flow cytometry. The percentage of cells in each cell cycle phase is expressed as mean ± SEM. (*, **) Statistically significant difference from vehicle control (0.1% DMSO) by Kruskal-Wallis (*$P < 0.05$; **$P < 0.01$). Each experiment was performed five times; per experiment each treatment was tested in triplicate.

3.7. SSRIs have no effect on hCG secretion in primary trophoblast cells

As expected, we observed a significant increase in hCG secretion ($P = 0.043$) by vehicle control trophoblast cells between 48 h and 72 h of primary culture (Fig. 7). After both 48 h and 72 h of SSRI treatments. None of the SSRIs affected hCG secretion in primary villous trophoblast after either 48 h or 72 h of treatment relative to their respective DMSO controls.

3.8. Fluoxetine and norfluoxetine increase hPL and syncytin-1 mRNA levels in primary villous trophoblast

None of the SSRIs had an effect on levels of *CGB*, *CSH*, *ERVW-1*, *ERVFRD-1* and *GJA1* gene expression relative to DMSO control in human villous trophoblast cells in primary culture (Fig. 8), although several genes were either increased (*CGB* for fluoxetine, *ERVW-1* for all SSRIs tested, *GJA1* for fluoxetine, sertraline and venlafaxine) or decreased (*CGB* for norfluoxetine and venlafaxine) in several individual placentas, underlining the great inter-individual variability in the responses to SSRIs.

4. Discussion

To our knowledge, the present study is the first to evaluate the effects of SSRIs (fluoxetine, norfluoxetine, sertraline, and venlafaxine) on the syncytialization *in vitro* of villous trophoblast cells. An overview of the effects of SSRI on BeWo and on primary villous trophoblast cells observed in the present study is presented in Table 2.

The 4 SSRIs evaluated in our study did not affect BeWo cell proliferation. Recent studies focused on the effect of fluoxetine on human cell proliferation found that fluoxetine decreased the proliferation of human adipose-derived stem cells (Sun et al., 2015), whereas it increased the proliferation of bone-marrow-derived mesenchymal stem cells (Sun et al., 2015) and of neuronal progenitor cells through modulation of GSK-3β/β-catenin signaling (Chang et al., 2010; Hui et al., 2014). These observations suggest that the mechanism(s) by which fluoxetine affects cell proliferation is cell-type dependent. The absence of cytotoxicity of the SSRIs within our tested concentration range (≤ no greater than 3 µM) is in agreement with Jacobsen et al., who observed that fluoxetine, paroxetine and sertraline reduced the viability of H295R adrenocortical carcinoma cells at 31.4, 20, and 15 µM, respectively after 48 h of SSRI exposure (Jacobsen et al., 2015). We observed that sertraline at 10 µM tended to decrease BeWo cell viability, which is consistent with previous observations that sertraline, between 20 and 50 µM, significantly decreased the viability of several different cell types at various times of exposure (Peng et al., 2012; Ghosh et al., 2015; Schmidt et al., 2016).

We found that our tested SSRIs had differential effects on the percentage of BeWo cells in the G0-G1 and G2-S phases. A study using hypoxic colon tumor cells treated with 20 µM of fluoxetine also found an increase of cells in the G0-G1 phase and a decrease in progression toward the S phase; the authors also observed that fluoxetine decreased the activation of the Akt-mTOR signaling pathway in these cells (Kannen et al., 2015). The same effects of fluoxetine (G0-G1 increase, S decrease) were observed in MDA-MB-231 human breast cancer and SiHa human cervical cancer cells; the authors proposed that the phenomenon arises from the functional inhibition of cyclin dependent kinase subunit 1 (CKS1), which leads to a fluctuation in p27, p21 mRNA levels (Krishnan et al., 2008). Interestingly, a recent article established a link between trophoblast cell cycle and cell fusion, in which fusion can only occur when cells are in the G0 phase (Lu et al., 2017). This phenomenon may be explained by the fact that the expression of p21 and GCM1, which regulate the fusogenic proteins syncytin-2; and syncytin-2 is restricted to the G0 phase.

In the present study *CSH* mRNA levels in BeWo cells remained stable or decreased during forskolin-induced differentiation, which is in contrast to the increased levels of *CSH* mRNA and hPL secretion during differentiation of primary trophoblast cells (Hoshina et al., 1984; Lanoix et al., 2008; Handwerger, 2010). Little is known about the effects of forskolin on *CSH* mRNA expression in BeWo cells. Although a recent study (Saryu Malhotra et al., 2015) reported an increase of *CSH* mRNA level in BeWo cells stimulated for 72 h with 25 µM forskolin, but the authors normalized their data with 18S, which can affect reliability of data (Lanoix et al., 2012). Consistent with our findings, Vargas et al. (Vargas et al., 2009) observed an increase in *ERVFRD-1*, but unlike us, also found an increase of *ERVW-1* mRNA levels, albeit using a greater forskolin concentration (50 µM) than in the present study (20 µM). The

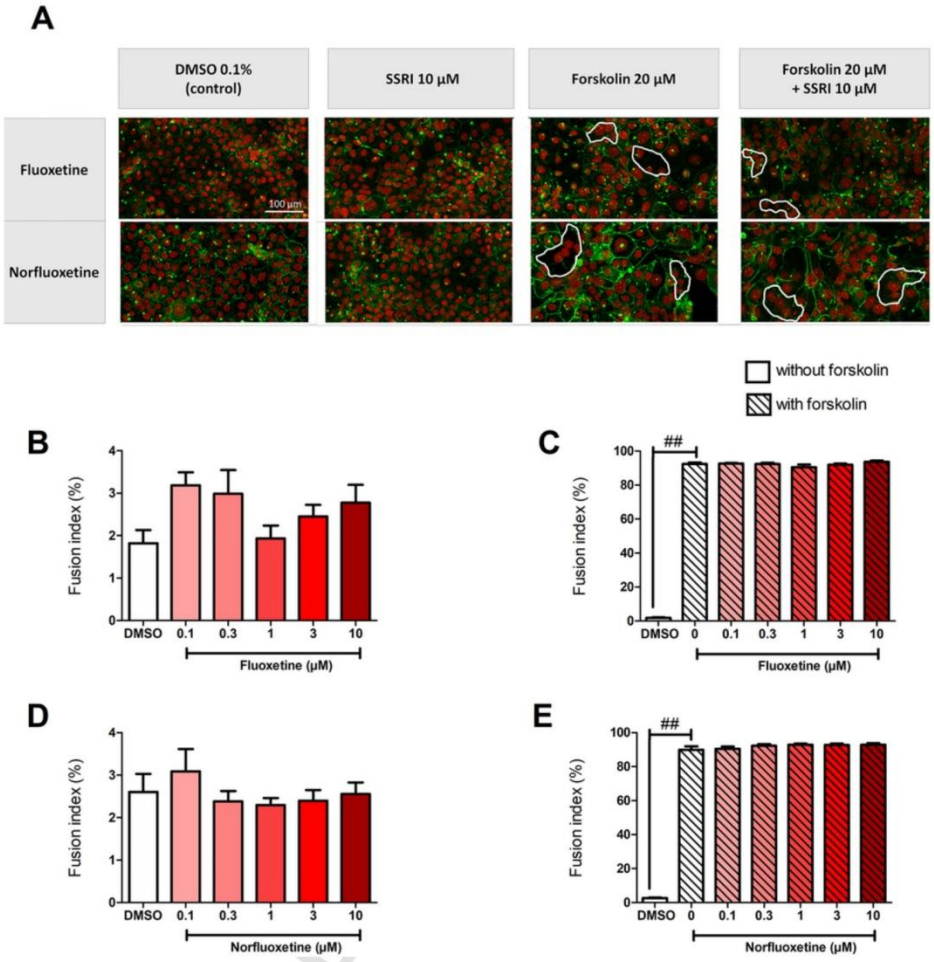

Fig. 3. Effects of fluoxetine and norfluoxetine on BeWo cells fusion. After 24 h in culture, cells were treated for 48 h with increasing concentrations of fluoxetine, norfluoxetine or vehicle control (0.1% DMSO), in the presence or absence of 20 µM forskolin. (A) Representative immunofluorescence images; cells were fixed in methanol, stained with desmoplakin for membranes (green), counterstained with propidium iodide for nuclei (red), and observed by confocal microscopy at 20× magnification. Scale bar: 100 µm. (B–E) The cellular fusion index was determined by counting about 200 cell nuclei per well, and expressed as the percentage of nuclei comprised in syncytia (mean ± SEM). (##) A statistically significant difference between cells treated with DMSO and cells treated with forskolin (Mann-Whitney *U* test; ## $P < 0.01$). No statistically significant differences were detected between SSRI treatments and vehicle control (Kruskal-Wallis test). Each experiment was performed three times; per experiment each treatment was tested in triplicate. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Robinson laboratory found similar results to us using 20 µM of forskolin on the BeWo cell line. After a 48 h treatment, they did not report any *ERVW-1* mRNA levels change compared to DMSO control, but after a 72 h treatment, they reported an increase (Omata et al., 2013).

We found that fluoxetine and its metabolite norfluoxetine did not affect the forskolin-induced fusion of BeWo cells, nor did they alter the biochemical differentiation of BeWo cells based on *CGB* gene expression and hCG secretion levels. Interestingly, in primary cell cultures treated with fluoxetine or norfluoxetine, *CSH*, *ERVW-1* and *GJA1* gene expression was increased compared to control. For syncytialization, we found an important inter-individual gene expression variability among placentas, with some primary cells very sensible to SSRIs, and others much less so. This inter-placental variability has also been observed in other studies and may be explained by differences in genetic, epigenetic and/or metabolic factors that influence placenta function (Pidoux et al., 2004; Hughes et al., 2015; Gundacker et al., 2016). Future studies, using a larger number of placentas, will be needed to confirm our results and would allow for the study of sex-specific differences in placental responses to SSRIs by comparing male and female placentas which are known to differ in physiology.

Syncytialization is a rare phenomenon in human physiology, undergone by only three cell types: cytotrophoblasts (into syncytiotrophoblast), myoblasts (into myotubes), and monocytes (into osteoclasts or giant cells) (Vignery, 2000; Huppertz et al., 2001). A study of several SSRIs (1 and 10 µM) demonstrated that, with the exception of citalopram, paroxetine fluoxetine and sertraline (in increasing potency) concentration-dependently inhibited osteoclast (OC) formation and resorption in primary human osteoblast and in colony forming unit-granulocyte/macrophage (CFU-GM)-derived OC precursor cells (Hodge et al., 2013). Our present study also found that SSRIs affected BeWo and primary trophoblast cell cycle, proliferation and syncytialization with different potencies dependent on their molecular structures, which are relatively diverse (see Fig. S1). This diversity may result in binding to a

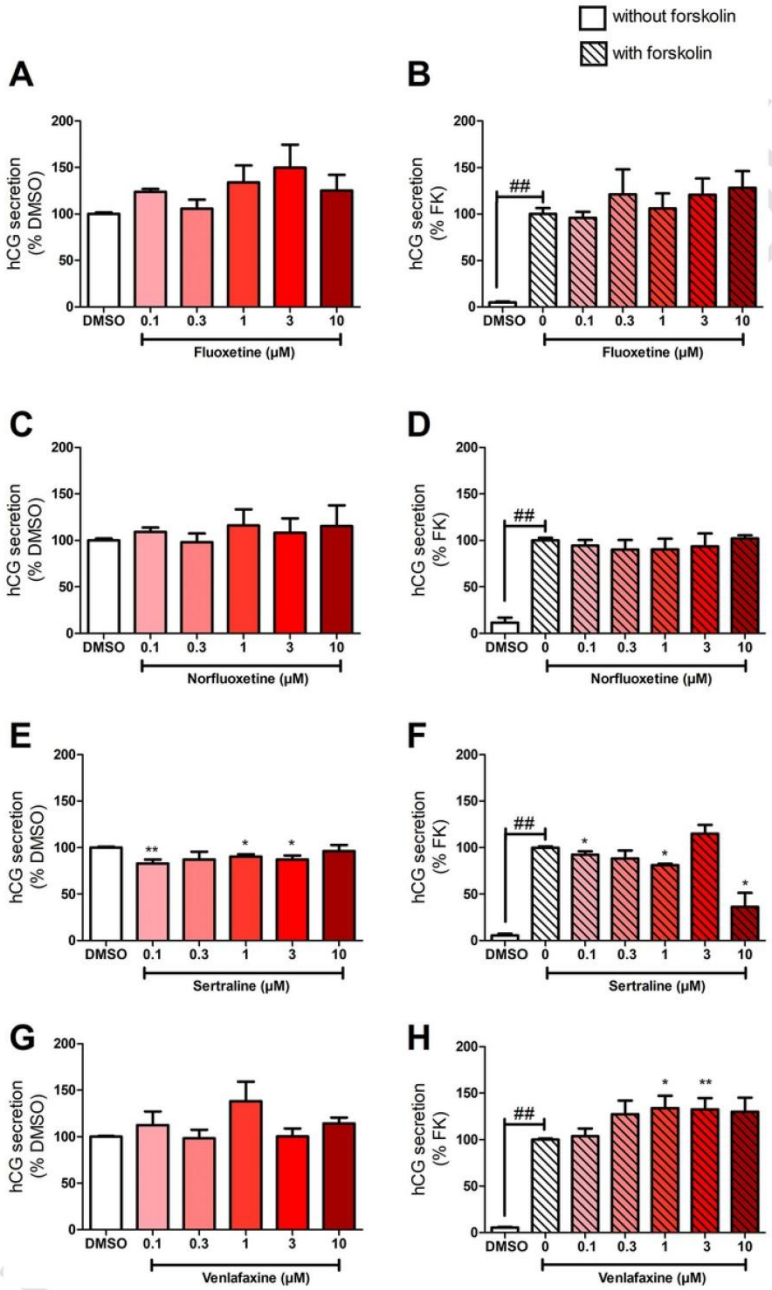

Fig. 4. Effects of fluoxetine, norfluoxetine, sertraline and venlafaxine on hCG secretion from BeWo cells. After 24 h in culture, cells were treated for 48 h with increasing SSRI concentrations or vehicle control (0.1% DMSO), in the presence or absence of 20 μM forskolin (FK). The amount (mean ± SEM) of hCG released was expressed as a percentage of DMSO or FK control. (##) A statistically significant difference between DMSO- and forskolin-treated cells (Mann-Whitney *U* test; $P < 0.01$). (*, **) A statistically significant difference between SSRI treatment and control (Kruskal-Wallis or Mood's median test; $^{*}P < 0.05$; $^{**}P < 0.01$). Each experiment was performed three times; per experiment each treatment was tested in triplicate.

variety of transporters and receptors other than their common SERT target, possibly explaining their differential effects found in trophoblast cells.

Our study highlights the differences between BeWo and primary trophoblast cells in their response to SSRIs. The BeWo cell line has been used extensively as a model of the villous cytotrophoblast (Pattillo and Gey, 1968). BeWo cells have the advantages of fusing in response to cAMP, producing hCG and hPL hormones similar to vCTBs, and being easy to manipulate. However, disadvantages are a gene expression profile that is cancerous in nature and insufficiently correlated

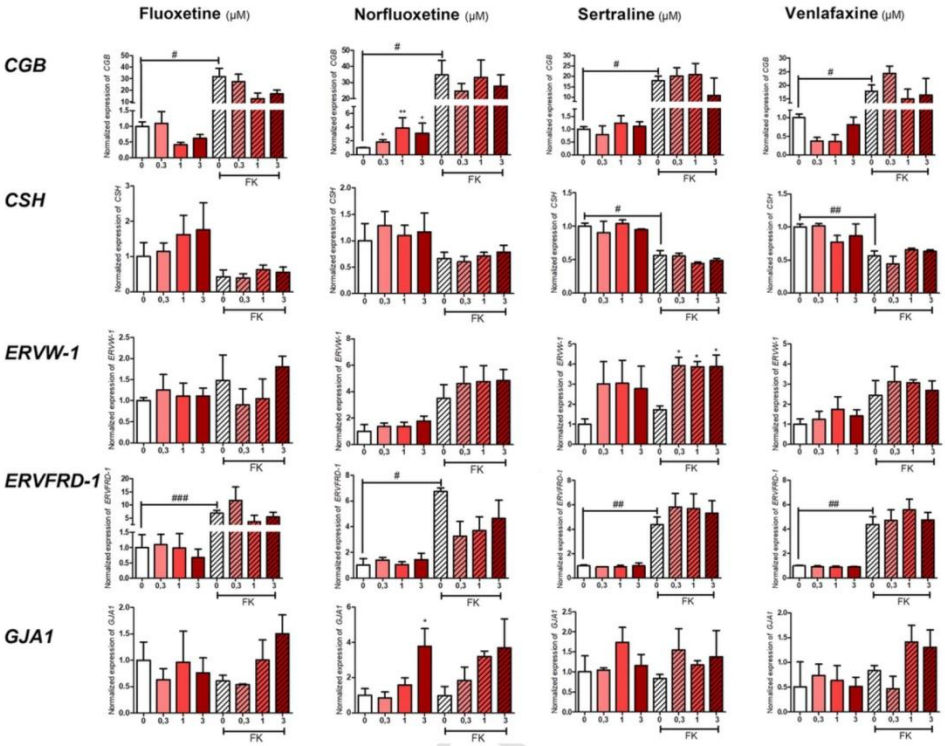

Fig. 5. Effects of fluoxetine, norfluoxetine, sertraline and venlafaxine on the mRNA level of *CGB*, *CSH*, *ERVW-1*, *ERVFRD-1*, *GJA1* genes in BeWo cells. After 24h in culture, cells were treated for 48h with increasing SSRI concentrations or vehicle control (0.1% DMSO), in the presence or absence of 20μM forskolin (FK). Levels of mRNA expression (mean±SEM) were determined by RT-qPCR using peptidylprolyl isomerase A (*PPIA*), topoisomerase 1 (*TOP-1*) and succinate dehydrogenase complex subunit A (*SDHA*) as reference genes. (#, ##, ###) A statistically significant difference between DMSO control and FK (Student t or Mann Whitney *U* test; #P < 0.05; ##P < 0.01; ###P < 0.001). (*, **) A statistically significant difference between SSRI treatment and DMSO or FK control (one-way ANOVA and Dunnett posteriori, Welch's or Kruskal-Wallis test; *P < 0.05; **P < 0.01). Each experiment was performed five times; per experiment each treatment was tested in triplicate.

with that of trophoblast cells in primary culture (Burleigh et al., 2007; Bilban et al., 2010; Novakovic et al., 2011). Isolated primary villous trophoblasts, although more difficult to obtain and manipulate (Sagrillo-Fagundes et al., 2016; Clabault et al., 2018), are physiologically closer to the *in vivo* situation. To better understand the molecular effects of SSRIs in primary trophoblast cells and the signaling pathways involved, further studies are required. These studies should also take into account the fetal sex of the placentas, to delineate possible sex-effects on trophoblast function.

5. Conclusion

This is the first study to show that SSRIs affect trophoblast differentiation. The four SSRIs tested (fluoxetine, norfluoxetine, citalopram and sertraline) did not have overt cytotoxic effects in villous trophoblasts at the therapeutically relevant concentrations tested, but more subtle effects were observed on trophoblast syncytialization, such as cell-cycle and expression of genes involved in the syncytialization process. By modifying syncytialization, SSRIs could alter villous trophoblast homeostasis, potentially altering the oxygen and nutrient exchange between mother and fetus, and the production of pregnancy hormones crucial for fetal development and physiological adaptation of the mother. Our study also shows the necessity to complement experiments using BeWo carcinoma cells with studies in primary villous trophoblasts to obtain results with greater *in vivo* relevance. Considering the increasing number of pregnant women taking SSRIs, it is of great importance to better understand the effects of SSRIs on placental development and function, in order to make improved, scientifically informed decisions on antidepressant therapies during pregnancy.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.taap.2018.04.018.

Conflict of interest

The authors have declared that no conflict of interest exists.

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Disclosure statement and conflict of interest

The authors declare no conflict of interest and have nothing to disclose.

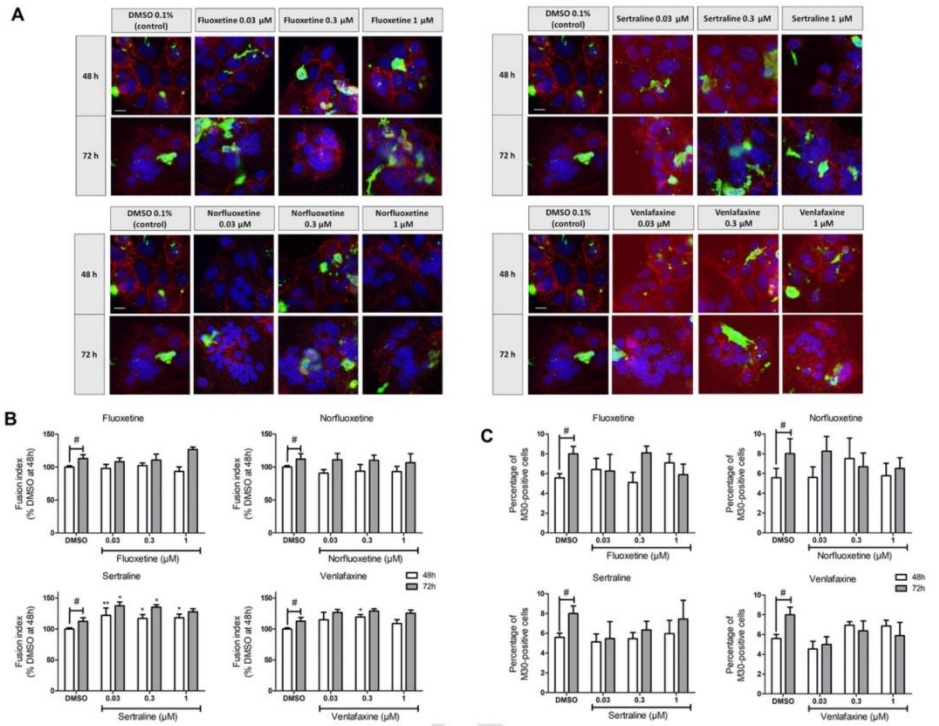

Fig. 6. Effects of fluoxetine, norfluoxetine, sertraline and venlafaxine on human normal term villous cytotrophoblast fusion. After 18h in culture, primary villous trophoblasts were treated for 48 or 72h with increasing SSRI concentrations or vehicle control (0.1% DMSO). (A) Representative immunofluorescence: cells were fixed in methanol, stained with anti-desmoplakin for membranes (red) and M30 CytoDEATH antibody for apoptotic cells (green), counterstained with DAPI (blue), and observed by confocal microscopy. Magnifications are 40×. Scale bar: 10µm. (B) Cellular fusion index was determined by counting about 200 cell nuclei per well and expressed as the percentage of nuclei comprised in syncytia (mean±SEM). (#) A statistically significant difference between vehicle control cells exposed to 0.1% DMSO for 48h or 72h (Student *t*-test; #$P < 0.05$); (*, **) A statistically significant difference between cells exposed to SSRIs and their respective DMSO control at either 48h or 72h (one-way ANOVA and Dunnett posteriori test; *$P < 0.05$; **$P < 0.01$). (C) Cytokeratin 18 neoepitope expression was quantified by counting M30-positive cells and presented as a percentage of the total cell number (mean±SEM). (#) A statistically significant difference between vehicle control cells exposed to 0.1% DMSO for 48h or 72h (Student *t*-test; #$P < 0.05$). No statistically significant difference was detected between cells exposed to SSRIs and their respective DMSO control at either 48h or 72h (one-way ANOVA or Welch test). Each experiment was performed five times; per experiment each treatment was tested in triplicate. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Role of the funding source

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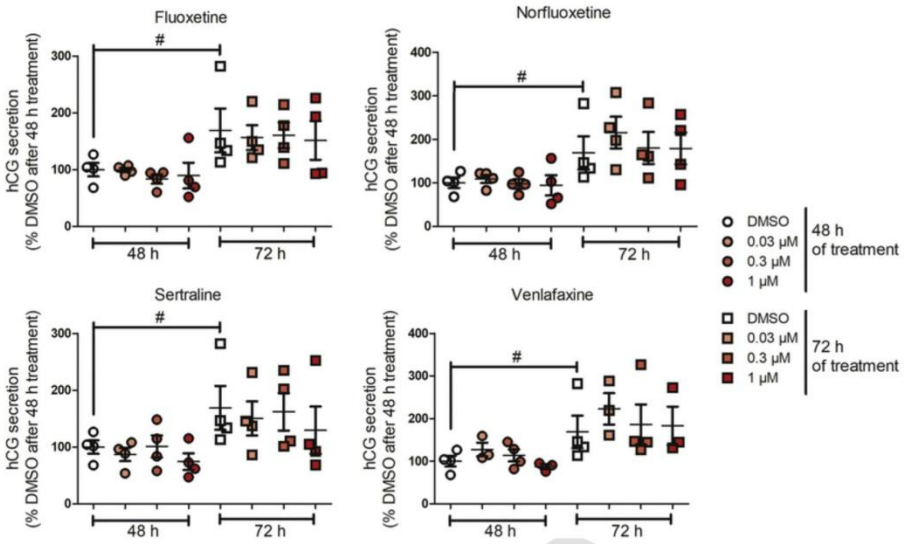


Fig. 7. Effects of fluoxetine, norfluoxetine, sertraline and venlafaxine on the hCG secretion from human normal term villous trophoblast cells. After 18 h in primary culture, trophoblast cells were exposed for 48 h and 72 h to increasing SSRI concentrations or vehicle control (0.1% DMSO). All treatments were replenished daily in fresh medium. Levels of hCG (mean ± SEM; n = 3–4) are expressed as a percentage of DMSO control. (#) A statistically significant difference between vehicle control cells exposed to 0.1% DMSO for 48 h or 72 h (Mann-Whitney *U* test; #$P < 0.05$). No statistically significant difference was detected between cells exposed to SSRIs and their respective DMSO control at either 48 h or 72 h (Kruskal-Wallis test).

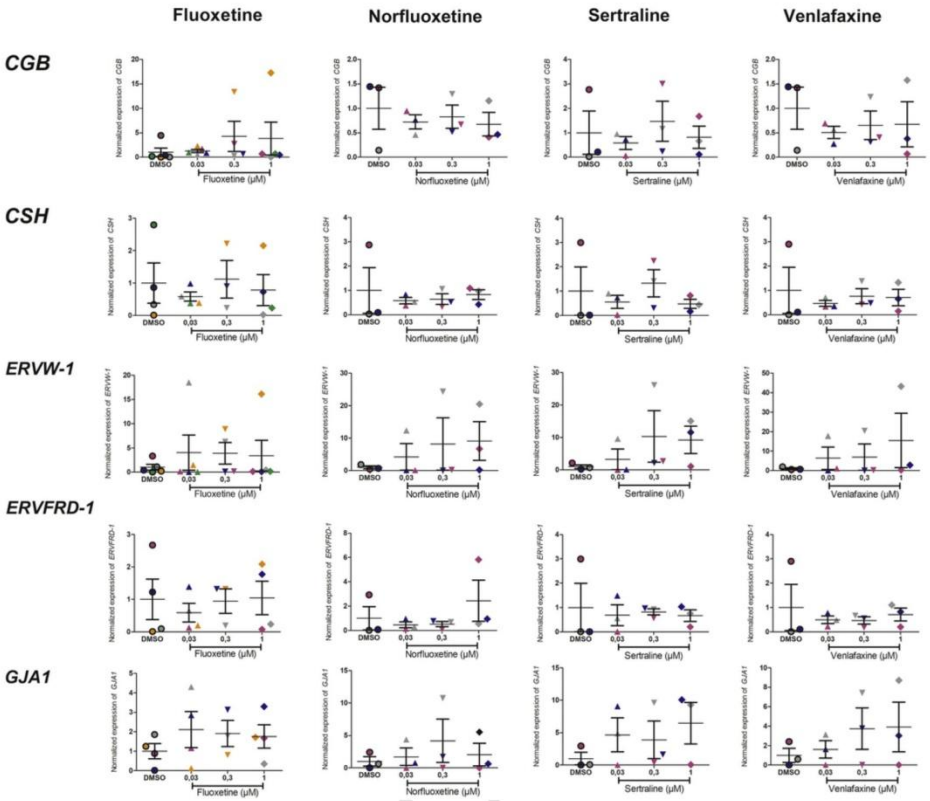


Fig. 8. Effects of fluoxetine, norfluoxetine, sertraline and venlafaxine on the mRNA levels of *CGB*, *CSH*, *ERVW-1*, *ERVFRD-1*, and *GJA1* genes levels in human normal term villous trophoblasts. Each placenta is indicated by a different colour. After 18h in primary culture, trophoblast cells were exposed for 72h to increasing SSRI concentrations or vehicle control (0.1% DMSO). Levels of mRNA (mean±SEM) were determined by RT-qPCR using peptidylprolyl isomerase A (*PPIA*) and topoisomerase 1 (*TOP-1*) as reference genes Between 3 and 5 placentas were used; per placenta each treatment was tested in triplicate. No statistically significant difference was detected between SSRI treatment and DMSO control (Kruskal-Wallis test).

Table 2
Summary of results.

		Fluoxetine	Norfluoxetine	Sertraline	Venlafaxine
Proliferation	BeWo	/	/	Tends to decrease at 10 µM	/
Cell cycle	BeWo	↑G0-G1 and ↓G2-M after 24h	G2-M after 24h	↑G0-G1 and ↓G2-M after 24h	/
M30-positive cells (apoptosis)	Primary cells	/	/	/	/
Cell fusion	BeWo	/	/	NT	NT
	Primary cells	/	/	↑ at 0.03, 0.3, 1 µM after 48h and 72h	↑ at 0.3 µM after 48h
hCG secretion	BeWo	/	/	↓ at 0.1, 1, 3 µM without FK, and ↓ at 0.1, 1, 10 µM with FK after 48h	↑ at 1 and 3 µM with FK after 48h
	Primary cells	/	/	/	/
mRNA expression levels	BeWo	/	↑ *CGB* at 0.3, 1, 3 µM without FK and ↑ *GJA1* at 3 µM without FK after 48h	↑ *ERVW-1* at 0.3, 1, 3 µM with FK after 48h	/
	Primary cells	/	/	/	/

/: no observed effect, FK: forskolin, NT: not tested, *CGB*: chorionic gonadotropin beta, *ERVW-1*: endogenous retrovirus group W member 1, and *GJA1*: gap junction protein alpha 1.

References

Adibi, J.J., Whyatt, R.M., Hauser, R., Bhat, H.K., Davis, B.J., Calafat, A.M., Hoepner, L.A., Perera, F.P., Tang, D., Williams, P.L., 2010. Transcriptional biomarkers of steroidogenesis and trophoblast differentiation in the placenta in relation to prenatal phthalate exposure. Environ. Health Perspect. 118, 291–296.

Albrecht, E.D., Pepe, G.J., 1990. Placental steroid hormone biosynthesis in primate pregnancy. Endocr. Rev. 11, 124–150.

Alsat, E., Haziza, J., Evain-Brion, D., 1993. Increase in epidermal growth factor receptor and its messenger ribonucleic acid levels with differentiation of human trophoblast cells in culture. J. Cell. Physiol. 154, 122–128.

Alsat, E., Malassiné, A., Tarrade, A., Merviel, P., Evain-Brion, D., 1999. Le cytotrophoblaste humain, un casse-tête pour le biologiste. Med. Sci. (Paris) 15, 1236–1243.

Alwan, S., Reefhuis, J., Rasmussen, S.A., Olney, R.S., Friedman, J.M., 2007. Use of selective serotonin-reuptake inhibitors in pregnancy and the risk of birth defects. N. Engl. J. Med. 356, 2684–2692.

Alwan, S., Reefhuis, J., Rasmussen, S.A., Friedman, J.M., 2011. Patterns of antidepressant medication use among pregnant women in a United States population. J. Clin. Pharmacol. 51, 264–270.

Andrade, S.E., Raebel, M.A., Brown, J., Lane, K., Livingston, J., Boudreau, D., Rolnick, S.J., Roblin, D., Smith, D.H., Willy, M.E., Staffa, J.A., Platt, R., 2008. Use of antidepressant medications during pregnancy: a multisite study. Am. J. Obstet. Gynecol. 198 (194), e191–195.

Aplin, J.D., 1991. Implantation, trophoblast differentiation and haemochorial placentation: mechanistic evidence in vivo and in vitro. J. Cell Sci. 99 (Pt 4), 681–692.

Bennett, H.A., Einarson, A., Taddio, A., Koren, G., Einarson, T.R., 2004. Depression during pregnancy : overview of clinical factors. Clin. Drug Investig. 24, 157–179.

Bennett, H.A., Einarson, A., Taddio, A., Koren, G., Einarson, T.R., 2004. Prevalence of depression during pregnancy: systematic review. Obstet. Gynecol. 103, 698–709.

Bilban, M., Tauber, S., Haslinger, P., Pollheimer, J., Saleh, L., Pehamberger, H., Wagner, O., Knofler, M., 2010. Trophoblast invasion: assessment of cellular models using gene expression signatures. Placenta 31, 989–996.

Balkovetz, D.F., Tiruppathi, C., Leibach, F.H., Mahesh, V.B., Ganapathy, V., 1989. Evidence for an imipramine-sensitive serotonin transporter in human placental brush-border membranes. J. Biol. Chem. 264, 2195–2198.

Bolte, A.C., Van Geijn, H.P., Dekker, G.A., 2001. Pathophysiology of preeclampsia and the role of serotonin. Eur. J. Obstet. Gynecol. Reprod. Biol. 95, 12–21.

Bonnin, A., Goeden, N., Chen, K., Wilson, M.L., King, J., Shih, J.C., Blakely, R.D., Deneris, E.S., Levitt, P., 2011. A transient placental source of serotonin for the fetal forebrain. Nature 472, 347–350.

Boukhris, T., Sheehy, O., Mottron, L., Berard, A., 2016. Antidepressant use during pregnancy and the risk of autism spectrum disorder in children. JAMA Pediatr. 170, 117–124.

Burleigh, D.W., Kendziorski, C.M., Choi, Y.J., Grindle, K.M., Grendell, R.L., Magness, R.R., Golos, T.G., 2007. Microarray analysis of BeWo and JEG3 trophoblast cell lines: identification of differentially expressed transcripts. Placenta 28, 383–389.

Byatt, N., Deligiannidis, K.M., Freeman, M.P., 2013. Antidepressant use in pregnancy: a critical review focused on risks and controversies. Acta Psychiatr. Scand. 127, 94–114.

Clabault, H., Laurent, L., Sanderson, J.T., Vaillancourt, C., 2018. Isolation and purification of villous cytotrophoblast cells from term human placenta. Methods Mol. Biol. 1710, 219–231.

Chambers, C.D., Johnson, K.A., Dick, L.M., Felix, R.J., Jones, K.L., 1996. Birth outcomes in pregnant women taking fluoxetine. N. Engl. J. Med. 335, 1010–1015.

Chang, E.A., Beyhan, Z., Yoo, M.S., Siripattarapravat, K., Ko, T., Lookingland, K.J., Madhukar, B.V., Cibelli, J.B., 2010. Increased cellular turnover in response to fluoxetine in neuronal precursors derived from human embryonic stem cells. Int. J. Dev. Biol. 54, 707–715.

Charlton, R.A., Jordan, S., Pierini, A., Garne, E., Neville, A.J., Hansen, A.V., Gini, R., Thayer, D., Tingay, K., Puccini, A., Bos, H.J., Nybo Andersen, A.M., Sinclair, M., Dolk, H., de Jong-van den Berg, L., 2015. Selective serotonin reuptake inhibitor prescribing before, during and after pregnancy: a population-based study in six European regions. BJOG 122, 1010–1020.

Chollangi, T., Clabault, H., Thibeault, A.H., Yong, H.E.J., Narula, S., Menkhorst, E., Sanderson, J.T., Vaillancourt, C., Murthi, P., 2018. An electrical impedance-based assay to examine functions of various placental cell types in vitro. Methods Mol. Biol. 1710, 267–276.

Cikos, S., Fabian, D., Makarevich, A.V., Chrenek, P., Koppel, J., 2011. Biogenic monoamines in preimplantation development. Hum. Reprod. 26, 2296–2305.

Daoud, G., Rassart, E., Masse, A., Lafond, J., 2006. Src family kinases play multiple roles in differentiation of trophoblasts from human term placenta. J. Physiol. 571, 537–553.

De Ocampo, M.P., Araneta, M.R., Macera, C.A., Alcaraz, J.E., Moore, T.R., Chambers, C.D., 2016. Risk of gestational hypertension and preeclampsia in women who discontinued or continued antidepressant medication use during pregnancy. Arch. Womens Ment. Health 19, 1051–1061.

Desforges, M., Sibley, C.P., 2010. Placental nutrient supply and fetal growth. Int. J. Dev. Biol. 54, 377–390.

Eke, A., Saccone, G., Berghella, V., **123**, 20016, 1900-1907. Selective serotonin reuptake inhibitor (SSRI) use during pregnancy and risk of preterm birth: a systematic review and meta-analysis. BJOG

Filippi, V., Chou, D., Ronsmans, C., Graham, W., Say, L., 2016. Levels and causes of maternal mortality and morbidity. In: Black, R.E., Laxminarayan, R., Temmerman, M., Walker, N. (Eds.), Reproductive, Maternal, Newborn, and Child Health: Disease Control Priorities, Third Edition (Volume 2). 2016 International Bank for Reconstruction and Development. The World Bank, Washington DC.

Fournier, T., 2016. Human chorionic gonadotropin: different glycoforms and biological activity depending on its source of production. Ann. Endocrinol. (Paris) 77, 75–81.

Frendo J.-L., Cronier, L., Bertin, G., Guibourdenche, J., Vidaud, M., Evain-Brion, D., Malassine, A., 2003a. Involvement of connexin 43 in human trophoblast cell fusion and differentiation. J. Cell Sci. 116, 3413–3421.

Frendo, J.-L., Olivier, D., Cheynet, V., Blond, J.-L., Bouton, O., Vidaud, M., Rabreau, M., Evain-Brion, D., Mallet, F., 2003b. Direct involvement of HERV-W Env glycoprotein in human trophoblast cell fusion and differentiation. Mol. Cell. Biol. 23, 3566–3574.

Garcia-Lloret, M.I., Yui, J., Winkler-Lowen, B., Guilbert, L.J., 1996. Epidermal growth factor inhibits cytokine-induced apoptosis of primary human trophoblasts. J. Cell. Physiol. 167, 324–332.

Gauster, M., Huppertz, B., 2008. Fusion of cytotrophoblast with syncytiotrophoblast in the human placenta: factors involved in syncytialization. J. Reproduktionsmed. Endokrinol. 5, 76–82.

Gavin, N.I., Gaynes, B.N., Lohr, K.N., Meltzer-Brody, S., Gartlehner, G., Swinson, T., 2005. Perinatal depression: a systematic review of prevalence and incidence. Obstet. Gynecol. 106, 1071–1083.

Gentile, S., 2014. Tricyclic antidepressants in pregnancy and puerperium. Expert Opin. Drug Saf. 13, 207–225.

Gentile, S., 2015. Untreated depression during pregnancy: Short- and long-term effects in offspring. A systematic review. Neuroscience.

Ghosh, C., Hossain, M., Spriggs, A., Ghosh, A., Grant, G.A., Marchi, N., Perucca, E., Janigro, D., 2015. Sertraline-induced potentiation of the CYP3A4-dependent neurotoxicity of carbamazepine: an in vitro study. Epilepsia 56, 439–449.

Grote, N.K., Bridge, J.A., Gavin, A.R., Melville, J.L., Iyengar, S., Katon, W.J., 2010. A meta-analysis of depression during pregnancy and the risk of preterm birth, low birth weight, and intrauterine growth restriction. Arch. Gen. Psychiatry 67, 1012–1024.

Gundacker, C., Neesen, J., Straka, E., Ellinger, I., Dolznig, H., Hengstschlager, M., 2016. Genetics of the human placenta: implications for toxicokinetics. Arch. Toxicol. 90, 2563–2581.

Hadden, C., Fahmi, T., Cooper, A., Savenka, A.V., Lupashin, V.V., Roberts, D.J., Maroteaux, L., Hauguel-de Mouzon, S., Kilic, F., **232**, 2017, 3520-3529. Serotonin transporter protects the placental cells against apoptosis in caspase 3-independent pathway. J. Cell. Physiol.

Handwerger, S., 2010. New insights into the regulation of human cytotrophoblast cell differentiation. Mol. Cell. Endocrinol. 323, 94–104.

Hendrick, V., Stowe, Z.N., Altshuler, L.L., Hwang, S., Lee, E., Haynes, D., 2003. Placental passage of antidepressant medications. Am. J. Psychiatry 160, 993–996.

Hodge, J.M., Wang, Y., Berk, M., Collier, F.M., Fernandes, T.J., Constable, M.J., Pasco, J.A., Dodd, S., Nicholson, G.C., Kennedy, R.L., Williams, L.J., 2013. Selective serotonin reuptake inhibitors inhibit human osteoclast and osteoblast formation and function. Biol. Psychiatry 74, 32–39.

Hoshina, M., Hussa, R., Pattillo, R., Camel, H.M., Boime, I., 1984. The role of trophoblast differentiation in the control of the hCG and hPL genes. Adv. Exp. Med. Biol. 176, 299–312.

Huang, H., Coleman, S., Bridge, J.A., Yonkers, K., Katon, W., 2014. A meta-analysis of the relationship between antidepressant use in pregnancy and the risk of preterm birth and low birth weight. Gen. Hosp. Psychiatry 36, 13–18.

Hudon Thibeault, A.A., Laurent, L., Duy, S.V., Sauve, S., Caron, P., Guillemette, C., Sanderson, J.T., Vaillancourt, C., **442**, 2016, 32-39. Fluoxetine and its active metabolite norfluoxetine disrupt estrogen synthesis in a co-culture model of the feto-placental unit. Mol. Cell. Endocrinol.

Hughes, D.A., Kircher, M., He, Z., Guo, S., Fairbrother, G.L., Moreno, C.S., Khaitovich, P., Stoneking, M., 2015. Evaluating intra- and inter-individual variation in the human placental transcriptome. Genome Biol. 16, 54.

Hui, J., Zhang, J., Kim, H., Tong, C., Ying, Q., Li, Z., Mao, X., Shi, G., Yan, J., Zhang, Z., Xi, G., 2014. Fluoxetine regulates neurogenesis in vitro through modulation of GSK-3beta/beta-catenin signaling. Int. J. Neuropsychopharmacol. 18.

Huppertz, B., Tews, D.S., Kaufmann, P., 2001. Apoptosis and syncytial fusion in human placental trophoblast and skeletal muscle. Int. Rev. Cytol. 205, 215–253.

Jacobsen, N.W., Hansen, C.H., Nellemann, C., Styrishave, B., Halling-Sorensen, B., 2015. Effects of selective serotonin reuptake inhibitors on three sex steroids in two versions of the aromatase enzyme inhibition assay and in the H295R cell assay. Toxicol. in Vitro 29, 1729–1735.

Ji, L., Brkic, J., Liu, M., Fu, G., Peng, C., Wang, Y.L., **34**, 2012, 981-1023 . Placental trophoblast cell differentiation: physiological regulation and pathological relevance to preeclampsia. Mol. Asp. Med.

Jordan, J.A., Butchko, A.R., 2002. Apoptotic activity in villous trophoblast cells during B19 infection correlates with clinical outcome: assessment by the caspase-related M30 Cytodeath antibody. Placenta 23, 547–553.

Kannen, V., Garcia, S.B., Silva Jr, W.A., Gasser, M., Monch, R., Alho, E.J., Heinsen, H., Scholz, C.J., Friedrich, M., Heinze, K.G., Waaga-Gasser, A.M., Stopper, H., 2015. Oncostatic effects of fluoxetine in experimental colon cancer models. Cell. Signal. 27, 1781–1788.

Kliman, H.J., Nestler, J.E., Sermasi, E., Sanger, J.M., Strauss 3rd, J.F., 1986. Purification, characterization, and in vitro differentiation of cytotrophoblasts from human term placentae. Endocrinology 118, 1567–1582.

Krishnan, A., Hariharan, R., Nair, S.A., Pillai, M.R., 2008. Fluoxetine mediates G0/G1 arrest by inducing functional inhibition of cyclin dependent kinase subunit (CKS)1. Biochem. Pharmacol. 75, 1924–1934.

Kulin, N.A., Pastuszak, A., Sage, S.R., Schick-Boschetto, B., Spivey, G., Feldkamp, M., Ormond, K., Matsui, D., Stein-Schechman, A.K., Cook, L., Brochu, J., Rieder, M., Koren, G., 1998. Pregnancy outcome following maternal use of the new selective serotonin reuptake inhibitors: a prospective controlled multicenter study. JAMA 279, 609–610.

Kumar, K., Gupta, M., 2014. Clinical and socio-demographic determinants of psychological health and burden in family caregivers of patients with unipolar depression. Asian J. Psychiatr. 9, 51–56.

Kwak, S.H., Park, B.L., Kim, H., German, M.S., Go, M.J., Jung, H.S., Koo, B.K., Cho, Y.M., Choi, S.H., Cho, Y.S., Shin, H.D., Jang, H.C., Park, K.S., 2012. Association of variations in TPH1 and HTR2B with gestational weight gain and measures of obesity. Obesity (Silver Spring) 20, 233–238.

Kwiecinska, P., Wisniewska, J., Gregoraszczuk, E.L., 2011. Effects of valproic acid (VPA) and levetiracetam (LEV) on proliferation, apoptosis and hormone secretion of the human choriocarcinoma BeWo cell line. Pharmacol. Rep. 63, 1195–1202.

Lanoix, D., Beghdadi, H., Lafond, J., Vaillancourt, C., 2008. Human placental trophoblasts synthesize melatonin and express its receptors. J. Pineal Res. 45, 50–60.

Lanoix, D., Lacasse, A.A., St-Pierre, J., Taylor, S.C., Ethier-Chiasson, M., Lafond, J., Vaillancourt, C., **52**, 2012, 234-243. Quantitative PCR pitfalls: the case of the human placenta. Mol. Biotechnol.

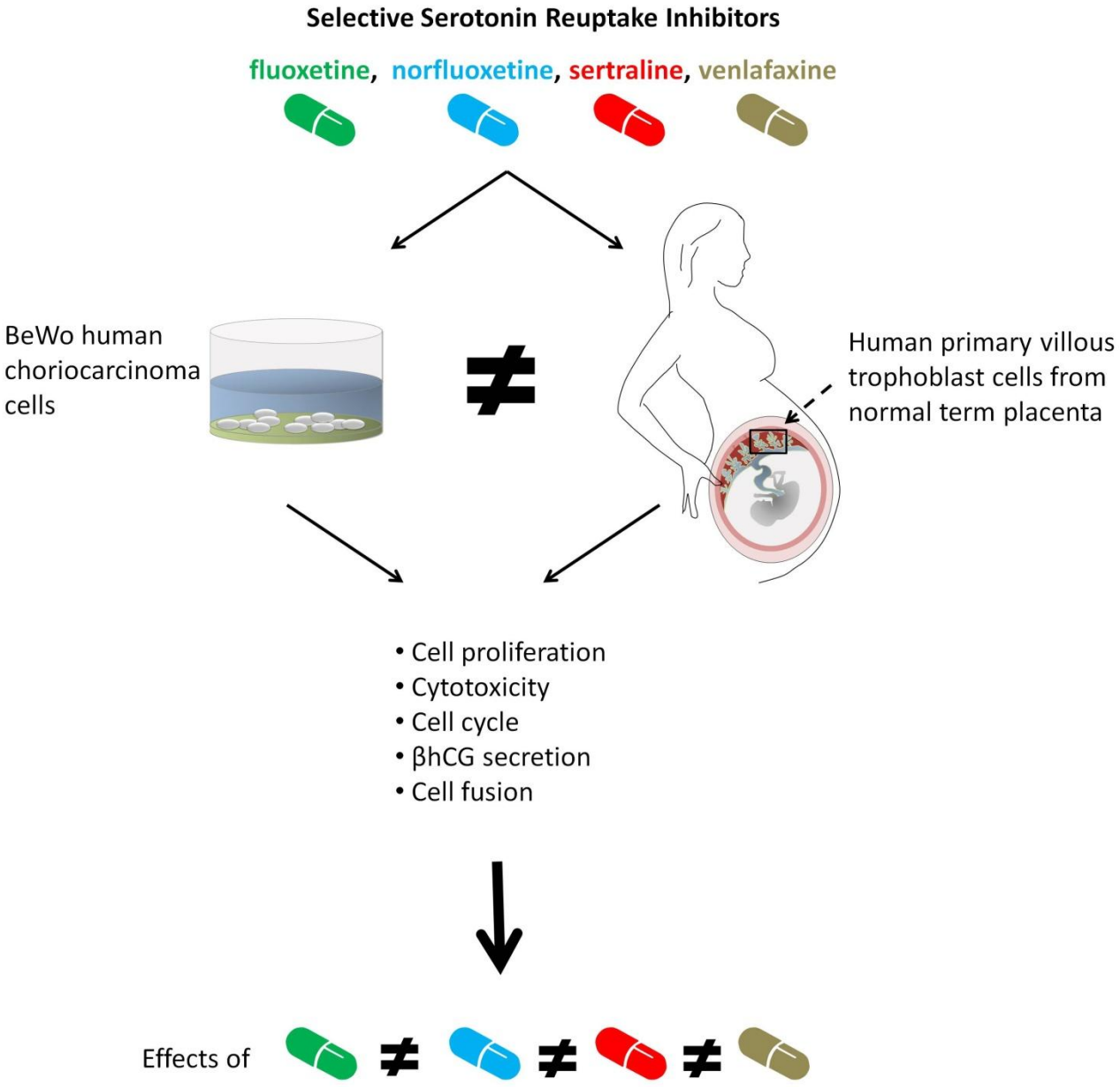
Laurent, L., Huang, C., Ernest, S.R., Berard, A., Vaillancourt, C., Hales, B.F., 2016. In utero exposure to venlafaxine, a serotonin-norepinephrine reuptake inhibitor, in-

creases cardiac anomalies and alters placental and heart serotonin signaling in the rat. Birth Defects Res. A Clin. Mol. Teratol.
Laurent, L., Deroy, K., St-Pierre, J., Cote, F., Sanderson, J.T., Vaillancourt, C., **140**, 2017, 159-165. Human placenta expresses both peripheral and neuronal isoform of tryptophan hydroxylase. Biochimie
Lu, X., Wang, R., Zhu, C., Wang, H., Lin, H.Y., Gu, Y., Cross, J.C., Wang, H., 2017. Fine-tuned and cell-cycle-restricted expression of Fusogenic protein Syncytin-2 maintains functional placental syncytia. Cell Rep. 21, 1150–1159.
Malassine, A., 2001. Morphological variability and placental function. Gynecol. Obstet. Fertil. 29, 489–496.
Montgomery, S.A., 2008. Tolerability of serotonin norepinephrine reuptake inhibitor antidepressants. CNS Spectr. 13, 27–33.
Morrish, D.W., Bhardwaj, D., Dabbagh, L.K., Marusyk, H., Siy, O., 1987. Epidermal growth factor induces differentiation and secretion of human chorionic gonadotropin and placental lactogen in normal human placenta. J. Clin. Endocrinol. Metab. 65, 1282–1290.
Novakovic, B., Gordon, L., Wong, N.C., Moffett, A., Manuelpillai, U., Craig, J.M., Sharkey, A., Saffery, R., 2011. Wide-ranging DNA methylation differences of primary trophoblast cell populations and derived cell lines: implications and opportunities for understanding trophoblast function. Mol. Hum. Reprod. 17, 344–353.
Omata, W., Ackerman, W.E., Vandre, D.D., Robinson, J.M., 2013. Trophoblast cell fusion and differentiation are mediated by both the protein kinase C and a pathways. PLoS One 8, e81003.
Oufkir, T., Arseneault, M., Sanderson, J.T., Vaillancourt, C., 2010. The 5-HT 2A serotonin receptor enhances cell viability, affects cell cycle progression and activates MEK-ERK1/2 and JAK2-STAT3 signalling pathways in human choriocarcinoma cell lines. Placenta 31, 439–447.
Pattillo, R.A., Gey, G.O., 1968. The establishment of a cell line of human hormone-synthesizing trophoblastic cells in vitro. Cancer Res. 28, 1231–1236.
Peng, Z.W., Xue, Y.Y., Wang, H.N., Wang, H.H., Xue, F., Kuang, F., Wang, B.R., Chen, Y.C., Zhang, L.Y., Tan, Q.R., 2012. Sertraline promotes hippocampus-derived neural stem cells differentiating into neurons but not glia and attenuates LPS-induced cellular damage. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 36, 183–188.
Pepe, G.J., Albrecht, E.D., 1995. Actions of placental and fetal adrenal steroid hormones in primate pregnancy. Endocr. Rev. 16, 608–648.
Perry, L.M., Goldstein-Piekarski, A.N., Williams, L.M., 2017. Sex differences modulating serotonergic polymorphisms implicated in the mechanistic pathways of risk for depression and related disorders. J. Neurosci. Res. 95, 737–762.
Pidoux, G., Gerbaud, P., Laurendeau, I., Guibourdenche, J., Bertin, G., Vidaud, M., Evain-Brion, D., Frendo, J.L., 2004. Large variability of trophoblast gene expression within and between human normal term placentae. Placenta 25, 469–473.
Pidoux, G., Gerbaud, P., Gnidehou, S., Grynberg, M., Geneau, G., Guibourdenche, J., Carette, D., Cronier, L., Evain-Brion, D., Malassine, A., Frendo, J.L., 2010. ZO-1 is involved in trophoblastic cell differentiation in human placenta. Am. J. Physiol. Cell Physiol. 298, C1517–1526.
Ram, D., Gandotra, S., 2015. Antidepressants, anxiolytics, and hypnotics in pregnancy and lactation. Indian J. Psychiatry 57, S354–371.
Ramos, E., St-Andre, M., Berard, A., 2010. Association between antidepressant use during pregnancy and infants born small for gestational age. Can. J. Psychiatr. 55, 643–652.
Sabolovic Rudman, S., Mustapic, M., Kosec, V., Pivac, N., Rudman, F., Muck-Seler, D., 2015. Serotonin risk factors for the development of hypertension in pregnancy. Arch. Gynecol. Obstet. 291, 779–785.
Sagrillo-Fagundes, L., Clabault, H., Laurent, L., Hudon-Thibeault, A.A., Salustiano, E.M., Fortier, M., Bienvenue-Pariseault, J., Wong Yen, P., Sanderson, J.T., Vaillancourt, C., (113), 2016. Human primary trophoblast cell culture model to study the protective effects of melatonin against hypoxia/reoxygenation-induced disruption. J. Vis. Exp.
Saryu Malhotra, S., Suman, P., Kumar Gupta, S., 2015. Alpha or beta human chorionic gonadotropin knockdown decrease BeWo cell fusion by down-regulating PKA and CREB activation. Sci. Rep. 5, 11210.
Schmidt, L., Baskaran, S., Johansson, P., Padhan, N., Matuszewski, D., Green, L.C., Elfineh, L., Wee, S., Haggblad, M., Martens, U., Westermark, B., Forsberg-Nilsson, K., Uhrbom, L., Claesson-Welsh, L., Andang, M., Sintorn, I.M., Lundgren, B., Lonnstedt, I., Krona, C., Nelander, S., 2016. Case-specific potentiation of glioblastoma drugs by pterostilbene. Oncotarget 7, 73200–73215.
Singh, A.T., Dharmarajan, A., Aye, I.L., Keelan, J.A., 2012. Sphingosine-sphingosine-1-phosphate pathway regulates trophoblast differentiation and syncytialization. Reprod. BioMed. Online 24, 224–234.
Sit, D., Perel, J.M., Wisniewski, S.R., Helsel, J.C., Luther, J.F., Wisner, K.L., 2011. Mother-infant antidepressant concentrations, maternal depression, and perinatal events. J. Clin. Psychiatry 72, 994–1001.
Sonier, B., Lavigne, C., Arseneault, M., Ouellette, R., Vaillancourt, C., 2005. Expression of the 5-HT2A serotoninergic receptor in human placenta and choriocarcinoma cells: mitogenic implications of serotonin. Placenta 26, 484–490.
, . tSci. Rep.
Sun, B.K., Kim, J.H., Choi, J.S., Hwang, S.J., Sung, J.H., 2015. Fluoxetine decreases the proliferation and Adipogenic differentiation of human adipose-derived stem cells. Int. J. Mol. Sci. 16, 16655–16668.
Toufaily, C., Vargas, A., Lemire, M., Lafond, J., Rassart, E., Barbeau, B., 2013. MFSD2a, the Syncytin-2 receptor, is important for trophoblast fusion. Placenta 34, 85–88.
Vaillancourt, C., Lanoix, D., Le Bellego, F., Daoud, G., Lafond, J., 2009. Involvement of MAPK signalling in human villous trophoblast differentiation. Mini Rev. Med. Chem. 9, 962–973.
Vargas, A., Moreau, J., Landry, S., LeBellego, F., Toufaily, C., Rassart, E., Lafond, J., Barbeau, B., 2009. Syncytin-2 plays an important role in the fusion of human trophoblast cells. J. Mol. Biol. 392, 301–318.
Viau, M., Lafond, J., Vaillancourt, C., 2009. Expression of placental serotonin transporter and 5-HT 2A receptor in normal and gestational diabetes mellitus pregnancies. Reprod. BioMed. Online 19, 207–215.
Vignery, A., 2000. Osteoclasts and giant cells: macrophage-macrophage fusion mechanism. Int. J. Exp. Pathol. 81, 291–304.
Wang, J.-m., Zhao, H.-x., Wang, L., Gao, Z.-y., Yao, Y.-q., 2013. The human leukocyte antigen G promotes trophoblast fusion and β-hCG production through the Erk1/2 pathway in human choriocarcinoma cell lines. Biochem. Biophys. Res. Commun. 434, 460–465.
Weikum, W.M., Oberlander, T.F., Hensch, T.K., Werker, J.F., 2012. Prenatal exposure to antidepressants and depressed maternal mood alter trajectory of infant speech perception. Proc. Natl. Acad. Sci. U. S. A. 109 (Suppl. 2), 17221–17227.
Wemakor, A., Casson, K., Garne, E., Bakker, M., Addor, M.C., Arriola, L., Gatt, M., Khoshnood, B., Klungsoyr, K., Nelen, V., O'Mahoney, M., Pierini, A., Rissmann, A., Tucker, D., Boyle, B., de Jong-van den Berg, L., Dolk, H., 2015. Selective serotonin reuptake inhibitor antidepressant use in first trimester pregnancy and risk of specific congenital anomalies: a European register-based study. Eur. J. Epidemiol. 30, 1187–1198.
Yui, J., Garcia-Lloret, M., Brown, A.J., Berdan, R.C., Morrish, D.W., Wegmann, T.G., Guilbert, L.J., 1994. Functional, long-term cultures of human term trophoblasts purified by column-elimination of CD9 expressing cells. Placenta 15, 231–246.

SSRIs were purchased from Sigma-Aldrich (Fig. S1). Norfluoxetine (a metabolite of fluoxetine) and venlafaxine were dissolved in H_2O miliQ; fluoxetine and sertraline in DMSO (Sigma-Aldrich). BeWo and primary trophoblast cells were treated with increasing concentrations of SSRIs (0 to 10 μM) in culture medium. The SSRI concentrations used corresponded to concentrations found in maternal (15–130 nM) and cord blood (65–250 nM) during treatment for depression (Hendrick et al., 2003; Sit et al., 2011). Control cells were exposed to a final concentration of 0.1% DMSO. To induce biochemical and morphological differentiation, BeWo cells were stimulated with 20 μM forskolin (Sigma-Aldrich). Cell culture medium with or without SSRI treatment was changed every 24 h.

Syncytialization is a rare phenomenon in human physiology, undergone by only three cell types: cytotrophoblasts (into syncytiotrophoblast), myoblasts (into myotubes), and monocytes (into osteoclasts or giant cells) (Vignery, 2000; Huppertz et al., 2001). A study of several SSRIs (1 and 10 μM) demonstrated that, with the exception of citalopram, paroxetine fluoxetine and sertraline (in increasing potency) concentration-dependently inhibited osteoclast (OC) formation and resorption in primary human osteoblast and in colony forming unit-granulocyte/macrophage (CFU-GM)-derived OC precursor cells (Hodge et al., 2013). Our present study also found that SSRIs affected BeWo and primary trophoblast cell cycle, proliferation and syncytialization with different potencies dependent on their molecular structures, which are relatively diverse (see Fig. S1). This diversity may result in binding to a variety of transporters and receptors other than their common SERT target, possibly explaining their differential effects found in trophoblast cells.

GRAPHICAL ABSTRACT

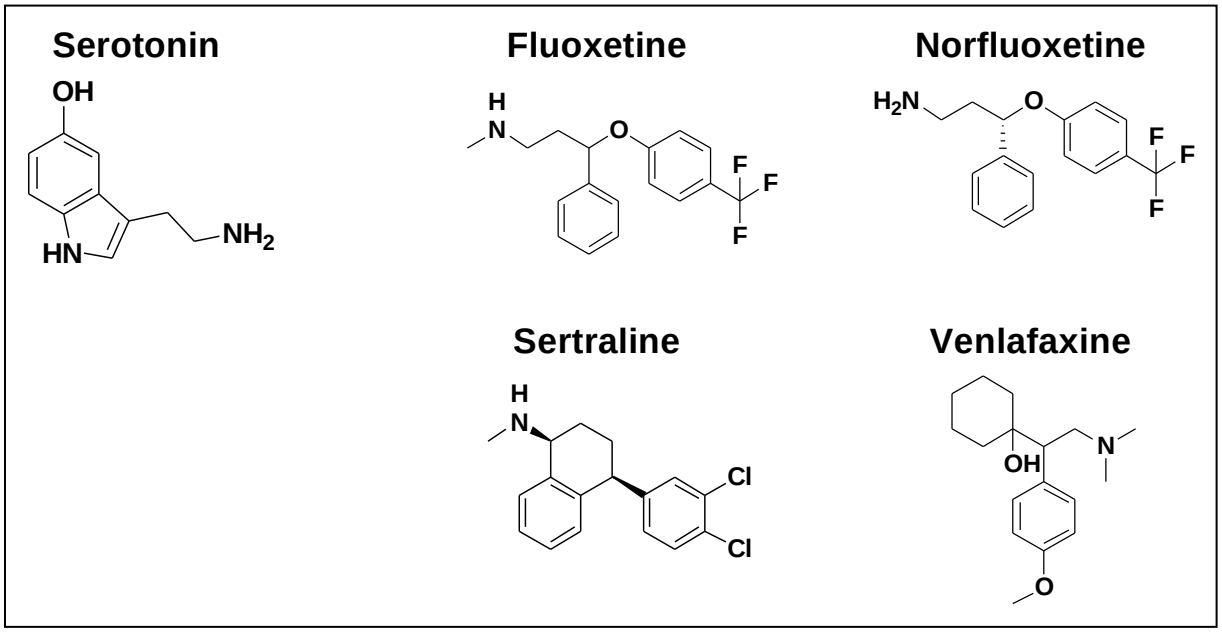


HIGHLIGHTS

- SSRIs affect villous trophoblast syncytialization in a structure- and concentration-dependent manner
- SSRIs affect syncytialization differently in BeWo cells than primary villous trophoblasts
- Sertraline increases morphological differentiation of primary villous trophoblast
- Sertraline at 10 µM affects BeWo proliferation

SUPPLEMENTARY FIGURES

Figure S1: Molecular structures of serotonin and the SSRIs used in the present study.



5 CHAPITRE 5 : EFFECTS OF SELECTIVE SEROTONIN-REUPTAKE INHIBITORS (SSRIs) ON JEG-3 AND HIPEC CELL MODELS OF THE EXTRAVILLOUS TROPHOBLAST

5.1 Présentation de l'article

Titre en français: Effets des antidépresseurs de la classe des inhibiteurs sélectifs de la recapture de la sérotonine (ISRS) sur les cellules JEG-3 et HIPEC, modèles de trophoblastes extravilleux.

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5.2 **Résumé de l'article en français**

La dépression est fréquente durant la grossesse, et de nombreuses femmes enceintes sont traitées par des antidépresseurs de la classe des inhibiteurs sélectifs de la recapture de la sérotonine (ISRS). Les cellules trophoblastiques extravilleuses (CTBev) placentaires migrent et envahissent les tissus maternels ce qui est crucial pour l'implantation embryonnaire et pour le remodelage des artères spiralées maternelles. Une migration/invasion insuffisante des TBev peut provoquer d'importantes complications de grossesse. Néanmoins, l'effet des ISRS sur l'homéostasie des CTBev n'est pas connu. Le but de cette étude est de déterminer les effets des ISRS sur la prolifération, le cycle cellulaire, la migration et les marqueurs d'invasion des cellules JEG-3 et HIPEC, lignées cellulaires de trophoblastes extravilleux. Les cellules JEG-3 et HIPEC ont été traitées avec des concentrations croissantes (0,03 à 10 µM) d'ISRS (fluoxétine, norfluoxétine, citalopram et venlafaxine). L'étude de la prolifération cellulaire et du cycle cellulaire a été réalisée, respectivement, en temps réel en utilisant le système xCELLigence© basé sur la mesure de l'impédance, et par cytométrie de flux. La migration a été déterminée en utilisant un test de cicatrisation. L'activité des métalloprotéinases MMP-2 et MMP-9 (marqueurs d'invasion) a été déterminée par zymographie sur gel de gélatine. La fluoxétine et la sertraline, à 10 µM, ont significativement diminué la prolifération par rapport au témoin véhicule (0,1% de DMSO) des cellules JEG-3 (respectivement par 94,3 % et par 99,6 %) and HIPEC (respectivement par 58,6 % et par 100 %). La norfluoxétine a augmenté l'activité de la MMP-9 par 2,0 % à 0,03 µM et par 43,9 % à 3 µM dans les cellules JEG-3, et diminué l'activité de la MMP-9 par 63,7 % à 3 µM dans les HIPEC. Cette étude suggère que les ISRS ont des effets structure-dépendants, et qu'ils affectent les cellules trophoblastiques extravilleuses JEG-3 et HIPEC à des doses thérapeutiques. Notre étude montre que certains ISRS pourraient affecter l'homéostasie des TBev. Elle est donc une base pour de futures recherches et peut aider à améliorer la prise de décisions cliniques dans le but d'améliorer la santé de la mère ainsi que celle du fœtus.

5.3 **Contribution de l’étudiante**

L’étudiante a participé à l’élaboration de l’étude, réalisé toutes les expériences et analysé les résultats. Elle a par ailleurs rédigé l’article et participé au choix du journal de publication.

5.4 **Article**

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HIGHLIGHTS

- SSRIs disrupt extravillous trophoblast-like cell homeostasis
- Fluoxetine and sertraline reduce JEG-3 and HIPEC cell proliferation
- Norfluoxetine increases MMP-9 activity in JEG-3 and decreases it in HIPEC cells
- Venlafaxine increases HIPEC cell migration and *ADAM10* expression
- Fluoxetine decreases, whereas NF increases JEG-3 cell migration

bEffects of selective serotonin-reuptake inhibitors (SSRIs) in JEG-3 and HIPEC cell models of the extravillous trophoblast

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Short title: SSRI effects on human extravillous trophoblasts

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ABSTRACT

Between 2 and 10% of pregnant women are treated with selective serotonin-reuptake inhibitors (SSRIs) for depression. The extravillous trophoblasts (evTBs), which migrate and invade maternal tissues, are crucial for embryo implantation and remodeling of maternal spiral arteries. Poor migration/invasion of evTBs can cause serious pregnancy complications, yet the effects of SSRIs on these processes has never been studied. To determine the effects of five SSRIs (fluoxetine, norfluoxetine, citalopram, sertraline and venlafaxine) on migration/invasion, we used JEG-3 and HIPEC cells as evTB models. Cells were treated with increasing concentrations (0.03-10 μM) of SSRIs. Cell proliferation was monitored using an impedance-based system and cell cycle by flow cytometry. Migration was determined using a scratch test and metalloproteinase (MMP) activities by zymography. Invasion markers were determined by RT-qPCR. Fluoxetine and sertraline (10 μM) significantly decreased cell proliferation by 94% and by 100%, respectively, in JEG-3 cells and by 58.6% and 100%, respectively, in HIPEC cells. Norfluoxetine increased MMP-9 activity in JEG-3 cells by 2.0% at 0.03 μM and by 43.9% at 3 μM, but decreased MMP-9 activity in HIPEC cells by 63.7% at 3 μM. Sertraline at 0.03 μM increased mRNA level of *TIMP-1* in JEG-3 cells by 36% and that of *ADAM-10* by 85% and 115% at 0.3 and 3 μM, respectively. In HIPEC cells, venlafaxine at 0.03 and 0.3 μM, increased *ADAM-10* mRNA levels by 156% and 167%, respectively. This study shows that SSRIs may affect evTBs homeostasis at therapeutic levels and provides guidance for future research.

KEYWORDS: human, placenta, migration, zymography, proliferation, matrix metalloproteinases, cell cycle, JEG-3, HIPEC.

INTRODUCTION

The extravillous trophoblast (evTB) cells play a key role in pregnancy by allowing embryo implantation and remodeling of the uterine arteries [1]. During the early first trimester, they penetrate the upper third of the myometrium and invade the maternal decidua (uterine cells) [2]. This invasion is permitted by the activity of matrix metalloproteinases (MMPs), mostly MMP-2 and MMP-9, which degrade the extracellular matrix of the uterine wall [3, 4]. Gradually, evTBs replace the endothelial cells of the uterine spiral arteries, which have become dilated [5]. This phenomenon allows maternal blood to smoothly enter the intervillous chamber, facilitating nutrient, oxygen and waste exchange between mother and fetus [6]. A consequence of defective evTB invasion and migration is poor spiral artery remodeling, followed by pregnancy complications such as miscarriage [7], fetal growth restriction [8, 9] and preeclampsia [9-11].

An estimated 10 to 20 % of women suffer from depression during their pregnancy [12-14]. This disease can be deleterious both for mother and fetus, and may cause poor maternal behaviors that result in malnutrition, drug/alcohol abuse and sometimes suicide. Depression may also lead to pregnancy complications such as spontaneous abortion, preeclampsia, preterm labor, and low birth weight [15-17]. In North America and Europe between 2 and 10 % of pregnant women are treated with selective serotonin-reuptake inhibitors (SSRIs) for depression [18-20]. This class of antidepressants blocks the serotonin transporter (SERT), thus increasing serotonin levels in the synaptic cleft of serotonergic neurons. SSRIs have shown benefits in the treatment of depression, but little is known about possible adverse effects on pregnancy, such as premature delivery and fetal disorders (withdraw syndrome, feeding difficulties, respiratory distress, low birth weight, and cardiac or pulmonary malformations) [21-27]. This is important as many women do not yet know

64 that they are pregnant during the early first trimester, and therefore expose their fetus and placenta
65 to xenobiotics, including SSRIs, without knowing it.

66 SSRIs cross the maternal-placental barrier [28] and are therefore in contact with placental
67 trophoblast cells. It has been established that these cells possess a serotonergic system comprising
68 SERT, serotonin receptors, tryptophan hydroxylases (TPHs) and monoamine oxidases (MAOs)
69 [29-32]. Serotonin plays a crucial role during pregnancy, in both fetal and placental development.
70 Indeed, the serotonergic system is involved in the proliferation and migration of fetal neuronal cells
71 [33-35], cardiac cells [36] and monocytes [37], as well as in trophoblast cell invasion and
72 proliferation [38]. Thus, it is possible that SSRIs affect placental homeostasis through its
73 serotonergic system, leading to, among other outcomes, to an improperly evTB function.

74 Despite the frequent prescription of SSRIs to pregnant women and the importance of evTB
75 function for healthy pregnancy, if and the mechanisms by which SSRIs may affect evTB have
76 never been studied. The objective of the present study was to determine the effects of fluoxetine,
77 its metabolite norfluoxetine, citalopram, sertraline and venlafaxine at therapeutic concentrations
78 on cell proliferation and migration as well as several markers of invasion in two cellular models of
79 the evTB: JEG-3 (human choriocarcinoma) and HIPEC (human invasive proliferative extravillous
80 cytotrophoblast) cells. [39, 40].

81

82 **MATERIAL AND METHODS**

83 ***Cell culture:*** JEG-3 cells were obtained from the American Type Culture Collection
84 (ATCC, Rockville, MD) and maintained in minimum essential medium (MEM) Eagle (Sigma-
85 Aldrich, Oakville, ON, Canada) supplemented with 1.1 g/L sodium bicarbonate, 1 mM sodium
86 pyruvate, 0.01 mM HEPES (Sigma-Aldrich) and 10% fetal bovine serum (FBS, Hyclone, Tempe,

AZ). Cells were cultured in 75 cm^2 culture flasks (Corning LifeSciences, Lowell, MA) in a humidified atmosphere containing 5% carbon dioxide (CO_2) at 37 °C, and then passaged when they reached about 90% confluence using 0.5% trypLE (cell dissociation buffer) (Thermo Fisher Scientific, Waltham, MA). HIPEC cells (gift from Thierry Fournier's laboratory, Université Paris Descartes, Paris, France) were maintained in Dulbecco's modified Eagle's medium (DMEM)/F12 Ham (Sigma-Aldrich) supplemented with 2 mM L-glutamine (Sigma-Aldrich) and 5% FBS [39].

Treatments: All SSRIs were purchased from Sigma-Aldrich (for structures, see supplementary Fig. S1). Norfluoxetine, citalopram and venlafaxine were dissolved in milliQ H_2O; fluoxetine and sertraline in DMSO (Sigma-Aldrich) as 1000-fold stock solutions. Cells were treated with increasing concentrations of the SSRIs (0 to 10 µM), completed with DMSO when SSRIs were dissolved in water, to obtain a final DMSO concentration of 0.1% in culture medium. Control cells were exposed to 0.1% DMSO.

Cell proliferation assay: Cell proliferation was monitored in real-time by measuring changes in cell impedance using an xCELLigence™ RTCA SP instrument (ACEA Biosciences, San Diego, CA) as described previously [41]. Briefly, electronic 96-well microtiter plates (E-Plate® 96; ACEA Biosciences) were filled with 100 µL of complete culture medium, and after 30 min background impedance was measured. Then, JEG-3 (5 x 10^4 cells/ml) or HIPEC cells (2.5 x 10^4 cells/ml) were added to the E-Plates, each in their respective complete culture medium. After 24 h, cells were treated with various concentrations of the SSRIs or with vehicle control (0.1% DMSO). Real-time cell adhesion and proliferation were determined quantitatively by measuring changes in impedance every 10 min over a period of 72 h, and data were analyzed using RTCA Data Analysis Software v1.0 (ACEA Biosciences) [41]. Cell indices were based on the slope of the normalized linear part of the proliferation curves.

110 ***Cell cycle analysis:*** JEG-3 (1 x 10^5 cells/ml) or HIPEC cells (5 x 10^5 cells/ml) were added
111 to 24-well plates (CellBind; Corning LifeSciences) in their complete culture medium, and allowed
112 to acclimatize for 24 h. The medium was then refreshed, and various concentrations of the SSRIs
113 were added. After 24 h, the supernatant containing dead cells and cellular debris were removed
114 prior to analysis, so only attached cells underwent cell cycle analysis. Cells were collected, washed
115 and fixed with ice-cold 70% ethanol (v/v), then stored at 4 °C overnight. The next morning, cells
116 were centrifuged at 311 x g for 10 min, then the cell pellets were resuspended in phosphate-
117 buffered saline (PBS) containing 50 µg/ml propidium iodide (Sigma-Aldrich) and 100 µg/ml
118 RNase A (Sigma-Aldrich), and were then incubated for 15 min in the dark at room temperature.
119 The cell cycle was analyzed using a FACSCalibur flow cytometer (Becton Dickinson, Franklin
120 Lakes, NJ). The percentage of cells in each phase was determined with Cell Quest Pro Software
121 (BD Biosciences, San Jose, CA). To evaluate cell cycle alterations, 3000 events were recorded.
122 ***Wound healing assay:*** Cells were cultured in 24-well CellBind plates (3.5 x 10^5 cells/ml)
123 in complete medium. After 24 h, when cells were confluent, each well was scratched with a sterile
124 200 µl pipette tip, which had been rinsed with PBS. Treatments were performed using medium
125 without FBS. Preliminary experiments were performed to determine wound closure rates, allowing
126 an estimation of the appropriate times to take photos. Cells were examined by light microscopy at
127 4X final magnification immediately after scratching the cells and then 8 h, 12 h and 24 h (JEG-3)
128 or 5 h, 24 h and 29 h (HIPEC) after treatment with the SSRIs. Wound closure times, which reflect
129 the ability of cells to migrate, were calculated using ImageJ software [42]. Results were expressed
130 as a percentage of wound closure compared to the initial area of the wound.
131 ***Gelatin zymogaphy:*** The secretion of gelatinases MMP-2 and MMP-9 by JEG-3 and
132 HIPEC cells was evaluated using zymography as described previously [43], with modifications.

JEG-3 (2 x 10^5 cells/ml) and HIPEC (3 x 10^5 cells/ml) cells were seeded in 6-well plates in their respective culture media containing 0.5% FBS and no other supplements. After 24 h, cells were treated with SSRIs. Supernatants were collected 24 h (JEG-3) or 48 h (HIPEC) after treatment. Then, supernatants containing the secreted proteins were loaded on 10% acrylamide gels containing 0.1% gelatin and 4.5% of acrylamide stacking gel. Proteins were separated by electrophoresis (200 V, 1 h) using a running buffer composed of Tris-glycine-SDS. Gels were washed for 1 h in a 2.5% Triton X100 solution and incubated overnight in digestion buffer at 37 °C. The next day, proteins were stained with 0.5% Coomasie brilliant blue R250 (Sigma-Aldrich) and unstained with a 5% methanol/7.5% acetic acid solution. Zymograms were scanned using a ChemiDocTM XRS1 System (Bio-Rad, Mississauga, ON, Canada), and analyzed with Image Lab software 5.2.1 (Bio-Rad).

mRNA level analysis of invasion markers by RT-qPCR: JEG-3 (2 x 10^5 cells/ml) and HIPEC cells (3 x 10^5 cells/ml) were seeded in 6-well plates and were treated 24 h later cells with SSRIs in their respective culture media containing 0.5% FBS and no other supplements. Total RNA was isolated 24 h (JEG-3) or 48 h (HIPEC) after treatment, using an AllPrep DNA/RNA/Protein Mini Kit (Qiagen, Toronto, ON, Canada), according to manufacturer instructions. RNA quality and quantity were analyzed using a NanoDrop spectrophotometer (Thermo Fisher Scientific). An Experion automated electrophoresis system (Bio-Rad) was used to measure RNA integrity. cDNA was synthesized using an iScript cDNA synthesis kit (Bio-Rad) and stored at -20 °C. Primer pair sequences used for amplification are shown in Table S1. Their selectivity was verified using Primer-Blast software (http://www.ncbi.nlm.nih.gov/tools/primer-blast/). Samples were amplified with SsoAdvanced Universal SYBR Green Supermix using a CFX-96 Real-Time PCR Detection System (Bio-Rad). The amplification response of the target gene was normalized using

156 *peptidylprolyl isomerase A* (*PPIA*) and *succinate dehydrogenase complex subunit A* (*SDHA*) as
157 reference genes (for JEG-3), or *PPIA* and *TATA box binding protein* (*TBP*) (for HIPEC), which
158 were selected using GeNorm software (BioGazelle, Zwijnaarde, Belgium) [44]. PCR products were
159 verified for specificity by electrophoresis on a 2% agarose gel containing ethidium bromide and
160 visualization under UV light.

161 ***Statistical analysis:*** Experiments were performed three to five times using different cell
162 passages; per experiment each treatment was performed in triplicate. Statistical analyses were
163 performed using IBM SPSS Statistics 21.0 software (Chicago, IL), and figures were made using
164 GraphPad Prism v5.01 (GraphPad Software, San Diego, CA). Dependent on the appropriate
165 statistical criteria for data variance, normality and homogeneity, one of the following tests was
166 used as specified in the figure legends: one-way ANOVA with a Dunnett posteriori test, Welch's
167 test, a Kruskal-Wallis test or Mood's median test, [45]. A value of $P < 0.05$ was considered
168 statistically significant.

169

170 **RESULTS**

171 ***Fluoxetine and sertraline decrease JEG-3 and HIPEC cell proliferation.*** Fluoxetine at
172 10 μM decreased the proliferation rate of JEG-3 cells by 94% (P=0.034) and that of HIPEC cells
173 by 59% (P=0.049), compared to vehicle control (0.1% DMSO) (Fig 1A, 1B and Fig. S2). Sertraline
174 decreased the proliferation rate of JEG-3 cells by almost 100% at 10 μM (P<0.001) (Fig. 1A and
175 Fig. S2), but did not affect proliferation at lower concentrations. Sertraline decreased HIPEC cell
176 proliferation by 23% at 0.3 μM, by 50% at 3 μM, and by 100% at 10 μM (P=0.043, P=0.034 and
177 P=0.021, respectively), compared to vehicle control (0.1% DMSO) (Fig. 1B and Fig. S2). None of

178 the other SSRIs (norfluoxetine, citalopram or venlafaxine) affected JEG-3 or HIPEC cell
179 proliferation at concentrations between 0.03 and 10 μM.

180 ***Norfluoxetine induces cell cycle arrest in the G0-G1 phase in HIPEC cells.*** None of the
181 SSRIs tested altered JEG-3 cell cycle after a 24 h exposure (Fig. 2A). However, a 24-h exposure
182 to 10 μM norfluoxetine increased the absolute fraction of HIPEC cells in the G0-G1 phase by
183 6.11% at 10 μM (P=0.027) (Fig. 2B).

184 ***Fluoxetine decreases the rate of JEG-3 cell migration.*** Fig. 3A and Fig. S3A show that a
185 12 h and 24 h of treatment with 0.3 μM of fluoxetine decreased JEG-3 cell migration by 9.9%
186 (P=0.022) and 13% (P=0.025), respectively, compared to vehicle control. A 24 h treatment with
187 3 μM of fluoxetine decreased JEG-3 cell migration by 16% (P=0.027), compared to control.
188 Interestingly, norfluoxetine had the opposite effect to fluoxetine on the migration rate of JEG-3
189 cells, with exposure to 0.3 μM norfluoxetine resulting in a 10% increase in cell migration 12 h after
190 scratching. Citalopram, sertraline and venlafaxine did not affect the migration rate of JEG-3 cells.
191 None of the SSRIs affected HIPEC cell migration (Fig. 3B and. Fig. S3B), except venlafaxine at
192 0.3 μM, which caused a 16% increase in cell migration, 24 h after the cells were scratched
193 (P=0.001).

194 ***Norfluoxetine has an opposite effect on MMP-9 activity in JEG-3 and HIPEC cells.***
195 Norfluoxetine increased MMP-9 secretion by JEG-3 cells by 29% at 0.03 μM (P=0.031) and by
196 44% at 3 μM (P=0.002) compared to vehicle control (Fig. 4A). In HIPEC cells, norfluoxetine
197 significantly decreased MMP-9 secretion by 64% at 3 μM (P=0.007) (Fig. 4B). None of the other
198 SSRIs affected MMP-2 or MMP-9 secretion by either cell line.

199 To further characterize the effects of SSRIs on extravillous trophoblast invasion and migration the
200 mRNA levels of various markers of the processes (Table S1) in JEG-3 and HIPEC cells were analyzed. In
201 JEG-3 cells exposed to fluoxetine, norfluoxetine, citalopram or venlafaxine mRNA levels of *MMP-2*, *-9*

202 and -*14*, *TIMP-1* and -*3,* or *ADAM-10* were not affected (Fig. 5). However, sertraline at 0.03 μM increased
203 *TIMP-1* expression in JEG-3 cells by 36% (P=0.048) and *ADAM-10 expression* by 85% at 0.3 μM
204 (P=0.036) and by 115% at 3 μM (P=0.005) compared to control. In HIPEC cells, venlafaxine at 0.03 μM
205 and 0.3 μM, increased ADAM-10 mRNA levels by 156% (P=0.040) and 167% (P=0.028), respectively
206 (Fig. 6). A summary of the effects of the SSRIs in JEG-3 and HIPEC cells is provided in Table 1.
207
208 **DISCUSSION**
209 This study investigated the ability of the fluoxetine, its metabolite norfluoxetine,
210 citalopram, sertraline and venlafaxine SSRIs to interfere with evTB homeostasis, by evaluating
211 their effects on proliferation, migration and several markers of invasion of JEG-3 and HIPEC cells,
212 which are established models of the evTB [39, 40]. The SSRI concentrations used were between
213 0.03 and 10 μM, which correspond to the mid to upper range of therapeutic concentrations found
214 in maternal blood (15-130 nM) or cord blood (65-250 nM) of pregnant women taking SSRI doses
215 as currently prescribed [46, 47]. To our knowledge, this is the first study to examine possible effects
216 of SSRIs commonly used during pregnancy on the proliferative, migratory and invasive properties
217 of these two cell models of the evTB.
218 Yavarone *et al.* observed that both fluoxetine and sertraline at 10 μM significantly
219 decreased cardiac cell (mesenchyme, endocardium and myocardium) proliferation [36], which is
220 in agreement with our results showing that fluoxetine and sertraline decreased JEG-3 and HIPEC
221 cell proliferation at the same concentration. The absence of effects on cell proliferation by the other
222 SSRIs (norfluoxetine, venlafaxine and citalopram), or by lower concentrations of fluoxetine and
223 sertraline, indicate that these effects are structure- and concentration-dependent. We have
224 previously shown that serotonin regulates the proliferation of BeWo and JEG-3 cells via activation
225 of the 5-HT_{2A} receptor and subsequent stimulation of the MEK-ERK1/2 and JAK2-STAT3

226 signaling pathways [38, 48]. It has been shown that sertraline and fluoxetine bind the 5-HT_{2A}
227 receptor [49] and it has been hypothesis that fluoxetine stimulates this receptor [50], but whether
228 this signaling pathway is involved in modulating cell proliferation in our extravillous trophoblast
229 models remains to be studied.

230 Among the SSRIs tested, only fluoxetine at 0.3 and 3 μM decreased JEG-3 (but not HIPEC)
231 cell migration. In agreement with our findings, Vichier-Guerre *et al.* reported no effects of 0.03,
232 0.3 and 3 μM sertraline on neural crest stem cell migration [51], and at 7 μM, sertraline had no
233 effect on the transwell membrane migration of glioblastoma cells [52]. Moreover, in human
234 colorectal cancer cells, Van Noort *et al.* found that citalopram did not affect HCT116 cell
235 migration, whereas it decreased HT29 cell migration, but only at the highest concentration tested
236 (100 μM), at which they also observed a decrease in cell proliferation [53]. With the exception of
237 fluoxetine, our results suggest that SSRIs commonly prescribed during pregnancy do not have
238 significant effects on JEG-3 and HIPEC cell migration.

239 In HIPEC cells, norfluoxetine caused a statistically significant increase in the number of
240 cells in the G0-G1 phase, and decreased the number of cells in the G2-S phase, although not
241 significantly. A study by Serafeim *et al.* found that a 24 h exposure to fluoxetine (20 μM) or
242 citalopram (100 μM) inhibited DNA synthesis in proliferating Burkitt lymphoma (BL) cells,
243 concurrent with an increase of the number of cells in the G0-G1 phase [54]. These cell cycle effects
244 of SSRIs have subsequently been demonstrated in hypoxic human colorectal adenocarcinoma cells
245 exposed to 20 μM fluoxetine for 24 h [55], and in MDA-MB-231 human breast cancer and SIHA
246 human cervical cancer cells exposed to 30 μM fluoxetine for 24 or 36 h [56]. It is suggested that
247 in MDA-MB-231 cells fluoxetine causes the accumulation of p21 through functional inhibition of
248 cyclin-dependent kinase subunit 1 (CKS1) [56]. In colorectal cells, a possible inhibition of the Akt-

mTOR signaling pathway by fluoxetine is suggested [55]. More studies are needed to determine the nature of the pathways involved in the effects of SSRIs on cell cycle in normal and cancerous cells.

Our data suggest that HIPEC cells, which are derived from human invasive proliferative extravillous cytotrophoblast cells, and JEG-3, which are choriocarcinoma cells, have different basal MMP-2 and MMP-9 activities which respond differently to SSRIs. With the exception of norfluoxetine, we observed a lack of effects of SSRIs on metalloproteinase expression in JEG-3 and HIPEC cells. Lee *et al.* found that fluoxetine (10 mg/kg) decreased *MMP-2*, *-9* and *-12* mRNA levels in mice after a spinal cord injury (an injury that increases MMP-2, -9 and -12 protein expression and activities), as well as pro-MMP-2, -9 and -12 activities, to near-normal levels measured in control mice [57]. The same investigators reported that in mice with ischemia, a condition known to increase MMP-2 and MMP-9 levels, exposure to 10 mg/kg fluoxetine decreased protein levels and activities of both MMPs to those found in non-ischemic mice. [58]. It would be interesting to study the effects of fluoxetine on pregnancy complications associated with altered placental MMP-2 and MMP-9 protein levels, such as preeclampsia [59-61] or placental ischemia [62].

There are very few studies of SSRIs other than fluoxetine on MMP expression and function. A study of the effects of citalopram (9.6 mg/kg/day for 2 or 6 weeks) in mice after myocardial infarction found no changes in *MMP-2* or *-9* gene expression, nor any alteration in tissue inhibitor of metalloproteinase (TIMP) isoforms TIMP-1 or TIMP-2; however, an increase in *MMP-13* gene expression was observed in the left ventricular infarcted myocardium after citalopram treatment [63]. In agreement, we also did not observe any differences in *MMP* gene expression or activity between citalopram treatment and control in our evTB cell models. Interestingly we found an

increased expression of the messenger RNA for protease ADAM10 in HIPEC cell treated with 0.3 and 3 µM of venlafaxine, which is consistent with the significant increase of HIPEC cell migration after exposure to 0.3 µM of venlafaxine. Tamasi and al. also found that neuron migration in rats after chronic venlafaxine treatment (3 weeks) was associated with changes in genes expression involved in cell migration [64]. Whether these SSRIs affect JEG-3 and HIPEC cell invasion remains to be studied.

Clinical relevance and perspectives: Extravillous trophoblast migration and invasion is crucial for a healthy pregnancy and a defect in these cellular processes can lead to pregnancy complications such as fetal growth restriction or preeclampsia. Moreover, these migration and invasion processes take place during the first trimester of pregnancy, when women often do not know they are pregnant. This is why it is important to study molecules such as SSRIs, which are widely used during pregnancy, in order to provide new insights that inform the scientific community and practitioners on the rationale for prescribing SSRIs for the treatment of depression in pregnant women. The results of the present study indicate that additional research is needed, using models with increased physiological relevance, such as freshly isolated extravillous trophoblast cells in primary culture, to identify the molecular mechanisms involved in the modulation of extravillous trophoblast function by SSRIs and possibly other medications commonly used during pregnancy.

CONCLUSION

Our study indicates that SSRIs affect various aspects of extravillous trophoblast function using JEG-3 and HIPEC cells as models. Our observations contribute to a better understanding of the potential impact SSRIs may have on extravillous trophoblasts homeostasis, thus providing

295 guidance for future research, as well as scientific evidence to support clinical decisions aimed at
296 improving maternal and fetal health.
297
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309
310 **REFERENCES**:

311 [1] J. Pollheimer, M. Knofler, The role of the invasive, placental trophoblast in human pregnancy,
312 Wien Med Wochenschr 162(9-10) (2012) 187-90.
313 [2] A. Malassine, [Morphological variability and placental function], Gynecol Obstet Fertil 29(7-
314 8) (2001) 489-96.
315 [3] K. Isaka, S. Usuda, H. Ito, Y. Sagawa, H. Nakamura, H. Nishi, Y. Suzuki, Y.F. Li, M.
316 Takayama, Expression and activity of matrix metalloproteinase 2 and 9 in human trophoblasts,
317 Placenta 24(1) (2003) 53-64.

318 [4] E. Staun-Ram, S. Goldman, D. Gabarin, E. Shalev, Expression and importance of matrix
319 metalloproteinase 2 and 9 (MMP-2 and -9) in human trophoblast invasion, Reprod Biol Endocrinol
320 2 (2004) 59.
321 [5] R. Pijnenborg, L. Vercruysse, M. Hanssens, The uterine spiral arteries in human pregnancy:
322 facts and controversies, Placenta 27(9-10) (2006) 939-58.
323 [6] D.R. Tessier, J. Yockell-Lelievre, A. Gruslin, Uterine Spiral Artery Remodeling: The Role of
324 Uterine Natural Killer Cells and Extravillous Trophoblasts in Normal and High-Risk Human
325 Pregnancies, Am J Reprod Immunol 74(1) (2015) 1-11.
326 [7] E. Ball, J.N. Bulmer, S. Ayis, F. Lyall, S.C. Robson, Late sporadic miscarriage is associated
327 with abnormalities in spiral artery transformation and trophoblast invasion, J Pathol 208(4) (2006)
328 535-42.
329 [8] L. Zuckerwise, J. Li, L. Lu, Y. Men, T. Geng, C.S. Buhimschi, I.A. Buhimschi, R. Bukowski,
330 S. Guller, M. Paidas, Y. Huang, H19 long noncoding RNA alters trophoblast cell migration and
331 invasion by regulating TbetaR3 in placentae with fetal growth restriction, Oncotarget 7(25) (2016)
332 38398-38407.
333 [9] F. Lyall, S.C. Robson, J.N. Bulmer, Spiral artery remodeling and trophoblast invasion in
334 preeclampsia and fetal growth restriction: relationship to clinical outcome, Hypertension 62(6)
335 (2013) 1046-54.
336 [10] Y. Uchikura, K. Matsubara, Y. Muto, Y. Matsubara, T. Fujioka, T. Matsumoto, T. Sugiyama,
337 Extranuclear Translocation of High-Mobility Group A1 Reduces the Invasion of Extravillous
338 Trophoblasts Involved in the Pathogenesis of Preeclampsia, Reprod Sci (2017)
339 1933719117697254.

340 [11] D. Evain-Brion, T. Fournier, P. Therond, A. Tarrade, L. Pavan, [Pathogenesis of pre-
341 eclampsia: role of gamma PPAR in trophoblast invasion], Bull Acad Natl Med 186(2) (2002) 409-
342 18; discussion 418-20.
343 [12] H.A. Bennett, A. Einarson, A. Taddio, G. Koren, T.R. Einarson, Prevalence of depression
344 during pregnancy: systematic review, Obstet Gynecol 103(4) (2004) 698-709.
345 [13] S. Gentile, Untreated depression during pregnancy: Short- and long-term effects in offspring.
346 A systematic review, Neuroscience (2015).
347 [14] N.I. Gavin, B.N. Gaynes, K.N. Lohr, S. Meltzer-Brody, G. Gartlehner, T. Swinson, Perinatal
348 depression: a systematic review of prevalence and incidence, Obstet Gynecol 106(5 Pt 1) (2005)
349 1071-83.
350 [15] L. Bonari, N. Pinto, E. Ahn, A. Einarson, M. Steiner, G. Koren, Perinatal risks of untreated
351 depression during pregnancy, Can J Psychiatry 49(11) (2004) 726-35.
352 [16] V. Filippi, D. Chou, C. Ronsmans, W. Graham, L. Say, Levels and Causes of Maternal
353 Mortality and Morbidity, in: R.E. Black, R. Laxminarayan, M. Temmerman, N. Walker (Eds.),
354 Reproductive, Maternal, Newborn, and Child Health: Disease Control Priorities, Third Edition
355 (Volume 2), 2016 International Bank for Reconstruction and Development / The World Bank.,
356 Washington DC, 2016.
357 [17] N.K. Grote, J.A. Bridge, A.R. Gavin, J.L. Melville, S. Iyengar, W.J. Katon, A meta-analysis
358 of depression during pregnancy and the risk of preterm birth, low birth weight, and intrauterine
359 growth restriction, Arch Gen Psychiatry 67(10) (2010) 1012-24.
360 [18] W.O. Cooper, M.E. Willy, S.J. Pont, W.A. Ray, Increasing use of antidepressants in
361 pregnancy, Am J Obstet Gynecol 196(6) (2007) 544 e1-5.

362 [19] R.A. Charlton, S. Jordan, A. Pierini, E. Garne, A.J. Neville, A.V. Hansen, R. Gini, D. Thayer,
363 K. Tingay, A. Puccini, H.J. Bos, A.M. Nybo Andersen, M. Sinclair, H. Dolk, L. de Jong-van den
364 Berg, Selective serotonin reuptake inhibitor prescribing before, during and after pregnancy: a
365 population-based study in six European regions, BJOG 122(7) (2015) 1010-20.
366 [20] S. Alwan, J. Reefhuis, S.A. Rasmussen, J.M. Friedman, Patterns of antidepressant medication
367 use among pregnant women in a United States population, J Clin Pharmacol 51(2) (2011) 264-70.
368 [21] A. Eke, G. Saccone, V. Berghella, Selective serotonin reuptake inhibitor (SSRI) use during
369 pregnancy and risk of preterm birth: a systematic review and meta-analysis, BJOG (2016).
370 [22] I. Torjesen, Antidepressants in late pregnancy are linked to respiratory disorder in newborns,
371 study finds, BMJ 350 (2015) h2980.
372 [23] E. Ramos, M. St-Andre, A. Berard, Association between antidepressant use during pregnancy
373 and infants born small for gestational age, Can J Psychiatry 55(10) (2010) 643-52.
374 [24] T. Boukhris, O. Sheehy, L. Mottron, A. Berard, Antidepressant Use During Pregnancy and
375 the Risk of Autism Spectrum Disorder in Children, JAMA Pediatr 170(2) (2016) 117-24.
376 [25] L. Laurent, C. Huang, S.R. Ernest, A. Berard, C. Vaillancourt, B.F. Hales, In utero exposure
377 to venlafaxine, a serotonin-norepinephrine reuptake inhibitor, increases cardiac anomalies and
378 alters placental and heart serotonin signaling in the rat, Birth Defects Res A Clin Mol Teratol
379 (2016).
380 [26] S. Alwan, J. Reefhuis, S.A. Rasmussen, R.S. Olney, J.M. Friedman, Use of selective
381 serotonin-reuptake inhibitors in pregnancy and the risk of birth defects, N Engl J Med 356(26)
382 (2007) 2684-92.
383 [27] A. Wemakor, K. Casson, E. Garne, M. Bakker, M.C. Addor, L. Arriola, M. Gatt, B.
384 Khoshnood, K. Klungsoyr, V. Nelen, M. O'Mahoney, A. Pierini, A. Rissmann, D. Tucker, B.

385 Boyle, L. de Jong-van den Berg, H. Dolk, Selective serotonin reuptake inhibitor antidepressant use
386 in first trimester pregnancy and risk of specific congenital anomalies: a European register-based
387 study, Eur J Epidemiol 30(11) (2015) 1187-98.
388 [28] J. Rampono, K. Simmer, K.F. Ilett, L.P. Hackett, D.A. Doherty, R. Elliot, C.H. Kok, A.
389 Coenen, T. Forman, Placental transfer of SSRI and SNRI antidepressants and effects on the
390 neonate, Pharmacopsychiatry 42(3) (2009) 95-100.
391 [29] M. Viau, J. Lafond, C. Vaillancourt, Expression of placental serotonin transporter and 5-HT
392 2A receptor in normal and gestational diabetes mellitus pregnancies, Reprod Biomed Online 19(2)
393 (2009) 207-15.
394 [30] A. Bonnin, N. Goeden, K. Chen, M.L. Wilson, J. King, J.C. Shih, R.D. Blakely, E.S. Deneris,
395 P. Levitt, A transient placental source of serotonin for the fetal forebrain, Nature 472(7343) (2011)
396 347-50.
397 [31] D.F. Balkovetz, C. Tiruppathi, F.H. Leibach, V.B. Mahesh, V. Ganapathy, Evidence for an
398 imipramine-sensitive serotonin transporter in human placental brush-border membranes, J Biol
399 Chem 264(4) (1989) 2195-8.
400 [32] L. Laurent, K. Deroy, J. St-Pierre, F. Cote, J.T. Sanderson, C. Vaillancourt, Human placenta
401 expresses both peripheral and neuronal isoform of tryptophan hydroxylase, Biochimie (2017).
402 [33] T. Vitalis, J.G. Parnavelas, The role of serotonin in early cortical development, Dev Neurosci
403 25(2-4) (2003) 245-56.
404 [34] O. Riccio, M. Jacobshagen, B. Golding, L. Vutskits, D. Jabaudon, J.P. Hornung, A.G. Dayer,
405 Excess of serotonin affects neocortical pyramidal neuron migration, Transl Psychiatry 1 (2011)
406 e47.

[35] M. Banasr, M. Hery, R. Printemps, A. Daszuta, Serotonin-induced increases in adult cell proliferation and neurogenesis are mediated through different and common 5-HT receptor subtypes in the dentate gyrus and the subventricular zone, Neuropsychopharmacology 29(3) (2004) 450-60.

[36] M.S. Yavarone, D.L. Shuey, H. Tamir, T.W. Sadler, J.M. Lauder, Serotonin and cardiac morphogenesis in the mouse embryo, Teratology 47(6) (1993) 573-84.

[37] R. Piga, Y. Naito, S. Kokura, O. Handa, T. Yoshikawa, Inhibitory effect of serotonin derivatives on high glucose-induced adhesion and migration of monocytes on human aortic endothelial cells, Br J Nutr 102(2) (2009) 264-72.

[38] T. Oufkir, M. Arseneault, J.T. Sanderson, C. Vaillancourt, The 5-HT 2A serotonin receptor enhances cell viability, affects cell cycle progression and activates MEK-ERK1/2 and JAK2-STAT3 signalling pathways in human choriocarcinoma cell lines, Placenta 31(5) (2010) 439-47.

[39] L. Pavan, A. Tarrade, A. Hermouet, C. Delouis, M. Titeux, M. Vidaud, P. Therond, D. Evain-Brion, T. Fournier, Human invasive trophoblasts transformed with simian virus 40 provide a new tool to study the role of PPARgamma in cell invasion process, Carcinogenesis 24(8) (2003) 1325-36.

[40] P.O. Kohler, W.E. Bridson, Isolation of hormone-producing clonal lines of human choriocarcinoma, J Clin Endocrinol Metab 32(5) (1971) 683-7.

[41] H. Clabault, L. Laurent, J.T. Sanderson, C. Vaillancourt, Isolation and Purification of Villous Cytotrophoblast Cells from Term Human Placenta, Methods Mol Biol 1710 (2018) 219-231.

[42] C.A. Schneider, W.S. Rasband, K.W. Eliceiri, NIH Image to ImageJ: 25 years of image analysis, Nat Methods 9(7) (2012) 671-5.

[43] M. Martelli, A. Campana, P. Bischof, Secretion of matrix metalloproteinases by human endometrial cells in vitro, J Reprod Fertil 98(1) (1993) 67-76.

[44] D. Lanoix, A.A. Lacasse, J. St-Pierre, S.C. Taylor, M. Ethier-Chiasson, J. Lafond, C. Vaillancourt, Quantitative PCR Pitfalls: The Case of the Human Placenta, Mol Biotechnol (2012).

[45] H.J. Zar, Biostatistical Analysis, 2010.

[46] D. Sit, J.M. Perel, S.R. Wisniewski, J.C. Helsel, J.F. Luther, K.L. Wisner, Mother-infant antidepressant concentrations, maternal depression, and perinatal events, J Clin Psychiatry 72(7) (2011) 994-1001.

[47] V. Hendrick, Z.N. Stowe, L.L. Altshuler, S. Hwang, E. Lee, D. Haynes, Placental passage of antidepressant medications, Am J Psychiatry 160(5) (2003) 993-6.

[48] T. Oufkir, C. Vaillancourt, Phosphorylation of JAK2 by serotonin 5-HT (2A) receptor activates both STAT3 and ERK1/2 pathways and increases growth of JEG-3 human placental choriocarcinoma cell, Placenta 32(12) (2011) 1033-40.

[49] M.J. Owens, W.N. Morgan, S.J. Plott, C.B. Nemeroff, Neurotransmitter receptor and transporter binding profile of antidepressants and their metabolites, J Pharmacol Exp Ther 283(3) (1997) 1305-22.

[50] A.A. Hudon Thibeault, L. Laurent, S.V. Duy, S. Sauve, P. Caron, C. Guillemette, J.T. Sanderson, C. Vaillancourt, Fluoxetine and its active metabolite norfluoxetine disrupt estrogen synthesis in a co-culture model of the feto-placental unit, Mol Cell Endocrinol (2016).

[51] C. Vichier-Guerre, M. Parker, Y. Pomerantz, R.H. Finnell, R.M. Cabrera, Impact of selective serotonin reuptake inhibitors on neural crest stem cell formation, Toxicol Lett (2017).

[52] L. Schmidt, S. Baskaran, P. Johansson, N. Padhan, D. Matuszewski, L.C. Green, L. Elfineh, S. Wee, M. Haggblad, U. Martens, B. Westermark, K. Forsberg-Nilsson, L. Uhrbom, L. Claesson-Welsh, M. Andang, I.M. Sintorn, B. Lundgren, I. Lonnstedt, C. Krona, S. Nelander, Case-specific potentiation of glioblastoma drugs by pterostilbene, Oncotarget 7(45) (2016) 73200-73215.

453 [53] V. van Noort, S. Scholch, M. Iskar, G. Zeller, K. Ostertag, C. Schweitzer, K. Werner, J. Weitz,
454 M. Koch, P. Bork, Novel drug candidates for the treatment of metastatic colorectal cancer through
455 global inverse gene-expression profiling, Cancer Res 74(20) (2014) 5690-9.
456 [54] A. Serafeim, M.J. Holder, G. Grafton, A. Chamba, M.T. Drayson, Q.T. Luong, C.M. Bunce,
457 C.D. Gregory, N.M. Barnes, J. Gordon, Selective serotonin reuptake inhibitors directly signal for
458 apoptosis in biopsy-like Burkitt lymphoma cells, Blood 101(8) (2003) 3212-9.
459 [55] V. Kannen, S.B. Garcia, W.A. Silva, Jr., M. Gasser, R. Monch, E.J. Alho, H. Heinsen, C.J.
460 Scholz, M. Friedrich, K.G. Heinze, A.M. Waaga-Gasser, H. Stopper, Oncostatic effects of
461 fluoxetine in experimental colon cancer models, Cell Signal 27(9) (2015) 1781-8.
462 [56] A. Krishnan, R. Hariharan, S.A. Nair, M.R. Pillai, Fluoxetine mediates G0/G1 arrest by
463 inducing functional inhibition of cyclin dependent kinase subunit (CKS)1, Biochem Pharmacol
464 75(10) (2008) 1924-34.
465 [57] J.Y. Lee, H.S. Kim, H.Y. Choi, T.H. Oh, T.Y. Yune, Fluoxetine inhibits matrix
466 metalloprotease activation and prevents disruption of blood-spinal cord barrier after spinal cord
467 injury, Brain 135(Pt 8) (2012) 2375-89.
468 [58] J.Y. Lee, H.E. Lee, S.R. Kang, H.Y. Choi, J.H. Ryu, T.Y. Yune, Fluoxetine inhibits transient
469 global ischemia-induced hippocampal neuronal death and memory impairment by preventing
470 blood-brain barrier disruption, Neuropharmacology 79 (2014) 161-71.
471 [59] S. Matsushita, H. Narumiya, M. Hirata, H. Yabushita, M. Noguchi, M. Nakanishi, Expression
472 of matrix metalloproteinase (MMP), tissue inhibitor of metalloproteinase (TIMP), and
473 transforming growth factor-β1 (TGF-β1) in human placenta during preeclampsia, Placenta
474 20(Supplement 1) (1999) 395-406.

475 [60] A.C. Palei, J.P. Granger, J.E. Tanus-Santos, Matrix metalloproteinases as drug targets in
476 preeclampsia, Curr Drug Targets 14(3) (2013) 325-34.
477 [61] J. Chen, R.A. Khalil, Matrix Metalloproteinases in Normal Pregnancy and Preeclampsia, Prog
478 Mol Biol Transl Sci 148 (2017) 87-165.
479 [62] W. Li, K.M. Mata, M.Q. Mazzuca, R.A. Khalil, Altered matrix metalloproteinase-2 and -9
480 expression/activity links placental ischemia and anti-angiogenic sFlt-1 to uteroplacental and
481 vascular remodeling and collagen deposition in hypertensive pregnancy, Biochem Pharmacol 89(3)
482 (2014) 370-85.
483 [63] A. Frey, V.M. Saxon, S. Popp, M. Lehmann, D. Mathes, C. Pachel, U. Hofmann, G. Ertl, K.P.
484 Lesch, S. Frantz, Early citalopram treatment increases mortality due to left ventricular rupture in
485 mice after myocardial infarction, J Mol Cell Cardiol 98 (2016) 28-36.
486 [64] V. Tamási, P. Petschner, C. Adori, E. Kirilly, R.D. Ando, L. Tothfalusi, G. Juhasz, G. Bagdy,
487 Transcriptional Evidence for the Role of Chronic Venlafaxine Treatment in Neurotrophic Signaling
488 and Neuroplasticity Including also Glutatmatergic- and Insulin-Mediated Neuronal Processes,
489 PLoS ONE 9(11) (2014) e113662.

490

FIGURE LEGENDS

Figure 1: Effects of fluoxetine, norfluoxetine, citalopram, sertraline and venlafaxine on JEG-3 (A) and HIPEC (B) cell proliferation. Relative cell proliferation rates are presented as mean ± SEM (n=3-4). (*, ***) Statistically significant difference between SSRI treatment and vehicle control (0.1% DMSO) (Welch or Kruskal-Wallis test, dependent on the appropriate statistical criteria; *P<0.05; ***P<0.001).

Figure 2: Effects of fluoxetine, norfluoxetine, citalopram, sertraline and venlafaxine on JEG-3 (A) and HIPEC (B) cell cycle. After 24 h in culture, cells (JEG-3: 1 x 10^5 cells/ml; HIPEC: 5 x 10^5 cells/ml) were treated for 24 h with the SSRIs, then stained using propidium iodide and analyzed by flow cytometry. The percentage of cells in each cell cycle phase is expressed as mean ± SEM (n=4-5). (*) Statistically significant difference from vehicle control (0.1% DMSO) (one-way ANOVA and Dunnett posteriori, or Kruskal-Wallis test, dependent on the appropriate statistical criteria, *P<0.05).

Figure 3: Effects of fluoxetine, norfluoxetine, citalopram, sertraline and venlafaxine on the migration rate of JEG-3 (A) and HIPEC (B) cells. After 24 h in culture, cells (3.5 x 10^5 cells/ml) exposed to SSRIs in medium without FBS were scratched. Photos were taken at 0 h, 8 h, 12 h and 24 h after scratching for JEG-3 and 0 h, 5 h, 24 h and 29 h after scratching for HIPEC cells (see Fig. S3). The area of wound closure (scratch area) is expressed as a percentage (mean ± SEM) of the initial area of the wound (n=3-5). (*, **) Statistically significant difference between SSRI treatment and vehicle control (0.1% DMSO) (one-way ANOVA and Dunnett posteriori test, or Welch's, Median or Kruskal-Wallis test, dependent on the appropriate statistical criteria *P<0.05, **P<0.01).

Figure 4: Effects of fluoxetine, norfluoxetine, citalopram, sertraline and venlafaxine on the activities of metalloproteinase (MMP)-2 and -9 secreted by JEG-3 (A) and HIPEC (B) cells. After 24 h in culture, JEG-3 (2 x 10^5 cells/ml) and HIPEC (3 x 10^5 cells/ml) cells were treated for 24 h (JEG-3) or for 48 h (HIPEC) with the SSRIs in medium containing 0.5 % FBS. Gelatinase (MMP) activity in supernatants was analyzed by zymography and expressed as a percentage (mean ± SEM; n=4) of vehicle control (0.1% DMSO). (*, **) Statistically significant difference between SSRI treatment and vehicle control (one-way ANOVA and Dunnett posteriori, or Welch's or Kruskal-Wallis test, dependent on the appropriate statistical criteria; *P<0.05, **P<0.01).

Figure 5: Effects of fluoxetine, norfluoxetine, citalopram, sertraline and venlafaxine on the expression of *matrix metalloproteinase (MMP)-2, -9* and *-14*, *tissue inhibitor of metalloproteinases (TIMP)-1* and *-3*, and *a disintegrin and metalloproteinase domain-containing protein* (*ADAM*)-*10* in JEG-3 cells. Cells were treated for 24 h with increasing concentrations of SSRIs or with vehicle control (0.1% DMSO) using culture medium with 0.5 % FBS but without supplements. Levels of mRNA expression were determined by reverse-transcription quantitative polymerase chain reaction (RT-qPCR) using peptidylprolyl isomerase A (PPIA) and succinate dehydrogenase complex subunit A (SDHA) as reference genes, and presented as mean ± standard error of the mean; n=3-5; per experiment each treatment was performed in triplicate. (*, **) Statistically significant difference between SSRI treatment and vehicle control (0.1% DMSO) (one-way ANOVA and Dunnett posteriori, Welch, Kruskal-Wallis or Mood's median test; dependent on the appropriate statistical criteria; *P<0.05, ** P<0.01).

Figure 6: Effects of fluoxetine, norfluoxetine, citalopram, sertraline and venlafaxine on the expression of *matrix metalloproteinase* (*MMP*)-*2* and -*14*, *tissue inhibitor of metalloproteinases* (*TIMP*)-*3*, and a *disintegrin and metalloproteinase domain-containing protein* (*ADAM*)-*10* in HIPEC cells. After 24 h in culture, cells were treated for 48 h with increasing concentrations of SSRIs or with vehicle control (0.1% DMSO), using a medium with 0.5 % FBS. Levels of mRNA expression were determined by reverse-transcription quantitative polymerase chain reaction (RT-qPCR) using *peptidylprolyl isomerase A* (*PPIA*) and *TATA box binding protein* (*TBP*) as reference genes, and presented as mean ± standard error of the mean; n=3-5; per experiment each treatment was performed in triplicate. (*) Statistically significant difference between SSRI treatment and vehicle control (0.1% DMSO) (one-way ANOVA and Dunnett posteriori, Welch, Kruskal-Wallis or Mood's median test, dependent on the appropriate statistical criteria; *$P<0.05$).

Table 1: Summary of the effects of SSRIs observed in JEG-3 and HIPEC extravillous trophoblast-like cells.

		Fluoxetine	Norfluoxetine	Citalopram	Sertraline	Venlafaxine
Cell proliferation	JEG-3	↓ at 10 µM	/	/	↓ at 10 µM	/
	HIPEC	↓ at 10 µM	/	/	↓ at 0.3, 3 and 10 µM	/
Cell cycle phase	JEG-3	/	/	/	/	/
	HIPEC	/	↑ G0-G1 at 10 µM	/	/	/
Wound healing assays	JEG-3	migration ↓ at 0.3 and 3 µM	migration ↓ at 0.3 at 12h	/	/	/
	HIPEC	/	/	/	/	/
Gelatin zyymography	JEG-3	/	↑ MMP-9 activity	/	/	/
	HIPEC	/	↓ MMP-9 activity	/	/	/
mRNA expression levels	JEG-3	/	/	/	↑ *TIMP-1* at 0.03 µM ↑ *ADAM-10* at 0.3 and 3 µM	/
	HIPEC	/	/	/	/	↑ *ADAM-10* at 0.03 and 0.3 µM

/: no effect; MMP: matrix metalloproteinase; TIMP: tissue inhibitor of metalloproteinases; ADAM-10: a desintegrin and metalloproteinase domain-containing protein 10.

Figure 1: Effects of fluoxetine, norfluoxetine, citalopram, sertraline and venlafaxine on JEG-3 (A) and HIPEC (B) cell proliferation.

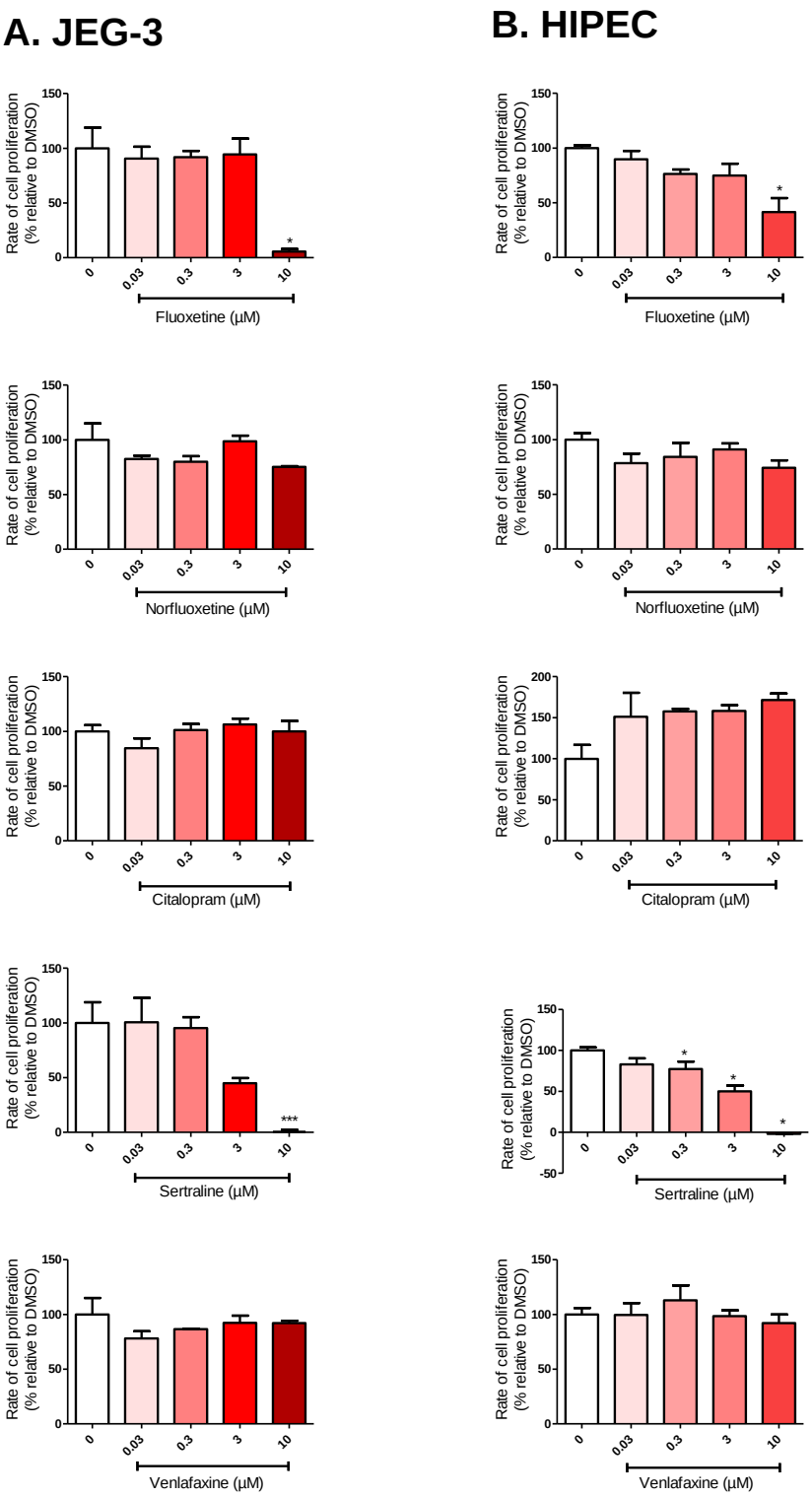


Figure 2: Effects of fluoxetine, norfluoxetine, citalopram, sertraline and venlafaxine on JEG-3 (A) and HIPEC (B) cell cycle.

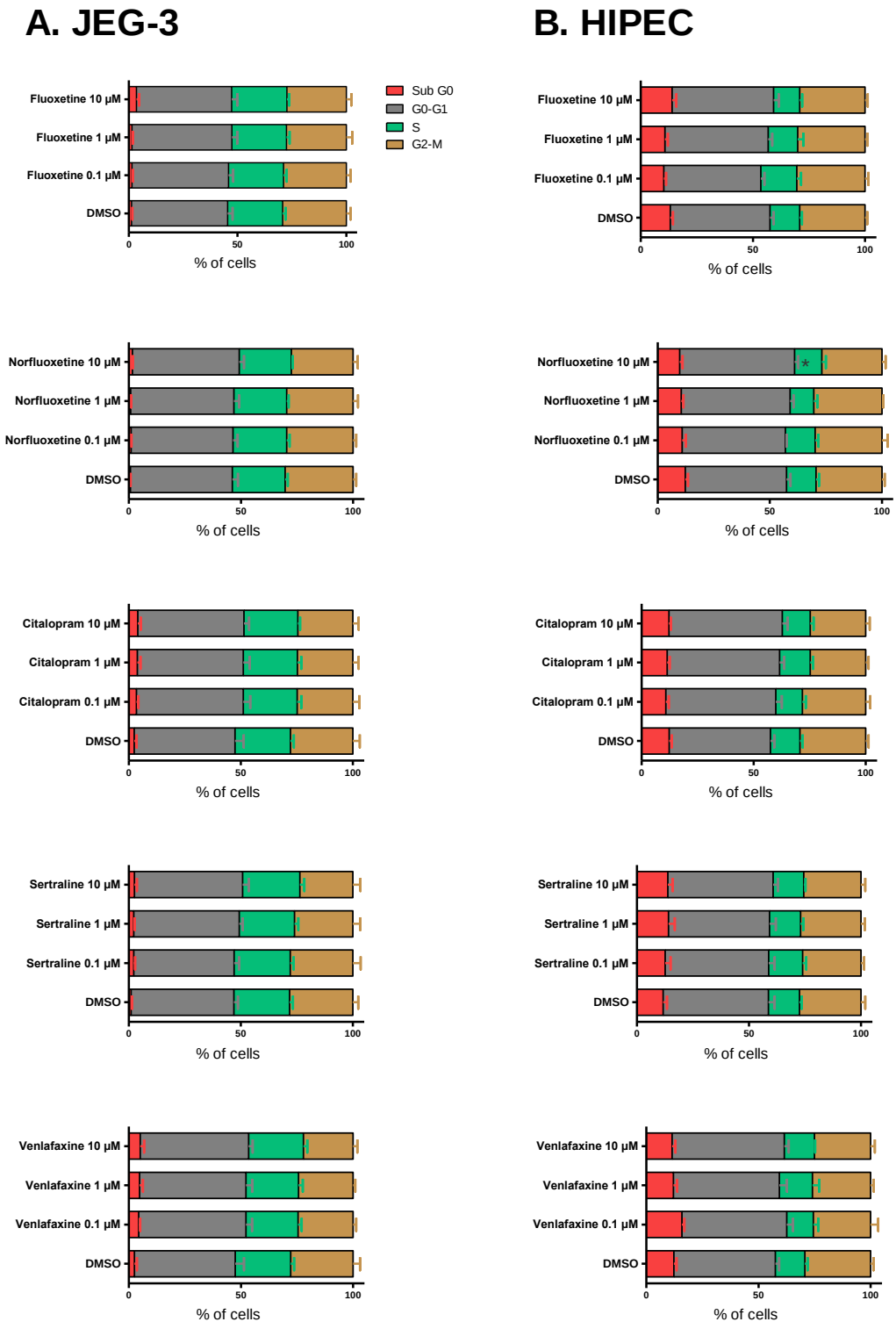


Figure 3: Effects of fluoxetine, norfluoxetine, citalopram, sertraline and venlafaxine on the migration rate of JEG-3 (A) and HIPEC (B) cells.

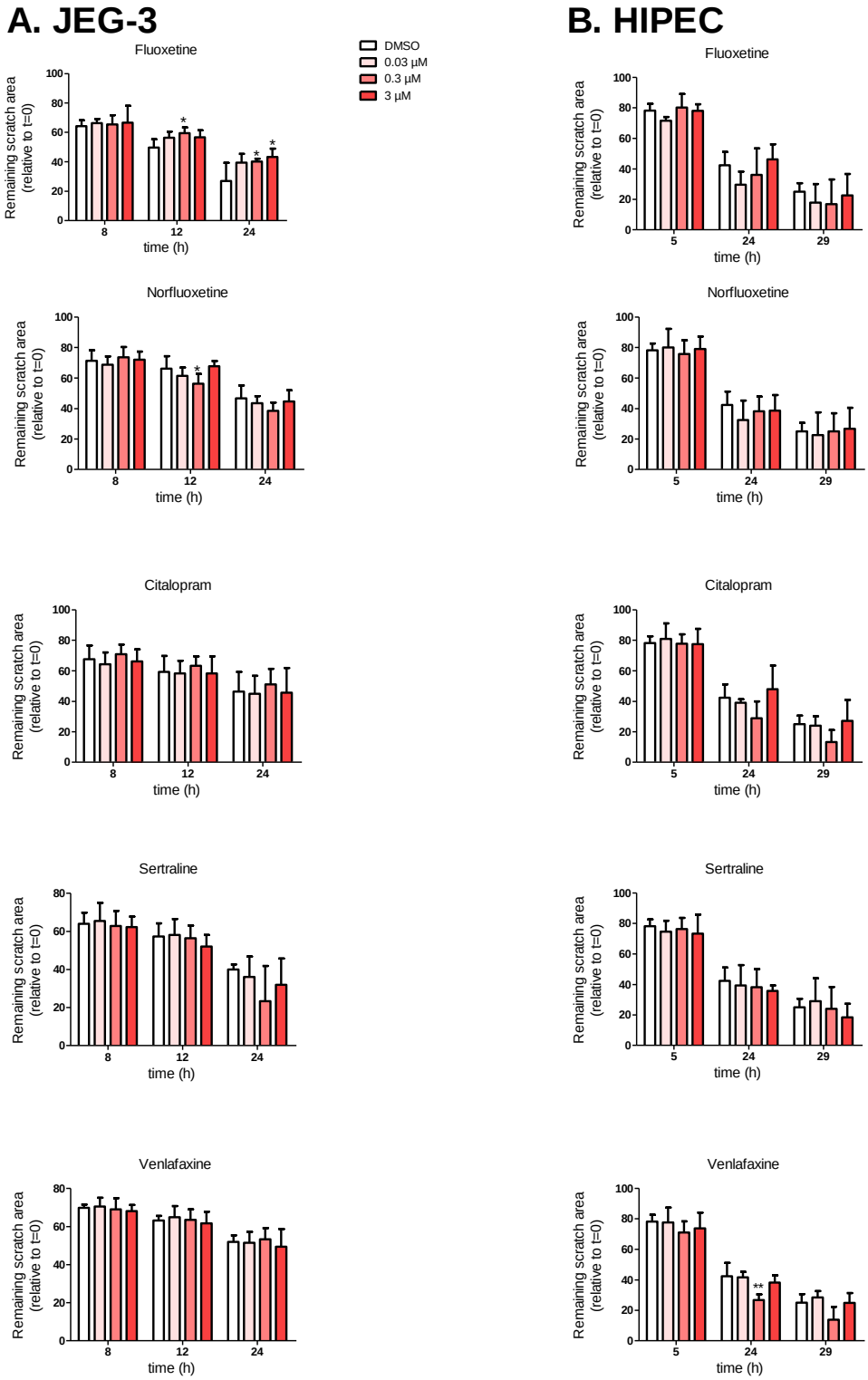


Figure 4: Effects of fluoxetine, norfluoxetine, citalopram, sertraline and venlafaxine on the activities of MMP-2 and MMP-9 secreted by JEG-3 (A) and HIPEC (B) cells.

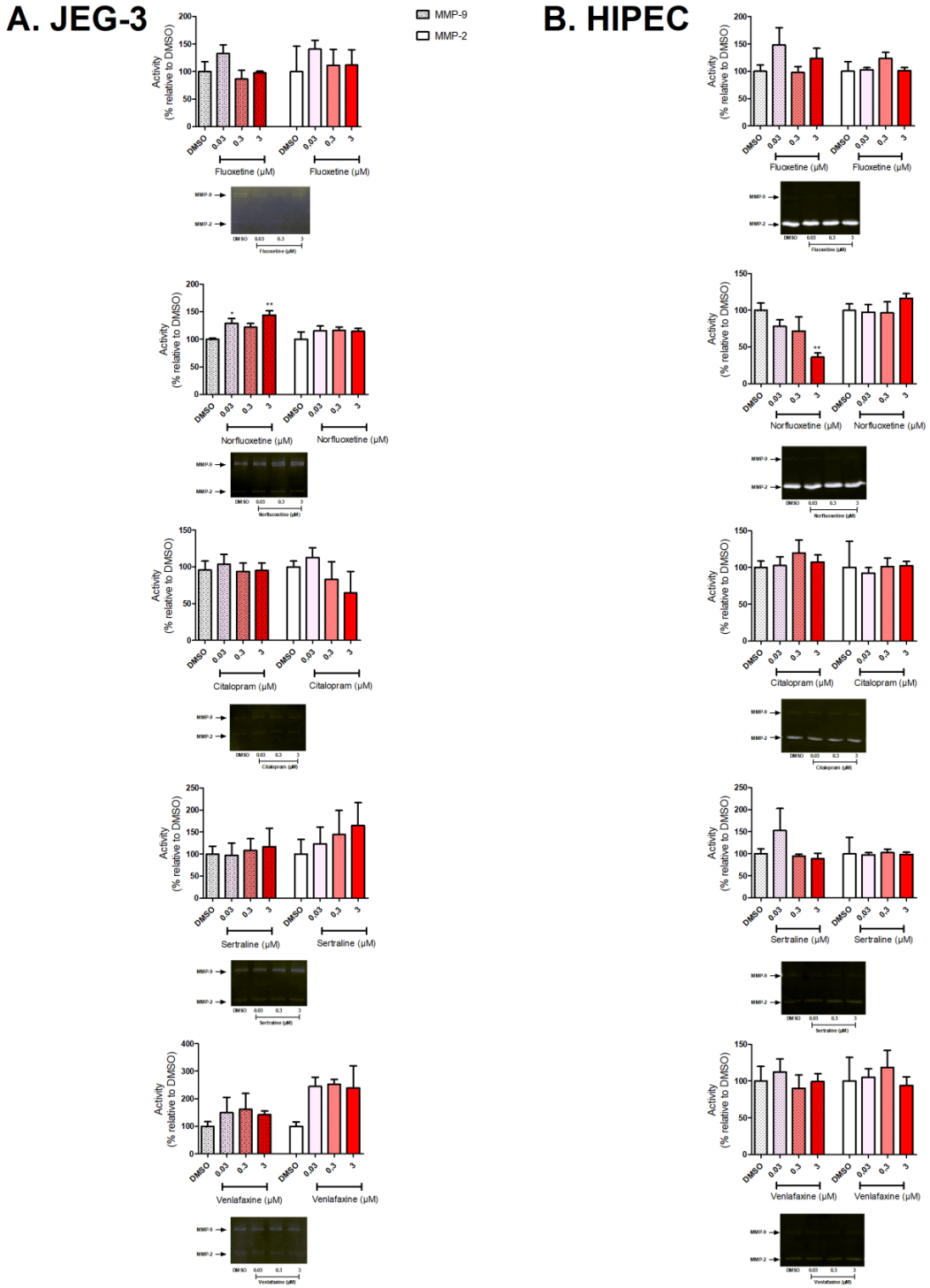


Figure 5: Effects of fluoxetine, norfluoxetine, citalopram, sertraline and venlafaxine on the expression of *matrix metalloproteinase (MMP)-2, -9* and *-14*, *tissue inhibitor of metalloproteinases (TIMP)-1* and *-3*, and *a disintegrin and metalloproteinase domain-containing protein* (*ADAM*)*-10* in JEG-3 cells.

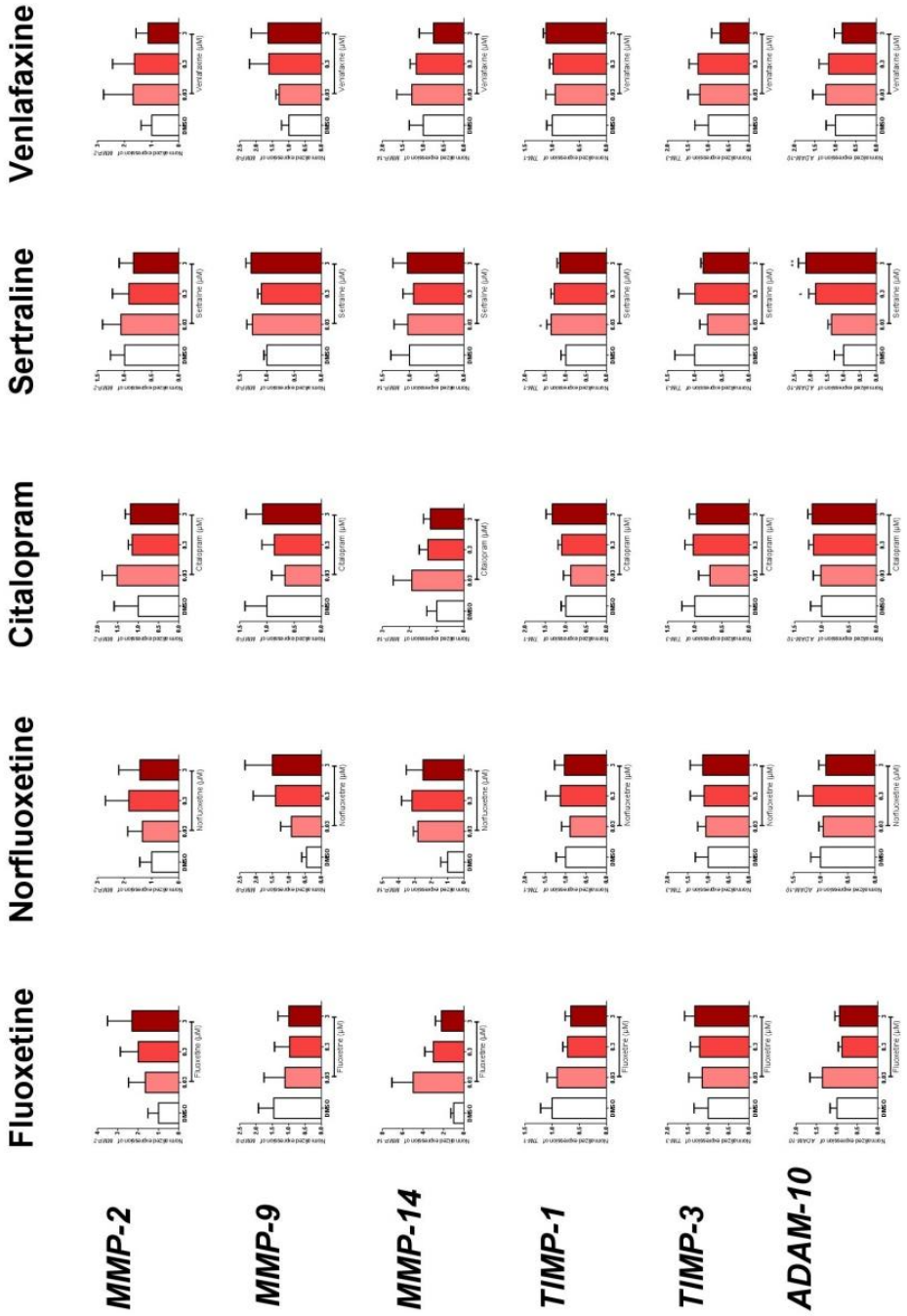
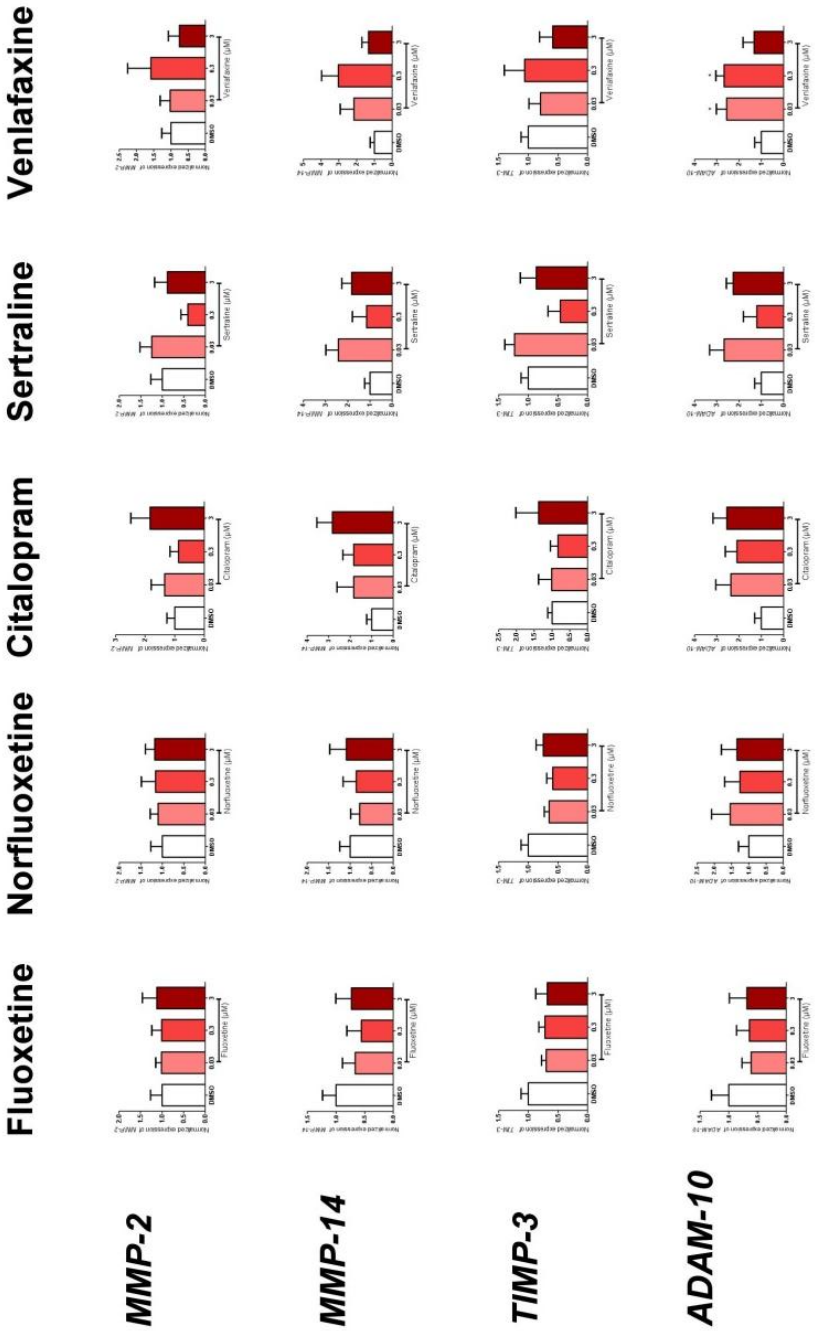


Figure 6: Effects of fluoxetine, norfluoxetine, citalopram, sertraline and venlafaxine on the expression of *matrix metalloproteinase* (*MMP)-2* and *-14*, *tissue inhibitor of metalloproteinases* (*TIMP)-3,* and a *disintegrin and metalloproteinase domain-containing protein* (*ADAM*)*-10* in HIPEC cells.



SUPPLEMENTAL TABLE AND FIGURES

Table S1: Oligonucleotide primer sequences used for gene amplification

Gene (used in cell line)	Accession number	Primer sequence (5'- to -3')	
		Sense	Antisense
MMP-2	NM_001302510.1 (B. Xu *et al.*, 2011)	TGCGACCACAGCCAACTACG	ACAGACGGAAGTTCTTGGTGTAGG
MMP-9	NM_004994.2 (Rasstrigina *et al.*, 2014)	CGACGTCTTCCAGTACCGAG	GTTGGTCCCAGTGGGGATTT
MMP-14	NM_004995.3 (Ma *et al.*, 2014)	CCCAACATCTGTGACGGGAACT	GAGCAGCATCAATCTTGTCGGTAG
TIMP-1	NM_003254.2	GTTGGCTGTGAGGAATGCAC	CGGGACTGGAAGCCCTTTTC
TIMP-3 (used for JEG-3)	NM_000362.4 (Godbole *et al.*, 2011)	CTGACAGGTCGCGTCTATGA	AGTCACAAAGCAAGGCAGGT
TIMP-3 (used for HIPEC)	NM_000362.4 (Hoyer *et al.*, 1994)	GCCTTCTGCAACTCCGACATC	CGTGTACATCTTGCCATCATA
ADAM-10 (used for JEG-3)	NM_001110.3 (H. Qiu *et al.*, 2015)	GACCACAGACTTCTCCGGAAT	TGAAGGTGCTCCAACCCAAG
ADAM-10 (used for HIPEC)	NM_001320570.1	TGCCCAGATCAATACAACTGCT	CCAGACCAAGTACGCCATCA
PPIA	NM_021130 (Lanoix *et al.*, 2012b)	GTTTGCAGACAAGGTCCCA	ACCCGTATGCTTTAGGATG
SDHA	NM_004168 (Lanoix *et al.*, 2012b)	TACAAGGTGCGGATTGATG	CGATCACGGGTCTATATTCAA
TBP	NM_003194 (Lanoix *et al.*, 2012b)	CACGAACCACGGCACTGAT	GTTGGTGGGTGAGCACAAGG

MMP: *matrix metalloproteinase* ; *TIMP*: *tissue inhibitor of metalloproteinases* ; *ADAM-10*: *a disintegrin and metalloproteinase domain-containing protein 10* ; *PPIA*: *peptidylprolyl isomerase A ; SDHA: succinate dehydrogenase complex subunit A, TBP: TATA-box binding protein*.

Figure S1: Molecular structures of serotonin and the SSRIs used in the present study

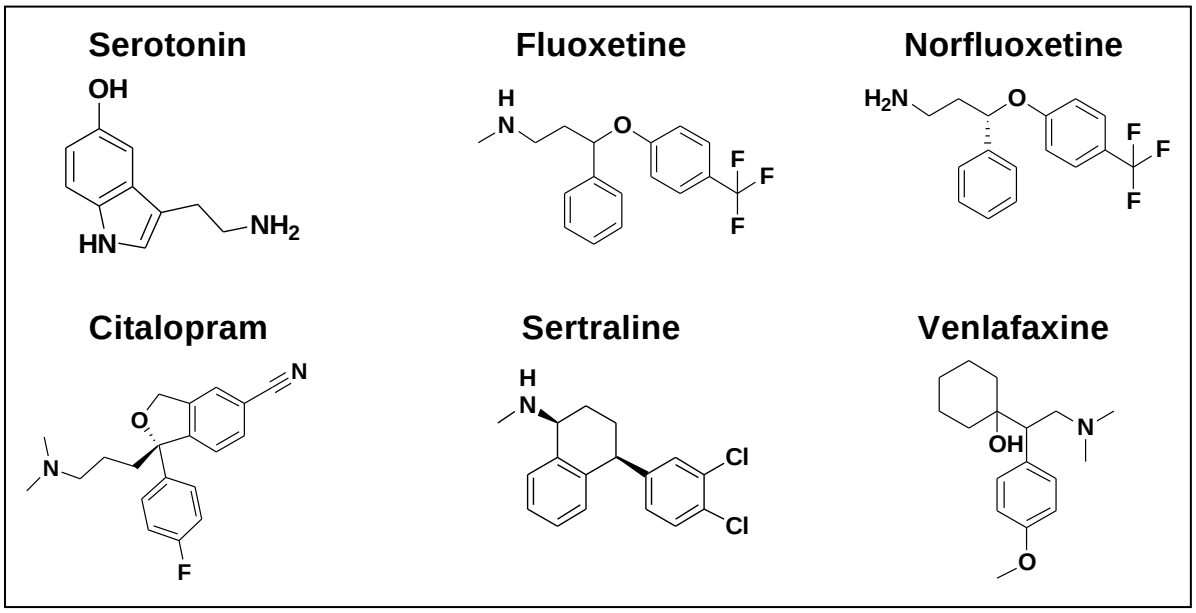


Figure S2: Representative graphs showing the effects of fluoxetine, norfluoxetine, citalopram, sertraline and venlafaxine on JEG-3 (A) and HIPEC (B) cell proliferation.

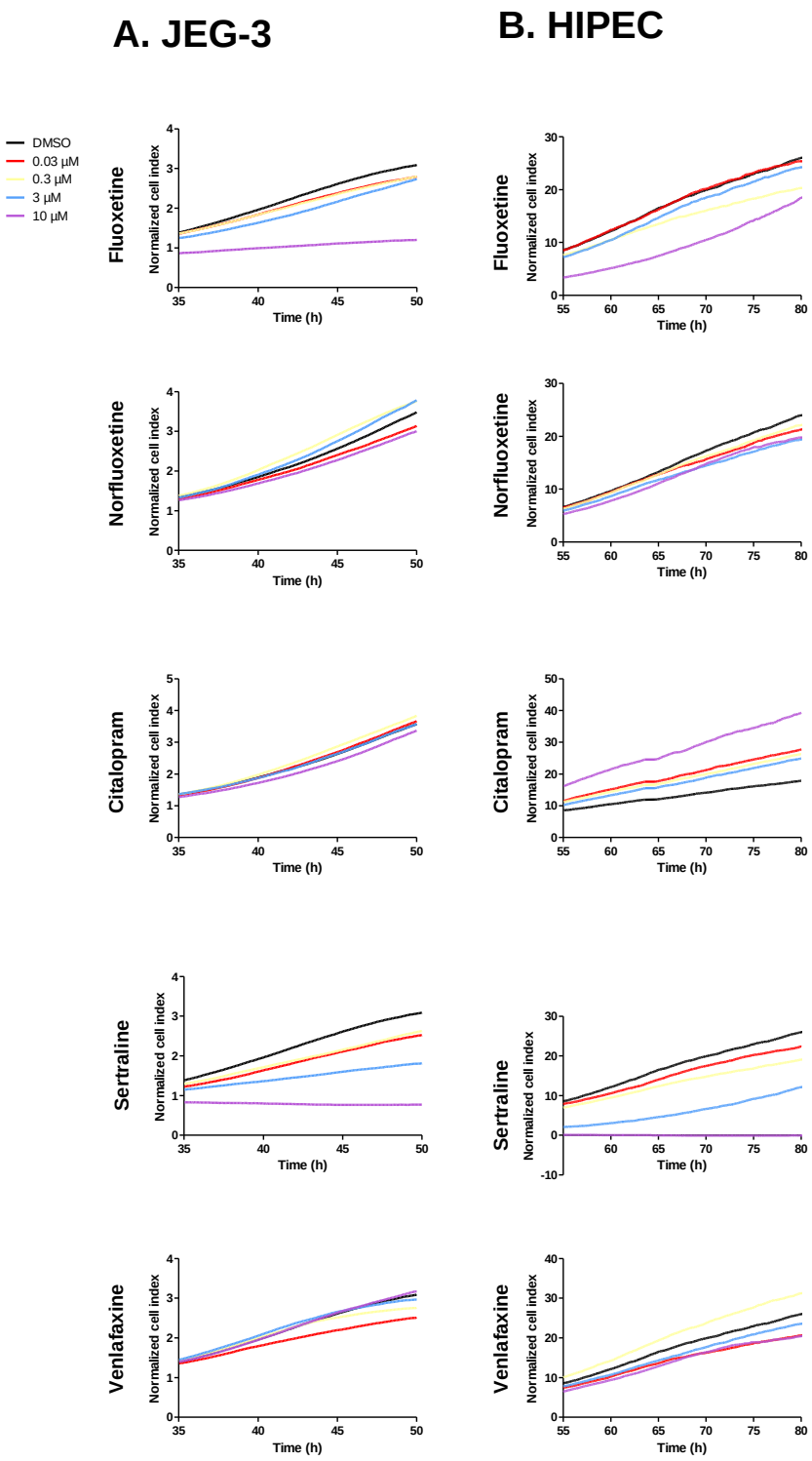
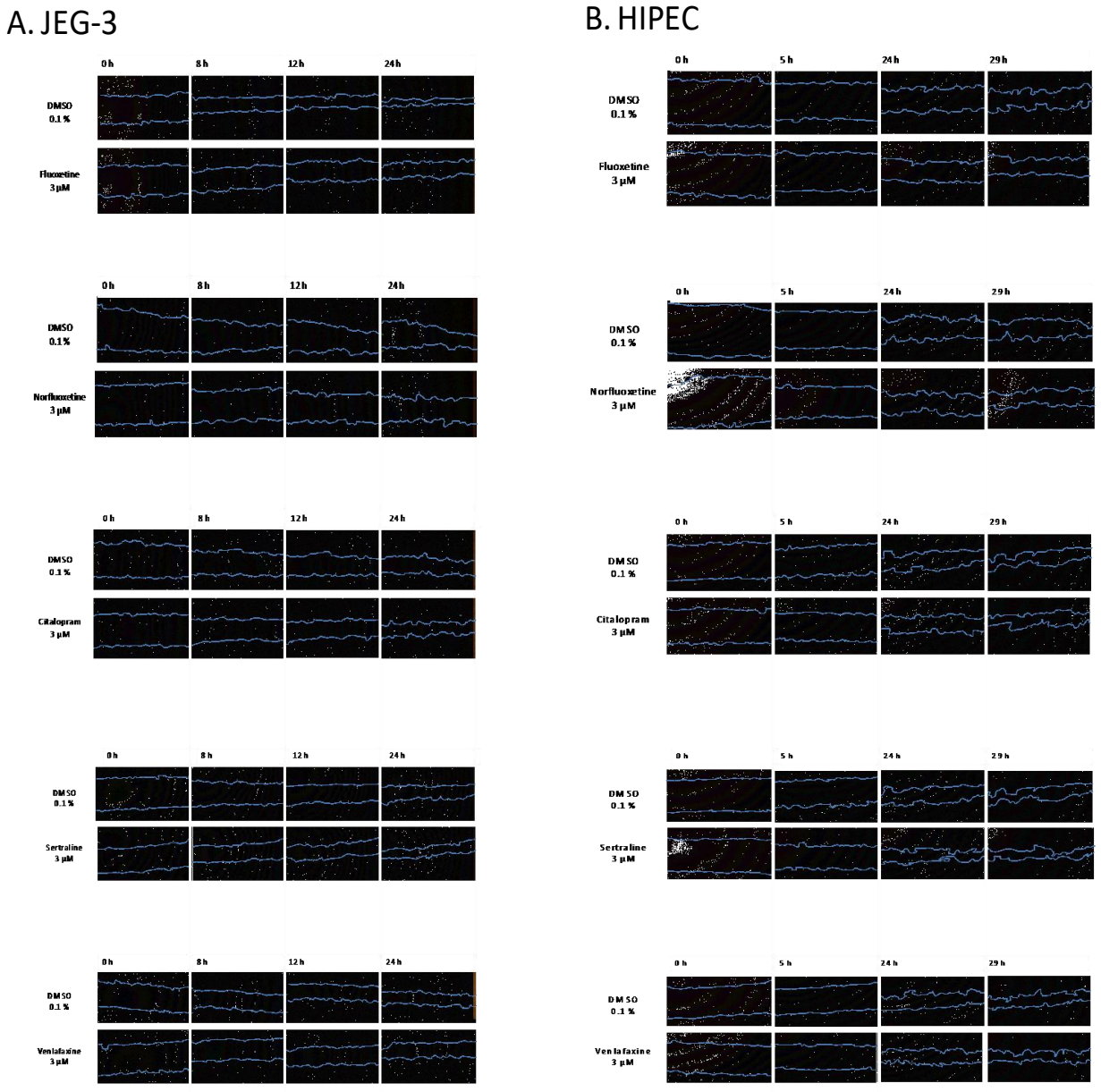


Figure S3: Representative images showing the effects of fluoxetine, norfluoxetine, citalopram, sertraline and venlafaxine on wound closure of JEG-3 (A) and HIPEC (B) cells.



6 CHAPITRE 6 : DISCUSSION

6.1 Avancées méthodologiques

Ce présent projet de doctorat a permis le perfectionnement et la mise en place de nouvelles méthodologies (Chapitres 2 et 3). Les primocultures de cytotrophoblastes villeux issus de placentas à terme représentent un bon modèle de la voie villeuse placentaire car, *in vitro comme in vivo*, elles se syncytialisent de façon spontanée avec une différenciation biochimique et une fusion pour former un syncytiotrophoblaste. L'isolement et la purification de ces cellules comportent plusieurs défis : obtenir une quantité de cellules suffisantes, la pureté et la durée. En 1986, Kliman *et al.* ont été les premiers à mettre au point la technique d'isolement des trophoblastes villeux, basée sur une série de digestions enzymatiques utilisant la trypsine et la DNase, suivie par une séparation des cellules d'intérêt sur un gradient de Percoll© (Kliman *et al.*, 1986). Si plusieurs chercheurs ont depuis adapté la technique de Kliman (Douglas *et al.*, 1989, Knofler *et al.*, 1998, Le Bellego *et al.*, 2009, Newby *et al.*, 2007, Petroff *et al.*, 2006, Potgens *et al.*, 2003, Yui *et al.*, 1994), c'est notre laboratoire qui a perfectionné les étapes de la congélation des cellules et de l'immunopurification. La congélation de ces cellules est un ajout majeur, qui permet de placer les primocultures en biobanque. La purification immunomagnétique avec des anticorps anti-HLA-ABC permet de purifier les cytotrophoblastes, ces anticorps n'étant pas exprimés à la surface des membranes des trophoblastes. Ces perfectionnement méthodologiques ont été publiés dans le livre *Preeclampsia: methods and protocols*, (voir chapitre 2 ; (Clabault *et al.*, 2018)) et dans *Journal of visualized experiments* (JOVE, Annexe II ; (Sagrillo-Fagundes *et al.*, 2016)).

Ce projet a également permis d'adapter la technologie xCELLigence© (ACEA Biosciences, Inc., San Diego, CA) pour l'analyse des cellules trophoblastiques. La majorité des expériences actuelles sont basées sur l'obtention de données à un temps ou à quelques temps précis. La technologie xCELLigence© révolutionne ce principe, en faisant l'acquisition de données, à une fréquence choisie par le chercheur (généralement toutes les 5 à 15 min), pendant la durée de l'expérience, et ce,

simultanément dans tous les puits d'une plaque de 16 ou de 96 puits (Chapitre 3, Table 1) (Chollangi *et al.*, 2018). Le principe est basé sur la mesure de l'impédance (conductivité électrique) en temps réel. La base des puits ou la base des inserts est recouverte de microélectrodes en or. Un courant électrique traverse les électrodes, ce qui permet de mesurer l'impédance, qui est modifiée selon la présence de cellules dans ces puits ou inserts. Les données fournies représentent l'index cellulaire en fonction du temps. Ce système permet donc de caractériser la prolifération, la cytotoxicité, la migration, ou encore l'invasion en temps réel. Il permet également des études plus poussées comme des essais en hypoxie, des tests d'interactions cellulaires (co-culture), l'étude de voies de signalisation récepteur-dépendantes, ou encore du criblage phénotypique (mode d'action de xénobiotiques). Le principe de fonctionnement du système xCELLigence©, ainsi que son application pour les études placentaires, font l'objet d'un chapitre de livre (Chapitre 3). Dans ce projet doctoral, la technologie xCELLigence© a permis d'analyser le comportement prolifératif des cellules trophoblastiques villeuses (la lignée BeWo) et extravilleuses (les lignées JEG-3 et HIPEC), ainsi que d'étudier l'effet des ISRS sur la prolifération de ces lignées cellulaires.

6.2 **Effets des ISRS sur le développement placentaire humain**

Dans la présente étude, des concentrations allant de 0,03 à 10 µM ont été utilisées, ce qui correspond aux doses prescrites aux femmes enceintes, ou plus précisément aux doses mesurées dans le sang maternel et dans le sang de cordon (Chapitre 1, Figure 5). L'avantage d'étudier des doses correspondant à une exposition réelle est que cela permet d'obtenir des données potentiellement plus pertinentes pour la clinique que celles de toxicité réalisées pour les mises en marché (utilisant 10 à 100 fois les doses thérapeutiques). Par ailleurs, la plupart des études publiées utilisent des doses beaucoup plus élevées que celles utilisées dans la pratique médicale, allant jusqu'à 100 µM (Bonnin *et al.*, 2012, Jacobsen *et al.*, 2015, Peng *et al.*, 2012), et parfois jusqu'à 1000 µM (Correia-Branco *et al.*, 2015) soit 10 à 100 fois plus élévé que notre dose maximale utilisée. Ceci explique en grande partie pourquoi ces études observent des effets beaucoup plus marqués que les nôtres (notamment pour ce qui est de la cytotoxicité). Notre étude est la première à étudier *in vitro* l'effet des ISRS aux doses pharmacologiques sur l'homéostasie des trophoblastes villeux et extravilleux.

L'effet des ISRS sur la prolifération a été analysé sur la lignée de trophoblastes villeux (BeWo) (Chapitre 4) et les lignées de trophoblastes extravilleux (JEG-3 et HIPEC) (Chapitre 5). Nous observons que la sertraline, à la concentration la plus forte testée (10 µM), diminue la prolifération dans ces trois lignées cellulaires. La fluoxétine, à 10 µM, diminue également la prolifération des cellules JEG-3 et HIPEC, sans avoir d'effets sur les cellules BeWo, tandis que la paroxétine, à la même concentration, diminue la prolifération des cellules HIPEC, mais n'a pas d'effets sur les cellules BeWo (ces expériences n'ont pas été réalisées avec la lignée JEG-3). Nos résultats, montrant un effet structure- et concentration-dépendant sur la prolifération, sont en accord avec la littérature. Un effet anti-prolifératif de la sertraline à 20 µM et 50 µM a été décrit sur les cellules souches neuronales (Peng *et al.*, 2012). De plus, des effets sur la prolifération de la fluoxétine et de la sertraline ont également été observés par Yavarone *et al.*, dans des cellules cardiaques d'embryon de souris (Yavarone *et al.*, 1993). Cela suggère que parmi les six ISRS testés, la sertraline, la fluoxétine et la paroxétine ont des effets antiprolifératifs et donc potentiellement plus à risque de causer

des altérations du fonctionnement du placenta, et par conséquent, du déroulement de la grossesse et du développement du fœtus. Pour confirmer ces résultats, des analyses sur les primocultures (cellules et explants) de trophoblastes villeux et extravilleux devront être effectuées. Des analyses plus approfondies devront aussi être réalisées afin de déterminer les voies de signalisation impliquées dans ces effets (antiprolifératif, cytotoxique, apoptose, nécrose, etc.).

Nous avons observé que les cellules BeWo sont plus sensibles que les lignées extravilleuses aux effets des ISRS au niveau du cycle cellulaire. D'une façon globale, une augmentation du nombre de cellules en phase G0-G1, et une diminution du nombre de cellules en phase G2-M, ont été observées dans les BeWo uniquement. La même tendance est observée dans les cellules HIPEC, mais n'est significative qu'à 10 µM de norfluoxétine et à 1 et 10 µM de citalopram. Cette sensibilité des cellules HIPEC, par rapport aux JEG-3, au niveau des effets sur le cycle cellulaire pourrait être expliquée par le fait que la lignée HIPEC dérive de primocultures de trophoblastes (primocultures humaines de CTBev immortalisées par transfection de gènes de virus Simien 40) tandis que les JEG-3 sont une lignée cancéreuse (choriocarcinomes placentaires humains) (Hertz, 1959, Pavan *et al.*, 2003). Il serait intéressant, dans une étude future, d'analyser l'effet des ISRS sur le cycle cellulaire des primocultures de trophoblastes extravilleux (car les primocultures de trophoblastes villeux ne prolifèrent pas).

Les résultats des expériences de cytométrie en flux et par la technologie xCELLigence® sont en adéquation car on observe, aux concentrations les plus fortes utilisées, une tendance à l'augmentation du nombre de cellules en phase Sub G0 (pour les BeWo traitées à 10 µM de sertraline, pour les JEG-3 traitées à 10 µM de fluoxétine, citalopram, sertraline et venlafaxine). Un effet des ISRS sur les phases G0-G1 et G2-M a déjà été remarqué à de nombreuses reprises dans la littérature, pour divers modèles cellulaires (Kannen *et al.*, 2015, Krishnan *et al.*, 2008, Serafeim *et al.*, 2003). La voie de signalisation Akt-mTOR (Kannen *et al.*, 2015) ou encore l'inhibition de la *cyclin dependent kinase subunit 1* (CKS1) (Krishnan *et al.*, 2008), ont été évoquées comme étant impliquées dans ce phénomène de modification des phases cellulaires par les ISRS. Il serait intéressant, dans une étude future, d'étudier quelle voie de signalisation est impliquée dans les effets observés dans notre étude.

Nos résultats montrent qu'uniquement la sertraline et la venlafaxine affectent la syncytialisation des primocultures de trophoblastes villeux et des cellules BeWo (fusion cellulaire et sécrétion de hCG). Ces données appuient notre hypothèse suggérant que les ISRS ont des effets structure- et concentration-dépendants. Nos résultats suggèrent que durant la grossesse, la prise de sertraline, comparée à d'autres ISRS (comme le citalopram), présente un risque d'altération de l'homéostasie de la voie trophoblastique villeuse, et donc de la placentation, et avoir des conséquences sur le court et sur le long terme pour la mère et le fœtus. D'autre part, la fluoxétine a un effet sur la migration des cellules JEG-3. Il est intéressant de noter que l'effet sur la migration des CTBev des ISRS testés diffère entre les cellules JEG-3 et HIPEC. On peut émettre l'hypothèse que cela vient du fait que les HIPEC dérivent de primocultures, alors que les JEG-3 sont une lignée cancéreuse. Le profil d'activité de MMP-2 et MMP-9 dans les cellules JEG-3 et HIPEC est également différent. Les cellules JEG-3 ont une forte activité de MMP-9 et une plus faible activité de MMP-2, tandis que les cellules HIPEC ont un profil inverse (activité de MMP-2 supérieure à celle de MMP-9) (Cohen *et al.*, 2006b, Lash *et al.*, 2007). Ces résultats sont en accord avec nos résultats d'analyse de l'ARNm, démontrant que l'expression de MMP-9 dans les cellules HIPEC est trop faible pour être détectée par RT-qPCR. Pour contourner cette limitation, l'utilisation de la technologie du Doplet Digital© PCR (ddPCR) permettrait de quantifier l'expression de MMP-9. Cette technique consiste à réaliser, à partir d'un échantillon, 20000 microgouttelettes par émulsion eau/huile, ce qui permet de quantifier l'expression de gènes faiblement exprimés. Dans la présente étude, on observe que l'activité de la MMP-9 est augmentée par la norfluoxétine et la paroxétine dans les cellules JEG-3, tandis qu'elle est diminuée par la norfluoxétine dans les cellules HIPEC. L'activité de la MMP-2 n'est modifiée par aucun des ISRS, dans les deux lignées. Comme l'expression de la MMP-9 commence à la 6^{e} semaine et augmente jusqu'à la 11^{e} semaine de grossesse, il serait important d'étudier l'effet des ISRS sur l'activité de la MMP-9 dans des primocultures de trophoblastes extravilleux issus de placentas de 1er trimestre tardif (entre la 6^{e} et la 12^{e} semaine de grossesse). De plus, dans une future étude sur les MMP-9, il serait intéressant d'étudier l'activité de leurs inhibiteurs : les TIMPS et ADAM. Nous avons analysé l'expression de *TIMP-1*, *TIMP-3*, *ADAM-10* et *-12* par RT-qPCR et observé une

augmentation du taux d'ARNm d'*ADAM-10* par la sertraline (JEG-3 et HIPEC) et la venlafaxine (HIPEC). Ces effets devront être confirmés au niveau protéique (expression protéique par immunobuvardage de type Western). Les résultats de la présente étude montrent que si certains ISRS semblent avoir un effet sur l'invasion, la migration et la prolifération du trophoblaste extravilleux, ils ne présentent pas tous ces effets, et ils ne les cumulent pas, validant ainsi l'hypothèse que les ISRS ont des effets structure-dépendants. Ces résultats prennent une importance d'autant plus grande que le phénomène d'invasion et de migration des CTBev a lieu au premier trimestre de la grossesse ; or, à ce moment, de nombreuses femmes ne savent encore qu'elles sont enceintes. Les femmes prenant habituellement des ISRS exposent donc leur fœtus et leur placenta à ces antidépresseurs sans le savoir.

Les résultats de cette étude permettent de mieux comprendre les effets des ISRS sur le trophoblaste villeux et extravilleux. Si aucun des ISRS analysés n'a des effets cytotoxiques sur les cellules trophoblastiques, des effets plus subtils sont observés, tels que sur la fusion des CTBv, sur la sécrétion de hCG ou encore sur l'activité des MMP-9. De plus, ces effets sont différents d'une molécule à l'autre. Les effets concentration- et structure-dépendants des ISRS étudiés sont intéressants. Même si les ISRS ont tous la propriété d'inhiber la recapture de la sérotonine, ils ont des affinités pour d'autres récepteurs et transporteurs (Chapitre 1, Tableau 6) et des propriétés physico-chimiques qui diffèrent (lipophilie/hydrophilie, électro-attractivité). La sertraline est l'ISRS qui a le plus d'effets dans notre étude. Une hypothèse pour expliquer ces effets est que la sertraline inhibe le transporteur de la dopamine avec le plus d'affinité, possède un log P supérieur aux autres ISRS (est plus lipophile) et son *polar surface area* (l'écartement entre ses atomes polaires) est inférieur à celui des autres ISRS, ce qui la rend plus susceptible de traverser les membranes cellulaires.

Les résultats de la présente étude permettent de faire un classement des ISRS de la molécule ayant le plus d'effet sur les lignées et primocultures trophoblastiques étudiées à celle en ayant le moins : sertraline > fluoxétine = norfluoxétine > venlafaxine > citalopram. La paroxétine n'est pas prise en compte ici, car cette molécule n'a été testée que pour un certain nombre d'expériences, mais les données préliminaires la placent au même niveau que la sertraline. Il est à noter que la paroxétine est de moins

en moins prescrite chez les femmes enceintes, du fait de sa cardiotoxicité fœtale avérée (Berard *et al.*, 2016, Huybrechts *et al.*, 2014, Nevels *et al.*, 2016). Récemment, Berard *et al.* ont publié une étude sur l'effet de la prise de sertraline au 1er trimestre de grossesse et le risque de malformations fœtales (Berard *et al.*, 2015). Ils ont trouvé que la sertraline augmente, mais de façon non significative, toutes les malformations fœtales par rapport aux femmes n'ayant pas pris d'antidépresseur, qu'il y a une augmentation du risque de malformations ventriculaires et atriales ainsi que de craniosténoses (déformations crâniennes). Ceci va dans le sens de nos résultats, et en direction de la nécessité de poursuivre les recherches sur l'impact des ISRS, et notamment de la sertraline, sur la grossesse et plus particulièrement sur le placenta.

En résumé, avec l'article sur les trophoblastes villeux, nous sommes à notre connaissance les premiers à avoir montré que les ISRS affectent la syncytialisation des trophoblastes de façon structure- et concentration-dépendante. Nous avons également montré que les ISRS affectent différemment la syncytialisation de la lignée cellulaire BeWo et celle des cellules primaires de trophoblastes villeux. Au niveau de la différenciation morphologique, nos résultats ont montré que la sertraline augmente cette différenciation dans les primocultures de trophoblastes villeux. Nous avons également mis en avant le fait que la sertraline à 10 µM affecte la prolifération des cellules BeWo. Avec l'article sur les lignées trophoblastiques extravilleuses JEG-3 et HIPEC, nous avons encore une fois montré pour la première fois que les ISRS affectent ces lignées de manière structure et concentration dépendante. Nous avons découvert que la fluoxétine et la sertraline à 10 µM diminuent la prolifération aussi bien dans la lignée HIPEC et la lignée JEG-3. Enfin, notre article met en avant le fait que la norfluoxétine augmente l'activité de la MMP-9 dans les cellules JEG-3 alors qu'elle diminue son activité dans les cellules HIPEC.

6.3 **Perspectives**

Notre étude devra être supportée par des études sur des primocultures de trophoblastes extravilleux et de trophoblastes villeux de 1er trimestre de grossesse, ainsi que sur des explants placentaires. Les études de 1er trimestre de grossesse sont particulièrement importantes, car comme évoqué précédemment, à cette période de nombreuses femmes enceintes ne savent pas qu'elles sont enceintes et donc exposent leur fœtus et placenta aux ISRS sans le savoir. Or, ce trimestre est crucial pour le développement du placenta puisque c'est à cette période qu'ont lieu l'invasion et la migration des CTBev permettant le remodelage des artères spiralées maternelles indispensable au bon déroulement de la grossesse. Des études *ex vivo* de perfusion du placenta humain permettraient de mieux comprendre la métabolisation placentaire des ISRS : quelle fraction de la molécule mère pénètre dans le placenta, quel(s) métabolites traversent le placenta et à quelle fraction. Des études dans ce sens ont été réalisées par le professeur Bonnin et son équipe. Ils ont utilisé un modèle murin et étudié, à différents stades gestationnels (jours de gestation 14 et 18), la métabolisation du citalopram et son passage au niveau du fœtus, en fonction des pharmacocinétiques maternelle et fœtale (Velasquez *et al.*, 2016). Ils ont constaté que la gestation affecte le profil pharmacocinétique du citalopram (volume de distribution augmente, clairance diminue), et que le citalopram traverse facilement la barrière placentaire pour atteindre rapidement la circulation et le cerveau du fœtus. De plus, ils ont montré que le passage du citalopram du compartiment maternel au compartiment fœtal est indépendant de la présence de SERT placentaire.

L'analyse de placentas d'une cohorte de femmes dépressives, ayant été traitées ou non avec des ISRS durant leur grossesse, apporterait des informations cruciales. En outre, étant dans les conditions physiologiques, toutes les interactions, aussi bien moléculaires que cellulaires, seraient prises en compte. Une étude longitudinale de cohorte permettrait également d'associer les impacts sur le développement de l'enfant. Les limitations d'une étude de cohorte sont que, pour des raisons éthiques évidentes, nous ne pouvons étudier le placenta qu'au terme de la grossesse (après la délivrance) ou suite à un avortement. Il serait également intéressant de poursuivre le projet en

utilisant un modèle animal dépressif ou non dépressif. Les avantages de l'utilisation du modèle animal sont que la durée de gestation est relativement courte comparée à celle de l'humain, qu'il est possible d'interrompre la gestation à tous les stades, et ainsi obtenir des placentas et des fœtus à tous les stades de développement. L'utilisation de modèles animaux dépressifs permet d'étudier l'effet des antidépresseurs avec une condition physiologique dépressive, tandis que l'utilisation d'animaux non dépressifs permet d'étudier le mécanisme d'action des ISRS en condition physiologique normale, ce qui n'est pas possible chez l'humain pour des raisons éthiques évidentes (ce qui permet de séparer l'effet de l'antidépresseur de l'effet de la dépression). Notre laboratoire a déjà fait des recherches en utilisant un modèle de rongeur non déprimé et publié un article sur l'effet de la venlafaxine lors d'une exposition *in utero* chez le rat (Laurent *et al.*, 2016). Cette étude montre que la venlafaxine augmente les anomalies cardiaques, augmente l'index placentaire (ratio poids du fœtus sur poids du placenta), et diminue l'expression du SERT placentaire (gène et protéine) alors même que cette expression est augmentée dans les cœurs des fœtus. Les limitations du modèle animal sont qu'il n'existe pas de modèle animal ayant un placenta identique à celui de l'humain ; il faudrait utiliser un des modèles s'en rapprochant le plus, tels que les modèles primates (difficiles à manipuler et dispendieux), rongeurs (les cochons d'Inde, les rats et les souris) ou lagomorphes (le lapin), qui possèdent une placentation hémochoriale comme celle de la femme (Chavatte Palmer *et al.*, 2008, Grigsby, 2016). L'autre difficulté serait d'avoir un modèle animal dépressif. S'il en existe certains tels que les modèles animaux de la dépression : pharmacologique, basé sur l'induction du stress (exemple : suspension caudale), basé sur la perturbation sociale (exemple : séparation maternelle), sensorielle (exemple : bulbectomie olfactive), basé sur les mutations génétiques ; il reste difficile de distinguer stress et dépression, et d'évaluer objectivement la dépression dans ce genre de modèle (Kalueff *et al.*, 2004).

7 CHAPITRE 7 : CONCLUSION

Dans ce projet doctoral, la méthode d'isolement et de purification de primocultures de cytotrophoblastes villeux issus de placentas à terme, et la méthode d'analyse de la prolifération en temps réel des cellules trophoblastiques par la technologie xCELLigence©, ont été mises au point et/ou perfectionnées afin analyser l'effet des ISRS sur le développement du trophoblaste placentaire. Ce projet a permis de déterminer l'effet de cinq ISRS (la fluoxétine (et son métabolite la norfluoxétine), la sertraline, le citalopram, la paroxétine et la venlafaxine), couramment prescrits durant la grossesse, sur : (1) la syncytialisation du trophoblaste villeux (cellules BeWo et primocultures de cytotrophoblastes villeux de placentas de grossesses normales à terme); et (2) l'invasion, la migration et la prolifération du trophoblaste extravilleux (cellules JEG-3 et HIPEC). Le Tableau 7.1 résume l'ensemble des résultats obtenus. Nous observons que des ISRS analysés, aux concentrations retrouvées dans le sang maternel et dans le sang de cordon, aucun n'a d'effet cytotoxique, mais qu'ils ont des effets concentration- et structure-dépendants sur la syncytialisation du trophoblaste villeux et l'invasion/migration du trophoblaste extravilleux. Cela suggère que le développement placentaire pourrait être compromis par ces antidépresseurs. Les résultats montrent également une différence de sensibilité aux ISRS entre le TBv et le TBev, ainsi qu'entre les lignées et les primocultures. Cela souligne l'importance, pour les études évaluant l'effet des médicaments sur le trophoblaste humain, d'utiliser des cellules primaires, plus près des conditions physiologiques, au lieu de lignées cellulaires. Ce projet doctoral a permis de mieux cerner quels ISRS ont le plus d'effets sur les trophoblastes villeux et extravilleux, et présente un premier classement de ces antidépresseurs les uns par rapport aux autres en fonction de l'impact qu'ils ont sur le développement placentaire. Cette étude a fourni de nouvelles connaissances afin d'éclairer la communauté scientifique et les praticiens sur cette classe d'antidépresseurs, pour le traitement des femmes enceintes, dans le but d'améliorer la santé de la mère et du fœtus.

Tableau 7.1 Tableau récapitulatif des résultats présentés dans le présent projet

		Fluoxétine	Norfluoxétine	Sertraline	Venlafaxine	Citalopram	Paroxétine
Prolifération	BeWo	/	/	tend à ↓ à 10 µM	/	/	/
Cycle cellulaire	BeWo	↑G0-G1 et ↓G2-M	↓G2-M	↑G0-G1 et ↓G2-M	/	/	/
Cellules positives au M30 (apoptose)	Cellules primaires villeuses	/	/	/	/	NT	NT
Fusion	BeWo	/	/	NT	NT	NT	NT
	Cellules primaires villeuses	/	/	↑ à 0,03 ; 0,3 ; 1 µM après 48 h et 72 h	↑ à 0,3 µM après 48 h	NT	NT
Sécrétion de hCG	BeWo	/	/	↓ à 0,1 ; 1 ; 3 µM sans FK ↓ à 0,1 ; 1 et 10 µM avec FK	↑ à 1 et 3 µM avec FK	/	/
	Cellules primaires villeuses	/	/	/	/	NT	NT
Niveaux d'expression d'ARNm	BeWo	/	↑ *β-hCG* à 0,3 ; 1 ; 3µM sans FK ↑ *CX*-43 à 3 µM sans FK	↑ *SYN-1* à 0,3 ; 1 et 3µM avec FK	/	NT	NT
	Cellules primaires villeuses	/	/	/	/	NT	NT

		Fluoxétine	Norfluoxétine	Sertraline	Venlafaxine	Citalopram	Paroxétine
Prolifération	JEG-3	↓ à 10 µM	/	↓ à 10 µM	/	/	↓ à 10 µM
	HIPEC	↓ à 10 µM	/	↓ à 0,3 ; 3 et 10 µM	/	/	NT
Cycle cellulaire	JEG-3	/	/	/	/	/	/
	HIPEC	/	↑G0-G1 à 10 µM	/	/	/	NT
Test de cicatrisation	JEG-3	migration ↓ à 0,3 et 3 µM	migration ↓ à 0.3 µM à 12 h	/	migration ↓ à 0.3 µM à 12	/	/
	HIPEC	/	/	/	/	/	NT
Zymographie en gel de gélatine	JEG-3	/	↑ activité MMP-9	/	/	/	↑ activité MMP-9
	HIPEC	/	↓ activité MMP-9	/	/	/	NT
RT-qPCR	JEG-3	/	/	↑ *TIMP-1* à 0,03 µM ↑ *ADAM-10* à 0,3 et 3 µM	/	/	NT
	HIPEC	/	/	*ADAM-10* à 0,03 µM tendance à ↑	↑ *ADAM-10* à 0,03 et 0,3 µM	/	NT
Invasion	JEG-3	/	/	NT	NT	NT	NT

/ : aucun effet observé*, ADAM-10* : *a desintegrin and metalloproteinase domain-containing protein 10, β-hCG : β-human chorionic gonadotropin* (gene *CGB*), *hPL : human placental lactogen* (gene *CSH*)*, CX-43 : connexine 43*, FK : forskoline, MMP : métalloprotéases matricielles, NT : non testé, *SYN-1 : syncytine-1* (gene *ERVW-1*), *SYN-2 : syncytine-2* (gene *ERVFRD-1*); *TIMP* : *tissue inhibitor of metalloproteinase*

REFERENCES

A. L, Dorado P, Berecz R, Gonzalez AP & Penas LEM (2004) Effect of CYP2D6 and CYP2C9 genotypes on fluoxetine and norfluoxetine plasma concentrations during steady-state conditions. *Eur J Clin Pharmacol* 59(12):869-873.

Doi: 10.1007/s00228-003-0707-y

Abe E, Mocharla H, Yamate T, Taguchi Y & Manolagas SC (1999) Meltrin-alpha, a fusion protein involved in multinucleated giant cell and osteoclast formation. *Calcif Tissue Int* 64(6):508-515.

Doi:

Ackerman WEt, Bulmer JN, Carter AM, Chaillet JR, Chamley L, Chen CP, Chuong EB, Coleman SJ, Collet GP, Croy BA, de Mestre AM, Dickinson H, Ducray J, Enders AC, Fogarty NM, Gauster M, Golos T, Haider S, Heazell AE, Holland OJ, Huppertz B, Husebekk A, John RM, Johnsen GM, Jones CJ, Kalionis B, Konig J, Lorenzon AR, Moffett A, Moreira de Mello JC, Nuzzo AM, Parham P, Parolini O, Petroff MG, Pidoux G, Ramirez-Pinilla MP, Robinson WP, Rolfo A, Sadovsky Y, Soma H, Southcombe JH, Tilburgs T & Lash GE (2012) IFPA Meeting 2011 workshop report III: Placental immunology; epigenetic and microRNA-dependent gene regulation; comparative placentation; trophoblast differentiation; stem cells. *Placenta* 33 Suppl:S15-22.

Doi: 10.1016/j.placenta.2011.11.022

Adibi JJ, Whyatt RM, Hauser R, Bhat HK, Davis BJ, Calafat AM, Hoepner LA, Perera FP, Tang D & Williams PL (2010) Transcriptional biomarkers of steroidogenesis and trophoblast differentiation in the placenta in relation to prenatal phthalate exposure. *Environ Health Perspect* 118(2):291-296.

Doi: 10.1289/ehp.0900788

Adler RR, Ng AK & Rote NS (1995) Monoclonal antiphosphatidylserine antibody inhibits intercellular fusion of the choriocarcinoma line, JAR. *Biol Reprod* 53(4):905-910.

Doi:

Agostini F, Neri E, Salvatori P, Dellabartola S, Bozicevic L & Monti F (2015) Antenatal depressive symptoms associated with specific life events and sources of social support among Italian women. *Matern Child Health J* 19(5):1131-1141.

Doi: 10.1007/s10995-014-1613-x

Alhusen JL, Ayres L & DePriest K (2016) Effects of Maternal Mental Health on Engagement in Favorable Health Practices During Pregnancy. *J Midwifery Womens Health* 61(2):210-216.

Doi: 10.1111/jmwh.12407

Allaman I, Fiumelli H, Magistretti PJ & Martin JL (2011) Fluoxetine regulates the expression of neurotrophic/growth factors and glucose metabolism in astrocytes. *Psychopharmacology (Berl)* 216(1):75-84.

Doi: 10.1007/s00213-011-2190-y

Alsat E, Malasiné A, Wyplosz P, Guibourdenche J & Evain-Brion D (1996a) Hypoxia impairs cell fusion and differentiation of human term cytotrophoblasts, In vitro. *Placenta* 17(5–6):A16.

Doi: http://dx.doi.org/10.1016/S0143-4004(96)90124-1

Alsat E, Malassiné A, Tarrade A, Merviel P & Evain-Brion D (1999) Le cytotrophoblaste humain, un casse-tête pour le biologiste. *Med Sci (Paris)* 15(11):1236-1243.

Doi:

Alsat E, Wyplosz P, Malassine A, Guibourdenche J, Porquet D, Nessmann C & Evain-Brion D (1996b) Hypoxia impairs cell fusion and differentiation process in human cytotrophoblast, in vitro. *Journal of cellular physiology* 168(2):346-353.

Doi: 10.1002/(SICI)1097-4652(199608)168:2<346::AID-JCP13>3.0.CO;2-1

Alwan S, Reefhuis J, Rasmussen SA & Friedman JM (2011) Patterns of antidepressant medication use among pregnant women in a United States population. *J Clin Pharmacol* 51(2):264-270.

Doi: 10.1177/0091270010373928

Anderson GD (2005) Pregnancy-induced changes in pharmacokinetics: a mechanistic-based approach. *Clin Pharmacokinet* 44(10):989-1008.

Doi: 10.2165/00003088-200544100-00001

Andrade SE, Raebel MA, Brown J, Lane K, Livingston J, Boudreau D, Rolnick SJ, Roblin D, Smith DH, Willy ME, Staffa JA & Platt R (2008) Use of antidepressant medications during pregnancy: a multisite study. *Am J Obstet Gynecol* 198(2):194 e191-195.

Doi: 10.1016/j.ajog.2007.07.036

Aplin JD (1991) Implantation, trophoblast differentiation and haemochorial placentation: mechanistic evidence in vivo and in vitro. *J Cell Sci* 99 (Pt 4):681-692.

Doi:

Association_canadienne_pour_la_santé_mentale (2018) *Men and Mental Illness.*), https://cmha.ca/documents/men-and-mental-illness

Auda GR, Kirk SH, Billett MA & Billett EE (1998) Localization of monoamine oxidase mRNA in human placenta. *J Histochem Cytochem* 46(12):1393-1400.

Doi: 10.1177/002215549804601208

Avalos LA, Chen H & Li DK (2015) Antidepressant medication use, depression, and the risk of preeclampsia. *CNS Spectr* 20(1):39-47.

Doi: 10.1017/s1092852915000024

Bach H, Huang Y-y, Underwood MD, Dwork AJ, Mann JJ & Arango V (2014) Elevated Serotonin and 5-HIAA in the Brainstem and Lower Serotonin Turnover in the Prefrontal Cortex of Suicides. *Synapse (New York, N.Y.)* 68(3):127-130.

Doi: 10.1002/syn.21695

Baker AH, Edwards DR & Murphy G (2002) Metalloproteinase inhibitors: biological actions and therapeutic opportunities. *J Cell Sci* 115(Pt 19):3719-3727.

Doi:

Balkovetz DF, Tiruppathi C, Leibach FH, Mahesh VB & Ganapathy V (1989) Evidence for an imipramine-sensitive serotonin transporter in human placental brush-border membranes. *J Biol Chem* 264(4):2195-2198.

Doi:

Banes AK, Shaw SM, Tawfik A, Patel BP, Ogbi S, Fulton D & Marrero MB (2005) Activation of the JAK/STAT pathway in vascular smooth muscle by serotonin. *Am J Physiol Cell Physiol* 288(4):C805-812.

Doi: 10.1152/ajpcell.00385.2004

Bansil P, Kuklina EV, Meikle SF, Posner SF, Kourtis AP, Ellington SR & Jamieson DJ (2010) Maternal and fetal outcomes among women with depression. *J Womens Health (Larchmt)* 19(2):329-334.

Doi: 10.1089/jwh.2009.1387

Bauer S, Pollheimer J, Hartmann J, Husslein P, Aplin JD & Knofler M (2004) Tumor necrosis factor-alpha inhibits trophoblast migration through elevation of plasminogen activator inhibitor-1 in first-trimester villous explant cultures. *J Clin Endocrinol Metab* 89(2):812-822.

Doi: 10.1210/jc.2003-031351

Bayrampour H, McDonald S & Tough S (2015) Risk factors of transient and persistent anxiety during pregnancy. *Midwifery* 31(6):582-589.

Doi: 10.1016/j.midw.2015.02.009

Benmansour S, Cecchi M, Morilak DA, Gerhardt GA, Javors MA, Gould GG & Frazer A (1999) Effects of chronic antidepressant treatments on serotonin transporter function, density, and mRNA level. *J Neurosci* 19(23):10494-10501.

Doi:

Benmansour S, Owens WA, Cecchi M, Morilak DA & Frazer A (2002) Serotonin clearance in vivo is altered to a greater extent by antidepressant-induced downregulation of the serotonin transporter than by acute blockade of this transporter. *J Neurosci* 22(15):6766-6772.

Doi: 20026626

Bennett HA, Einarson A, Taddio A, Koren G & Einarson TR (2004) Prevalence of depression during pregnancy: systematic review. *Obstet Gynecol* 103(4):698-709.

Doi: 10.1097/01.AOG.0000116689.75396.5f

Berard A, Chaabane S & Boukhris T (2014) Antidepressant use in pregnancy and the risk of cardiac defects. *N Engl J Med* 371(12):1167-1168.

Doi: 10.1056/NEJMc1409203#SA2

Berard A, Iessa N, Chaabane S, Muanda FT, Boukhris T & Zhao JP (2016) The risk of major cardiac malformations associated with paroxetine use during the first trimester of pregnancy: a systematic review and meta-analysis. *Br J Clin Pharmacol* 81(4):589-604.

Doi: 10.1111/bcp.12849

Berard A, Zhao JP & Sheehy O (2015) Sertraline use during pregnancy and the risk of major malformations. *Am J Obstet Gynecol* 212(6):795 e791-795 e712.

Doi: 10.1016/j.ajog.2015.01.034

Biaggi A, Conroy S, Pawlby S & Pariante CM (2016) Identifying the women at risk of antenatal anxiety and depression: A systematic review. *J Affect Disord* 191:62-77.

Doi: 10.1016/j.jad.2015.11.014

Billett EE (2004) Monoamine oxidase (MAO) in human peripheral tissues. *Neurotoxicology* 25(1-2):139-148.

Doi: 10.1016/s0161-813x(03)00094-9

Billon S, Ripoll L & Drouet L (2000) Les métalloprotéases de la matrice extracellulaire : présentation générale et rôles dans la pathologie cardiovasculaire. *Sang Thrombose Vaisseaux* 12(3):135-143.

Doi:

Bischof P, Meisser A & Campana A (2000) Paracrine and autocrine regulators of trophoblast invasion--a review. *Placenta* 21 Suppl A:S55-60.

Doi: S0143400400905216 [pii]

Bjorn SF, Hastrup N, Larsen JF, Lund LR & Pyke C (2000) Messenger RNA for membrane-type 2 matrix metalloproteinase, MT2-MMP, is expressed in human placenta of first trimester. *Placenta* 21(2-3):170-176.

Doi: 10.1053/plac.1999.0447

Black S, Kadyrov M, Kaufmann P, Ugele B, Emans N & Huppertz B (2004) Syncytial fusion of human trophoblast depends on caspase 8. *Cell Death Differ* 11(1):90-98.

Doi: 10.1038/sj.cdd.4401307

Blaise S, de Parseval N, Benit L & Heidmann T (2003) Genomewide screening for fusogenic human endogenous retrovirus envelopes identifies syncytin 2, a gene conserved on primate evolution. *Proc Natl Acad Sci U S A* 100(22):13013-13018.

Doi: 10.1073/pnas.2132646100

Blakeley PM, Capron LE, Jensen AB, O'Donnell KJ & Glover V (2013) Maternal prenatal symptoms of depression and down regulation of placental monoamine oxidase A expression. *J Psychosom Res* 75(4):341-345.

Doi: 10.1016/j.jpsychores.2013.07.002

Blond JL, Lavillette D, Cheynet V, Bouton O, Oriol G, Chapel-Fernandes S, Mandrand B, Mallet F & Cosset FL (2000) An envelope glycoprotein of the human endogenous retrovirus HERV-W is expressed in the human placenta and fuses cells expressing the type D mammalian retrovirus receptor. *J Virol* 74(7):3321-3329.

Doi:

Bodecs T, Szilagyi E, Cholnoky P, Sandor J, Gonda X, Rihmer Z & Horvath B (2013) Prevalence and psychosocial background of anxiety and depression emerging during the first trimester of pregnancy: data from a Hungarian population-based sample. *Psychiatr Danub* 25(4):352-358.

Doi:

Bolden-Watson C & Richelson E (1993) Blockade by newly-developed antidepressants of biogenic amine uptake into rat brain synaptosomes. *Life Sci* 52(12):1023-1029.

Doi:

Bolte AC, Van Geijn HP & Dekker GA (2001) Pathophysiology of preeclampsia and the role of serotonin. *Eur J Obstet Gynecol Reprod Biol* 95(1):12-21.

Doi:

Bonnin A, Goeden N, Chen K, Wilson ML, King J, Shih JC, Blakely RD, Deneris ES & Levitt P (2011a) A transient placental source of serotonin for the fetal forebrain. *Nature* 472(7343):347-350.

Doi: 10.1038/nature09972

Bonnin A & Levitt P (2011b) Fetal, maternal, and placental sources of serotonin and new implications for developmental programming of the brain. *Neuroscience* 197:1-7.

Doi: 10.1016/j.neuroscience.2011.10.005

Bonnin A, Zhang L, Blakely RD & Levitt P (2012) The SSRI citalopram affects fetal thalamic axon responsiveness to netrin-1 in vitro independently of SERT antagonism. *Neuropsychopharmacology* 37(8):1879-1884.

Doi: 10.1038/npp.2012.35

Bottalico B, Larsson I, Brodszki J, Hernandez-Andrade E, Casslen B, Marsal K & Hansson SR (2004) Norepinephrine transporter (NET), serotonin transporter (SERT), vesicular monoamine transporter (VMAT2) and organic cation transporters (OCT1, 2 and EMT) in human placenta from pre-eclamptic and normotensive pregnancies. *Placenta* 25(6):518-529.

Doi: 10.1016/j.placenta.2003.10.017

Boukhris T, Sheehy O, Mottron L & Berard A (2016) Antidepressant Use During Pregnancy and the Risk of Autism Spectrum Disorder in Children. *JAMA Pediatr* 170(2):117-124.

Doi: 10.1001/jamapediatrics.2015.3356

Branco-de-Almeida LS, Franco GC, Castro ML, Dos Santos JG, Anbinder AL, Cortelli SC, Kajiya M, Kawai T & Rosalen PL (2012) Fluoxetine inhibits inflammatory response and bone loss in a rat model of ligature-induced periodontitis. *J Periodontol* 83(5):664-671.

Doi: 10.1902/jop.2011.110370

Brittain K, Myer L, Koen N, Koopowitz S, Donald KA, Barnett W, Zar HJ & Stein DJ (2015) Risk Factors for Antenatal Depression and Associations with Infant Birth Outcomes: Results From a South African Birth Cohort Study. *Paediatr Perinat Epidemiol* 29(6):505-514.

Doi: 10.1111/ppe.12216

Bunevicius R, Kusminskas L, Bunevicius A, Nadisauskiene RJ, Jureniene K & Pop VJ (2009) Psychosocial risk factors for depression during pregnancy. *Acta Obstet Gynecol Scand* 88(5):599-605.

Doi: 10.1080/00016340902846049

Buxeraud J & Faure S (2017) Les nouveaux médicaments en cardiologie, gastro-entérologie, psychiatrie et urologie. *Actualités Pharmaceutiques* 56(567, Supplement):37-45.

Doi: https://doi.org/10.1016/j.actpha.2017.03.021

Caniggia I, Grisaru-Gravnosky S, Kuliszewsky M, Post M & Lye SJ (1999) Inhibition of TGF-beta 3 restores the invasive capability of extravillous trophoblasts in preeclamptic pregnancies. *J Clin Invest* 103(12):1641-1650.

Doi: 10.1172/JCI6380

Caniggia I, Lye SJ & Cross JC (1997) Activin is a local regulator of human cytotrophoblast cell differentiation. *Endocrinology* 138(9):3976-3986.

Doi:

Caniggia I, Mostachfi H, Winter J, Gassmann M, Lye SJ, Kuliszewski M & Post M (2000) Hypoxia-inducible factor-1 mediates the biological effects of oxygen on human trophoblast differentiation through TGFbeta(3). *J Clin Invest* 105(5):577-587.

Doi: 10.1172/jci8316

Carino C, Hirchenhain J, Krussel J, Schanz A, Hess A & Larrea F (2003) Estradiol stimulation increases syncytin expression in normal trophoblasts in vitro. *Placenta* (24):A58.

Doi:

Castellucci M, De Matteis R, Meisser A, Cancello R, Monsurro V, Islami D, Sarzani R, Marzioni D, Cinti S & Bischof P (2000) Leptin modulates extracellular matrix molecules and metalloproteinases: possible implications for trophoblast invasion. *Mol Hum Reprod* 6(10):951-958.

Doi:

Challier JC & Uzan S (2003) [The human placenta and its pathologies: focus on oxygen]. *Med Sci (Paris)* 19(11):1111-1120.

Doi: 10.1051/medsci/200319111111

Challis JR, Lockwood CJ, Myatt L, Norman JE, Strauss JF, 3rd & Petraglia F (2009) Inflammation and pregnancy. *Reprod Sci* 16(2):206-215.

Doi: 10.1177/1933719108329095

Chambers AF & Matrisian LM (1997) Changing views of the role of matrix metalloproteinases in metastasis. *J Natl Cancer Inst* 89(17):1260-1270.

Doi:

Charlton RA, Jordan S, Pierini A, Garne E, Neville AJ, Hansen AV, Gini R, Thayer D, Tingay K, Puccini A, Bos HJ, Nybo Andersen AM, Sinclair M, Dolk H & de Jong-van den Berg L (2015) Selective serotonin reuptake inhibitor prescribing before, during and after pregnancy: a population-based study in six European regions. *BJOG* 122(7):1010-1020.

Doi: 10.1111/1471-0528.13143

Chavatte Palmer P, Morel O, Pachy F, Tsatsaris V & Bonneau M (2008) Modèles animaux de retard de croissance intra-utérin d'origine vasculaire. *Bulletin de l'Académie Vétérinaire de France* 161(5):461-466.

Doi: http://prodinra.inra.fr/record/31414

Chen B, Longtine MS, Sadovsky Y & Nelson DM (2010) Hypoxia downregulates p53 but induces apoptosis and enhances expression of BAD in cultures of human syncytiotrophoblasts. *Am J Physiol Cell Physiol* 299(5):C968-976.

Doi: 10.1152/ajpcell.00154.2010

Chen B, Tuuli MG, Longtine MS, Shin JS, Lawrence R, Inder T & Michael Nelson D (2012) Pomegranate juice and punicalagin attenuate oxidative stress and apoptosis in human placenta and in human placental trophoblasts. *Am J Physiol Endocrinol Metab* 302(9):E1142-1152.

Doi: 10.1152/ajpendo.00003.2012

Chen S, Xuan J, Couch L, Iyer A, Wu Y, Li QZ & Guo L (2014a) Sertraline induces endoplasmic reticulum stress in hepatic cells. *Toxicology* 322:78-88.

Doi: 10.1016/j.tox.2014.05.007

Chen S, Xuan J, Wan L, Lin H, Couch L, Mei N, Dobrovolsky VN & Guo L (2014b) Sertraline, an antidepressant, induces apoptosis in hepatic cells through the mitogen-activated protein kinase pathway. *Toxicol Sci* 137(2):404-415.

Doi: 10.1093/toxsci/kft254

Chollangi T, Clabault H, Thibeault AH, Yong HEJ, Narula S, Menkhorst E, Sanderson JT, Vaillancourt C & Murthi P (2018) An Electrical Impedance-Based Assay to Examine Functions of Various Placental Cell Types In Vitro. *Methods Mol Biol* 1710:267-276.

Doi: 10.1007/978-1-4939-7498-6_20

Christian LM, Franco A, Glaser R & Iams JD (2009) Depressive symptoms are associated with elevated serum proinflammatory cytokines among pregnant women. *Brain Behav Immun* 23(6):750-754.

Doi: S0889-1591(09)00055-5 [pii]

10.1016/j.bbi.2009.02.012

Clabault H, Laurent L, Sanderson JT & Vaillancourt C (2018) Isolation and Purification of Villous Cytotrophoblast Cells from Term Human Placenta. *Methods Mol Biol* 1710:219-231.

Doi: 10.1007/978-1-4939-7498-6_17

Cleare AJ (1997) Reduced whole blood serotonin in major depression. *Depression and Anxiety* 5(2):108-111.

Doi: doi:10.1002/(SICI)1520-6394(1997)5:2<108::AID-DA8>3.0.CO;2-B

Cohen M, Meisser A & Bischof P (2006a) Metalloproteinases and human placental invasiveness. *Placenta* 27(8):783-793.

Doi: 10.1016/j.placenta.2005.08.006

Cohen M, Meisser A, Haenggeli L & Bischof P (2006b) Involvement of MAPK pathway in TNF-alpha-induced MMP-9 expression in human trophoblastic cells. *Mol Hum Reprod* 12(4):225-232.

Doi: 10.1093/molehr/gal023

Cole JA, Ephross SA, Cosmatos IS & Walker AM (2007) Paroxetine in the first trimester and the prevalence of congenital malformations. *Pharmacoepidemiol Drug Saf* 16(10):1075-1085.

Doi: 10.1002/pds.1463

Cool DR, Leibach FH & Ganapathy V (1990) Modulation of serotonin uptake kinetics by ions and ion gradients in human placental brush-border membrane vesicles. *Biochemistry* 29(7):1818-1822.

Doi:

Cooper WO, Willy ME, Pont SJ & Ray WA (2007) Increasing use of antidepressants in pregnancy. *Am J Obstet Gynecol* 196(6):544 e541-545.

Doi: 10.1016/j.ajog.2007.01.033

Correia-Branco A, Azevedo CF, Araujo JR, Guimaraes JT, Faria A, Keating E & Martel F (2015) Xanthohumol impairs glucose uptake by a human first-trimester extravillous trophoblast cell line (HTR-8/SVneo cells) and impacts the process of placentation. *Mol Hum Reprod* 21(10):803-815.

Doi: 10.1093/molehr/gav043

Costa MA (2016) The endocrine function of human placenta: an overview. *Reprod Biomed Online* 32(1):14-43.

Doi: 10.1016/j.rbmo.2015.10.005

Costentin J (2009) Les cibles primaires de l'action des médicaments antidépresseurs. *PSN* 7(3-4):140-144.

Doi: 10.1007/s11836-009-0097-z

CRAT (2017) *Quel antidépresseur choisir en cours de grossesse ou d'allaitement.*), http://www.lecrat.org/article.php3?id_article=20 (Consulté le Consulté le 05 août 2017)

Crocker IP, Strachan BK, Lash GE, Cooper S, Warren AY & Baker PN (2001) Vascular endothelial growth factor but not placental growth factor promotes trophoblast syncytialization in vitro. *J Soc Gynecol Investig* 8(6):341-346.

Doi:

Cronier, Bastide B, Hervé JC, Délèze J & Malassiné A (1994) Gap junctional communication during human trophoblast differentiation: influence of human chorionic gonadotropin. *Endocrinology* 135(1):402-408.

Doi: 10.1210/en.135.1.402

Cronier, Defamie N, Dupays L, Theveniau-Ruissy M, Goffin F, Pointis G & Malassine A (2002) Connexin expression and gap junctional intercellular communication in human first trimester trophoblast. *Mol Hum Reprod* 8(11):1005-1013.

Doi:

Cronier, Guibourdenche J, Niger C & Malassine A (1999) Oestradiol stimulates morphological and functional differentiation of human villous cytotrophoblast. *Placenta* 20(8):669-676.

Doi: 10.1053/plac.1999.0423

Cronier L, Alsat E, Hervé J-C, Délèze J & Malassiné A (1998) Dexamethasone stimulates gap junctional communication, peptide hormones production and differentiation in human term trophoblast. *Placenta* 19, Supplement 1(0):35-49.

Doi: http://dx.doi.org/10.1016/S0143-4004(98)80005-2

CTD (2017a) *Citalopram.*), http://ctdbase.org/detail.go?type=chem&acc=D015283 (Consulté le Consulté le 5 janvier 2017)

CTD (2017b) *Fluoxetine.*), http://ctdbase.org/detail.go?page=1&view=gene&type=chem&acc=D005473 (Consulté le Consulté le 5 janvier 2017)

CTD (2017c) *Paroxétine.*), http://ctdbase.org/detail.go?type=chem&acc=D017374 (Consulté le Consulté le 5 janvier 2017)

CTD (2017d) *Sertraline.*), http://ctdbase.org/detail.go?type=chem&acc=D020280 (Consulté le Consulté le 5 janvier 2017)

CTD (2017e) *Venlafaxine.*), http://ctdbase.org/detail.go?type=chem&acc=C047426 (Consulté le Consulté le 5 janvier 2017)

Cusack B, Nelson A & Richelson E (1994) Binding of antidepressants to human brain receptors: focus on newer generation compounds. *Psychopharmacology (Berl)* 114(4):559-565.

Doi:

Dalton P, Christian H, Redman C, Sargent I & Boyd C (2007) Membrane trafficking of CD98 and its ligand galectin 3 in BeWo cells--implication for placental cell fusion. *FEBS J.* 274(11):2715-2727.

Doi: 17451431

Damsky CH, Librach C, Lim KH, Fitzgerald ML, McMaster MT, Janatpour M, Zhou Y, Logan SK & Fisher SJ (1994) Integrin switching regulates normal trophoblast invasion. *Development* 120(12):3657-3666.

Doi:

Daoud G, Amyot M, Rassart E, Masse A, Simoneau L & Lafond J (2005) ERK1/2 and p38 regulate trophoblasts differentiation in human term placenta. *J Physiol* 566(Pt 2):409-423.

Doi: 10.1113/jphysiol.2005.089326

Daszuta A, Ban M, Sr., Soumier A, Hery M & Mocaer E (2005) [Depression and neuroplasticity: implication of serotoninergic systems]. *Therapie* 60(5):461-468.

Doi:

Davalos DB, Yadon CA & Tregellas HC (2012) Untreated prenatal maternal depression and the potential risks to offspring: a review. *Arch Womens Ment Health* 15(1):1-14.

Doi: 10.1007/s00737-011-0251-1

de Paz NC, Sanchez SE, Huaman LE, Chang GD, Pacora PN, Garcia PJ, Ananth CV, Qiu C & Williams MA (2011) Risk of placental abruption in relation to maternal depressive, anxiety and stress symptoms. *J Affect Disord* 130(1-2):280-284.

Doi: S0165-0327(10)00505-7 [pii]

10.1016/j.jad.2010.07.024

Deave T, Heron J, Evans J & Emond A (2008) The impact of maternal depression in pregnancy on early child development. *BJOG* 115(8):1043-1051.

Doi: 10.1111/j.1471-0528.2008.01752.x

Dempsey CM, Mackenzie SM, Gargus A, Blanco G & Sze JY (2005) Serotonin (5HT), fluoxetine, imipramine and dopamine target distinct 5HT receptor signaling to modulate Caenorhabditis elegans egg-laying behavior. *Genetics* 169(3):1425-1436.

Doi: 10.1534/genetics.104.032540

DeVane CL, Stowe ZN, Donovan JL, Newport DJ, Pennell PB, Ritchie JC, Owens MJ & Wang JS (2006) Therapeutic drug monitoring of psychoactive drugs during pregnancy in the genomic era: challenges and opportunities. *J Psychopharmacol* 20(4 Suppl):54-59.

Doi: 10.1177/1359786806066054

Dong JC, Dong H, Campana A & Bischof P (2002) Matrix metalloproteinases and their specific tissue inhibitors in menstruation. *Reproduction* 123(5):621-631.

Doi:

Dorell K, Cohen MA, Huprikar SS, Gorman JM & Jones M (2005) Citalopram-induced diplopia. *Psychosomatics* 46(1):91-93.

Doi: 10.1176/appi.psy.46.1.91

Douglas GC & King BF (1989) Isolation of pure villous cytotrophoblast from term human placenta using immunomagnetic microspheres. *J Immunol Methods* 119(2):259-268.

Doi:

Du L, Bakish D, Lapierre YD, Ravindran AV & Hrdina PD (2000) Association of polymorphism of serotonin 2A receptor gene with suicidal ideation in major depressive disorder. *Am J Med Genet* 96(1):56-60.

Doi:

Eke A, Saccone G & Berghella V (2016) Selective serotonin reuptake inhibitor (SSRI) use during pregnancy and risk of preterm birth: a systematic review and meta-analysis. *BJOG*.

Doi: 10.1111/1471-0528.14144

Englander MT, Dulawa SC, Bhansali P & Schmauss C (2005) How stress and fluoxetine modulate serotonin 2C receptor pre-mRNA editing. *J Neurosci* 25(3):648-651.

Doi: 10.1523/jneurosci.3895-04.2005

Etienne N, Schaerlinger B, Jaffre F & Maroteaux L (2004) [The 5-HT2B receptor: a main cardio-pulmonary target of serotonin]. *J Soc Biol* 198(1):22-29.

Doi:

Evain-Brion D (2001) [The 2 differentiation pathways of the human trophoblast]. *Gynecol Obstet Fertil* 29(7-8):497-502.

Doi:

Evseenko D, Paxton JW & Keelan JA (2006) Active transport across the human placenta: impact on drug efficacy and toxicity. *Expert Opin Drug Metab Toxicol* 2(1):51-69.

Doi: 10.1517/17425255.2.1.51

Ewing G, Tatarchuk Y, Appleby D, Schwartz N & Kim D (2015) Placental transfer of antidepressant medications: implications for postnatal adaptation syndrome. *Clin Pharmacokinet* 54(4):359-370.

Doi: 10.1007/s40262-014-0233-3

Fallet C (2010) Développement placentaire et hCG d'après la communication de T. Fournier. in *J Gynecol Obstet Biol Reprod (Paris)*), p H9-H10.

Fatemi SH, Folsom TD, Reutiman TJ, Pandian T, Braun NN & Haug K (2008) Chronic psychotropic drug treatment causes differential expression of connexin 43 and GFAP in frontal cortex of rats. *Schizophr Res* 104(1-3):127-134.

Doi: 10.1016/j.schres.2008.05.016

FDA (2011) *FDA Drug Safety Communication: Selective serotonin reuptake inhibitor (SSRI) antidepressant use during pregnancy and reports of a rare heart and lung condition in newborn babies.*), http://www.fda.gov/drugs/drugsafety/ucm283375.htm (Consulté le Consulté le 5 août 2017)

Fellenzer JL & Cibula DA (2014) Intendedness of pregnancy and other predictive factors for symptoms of prenatal depression in a population-based study. *Matern Child Health J* 18(10):2426-2436.

Doi: 10.1007/s10995-014-1481-4

Ferretti C, Bruni L, Dangles-Marie V, Pecking AP & Bellet D (2007) Molecular circuits shared by placental and cancer cells, and their implications in the proliferative, invasive and migratory capacities of trophoblasts. *Hum Reprod Update* 13(2):121-141.

Doi: 10.1093/humupd/dml048

Field T, Diego M, Delgado J & Medina L (2013a) Peer support and interpersonal psychotherapy groups experienced decreased prenatal depression, anxiety and cortisol. *Early Hum Dev* 89(9):621-624.

Doi: 10.1016/j.earlhumdev.2013.04.006

Field T, Diego M, Delgado J & Medina L (2013b) Yoga and social support reduce prenatal depression, anxiety and cortisol. *J Bodyw Mov Ther* 17(4):397-403.

Doi: 10.1016/j.jbmt.2013.03.010

Filippi V, Chou D, Ronsmans C, Graham W & Say L (2016) Levels and Causes of Maternal Mortality and Morbidity. *Reproductive, Maternal, Newborn, and Child Health: Disease Control Priorities, Third Edition (Volume 2)*, Black RE, Laxminarayan R, Temmerman M & Walker N (Édit.) 2016 International Bank for Reconstruction and Development / The World Bank., Washington DC.

Fischer U, Janicke RU & Schulze-Osthoff K (2003) Many cuts to ruin: a comprehensive update of caspase substrates. *Cell Death Differ* 10(1):76-100.

Doi: 10.1038/sj.cdd.4401160

Fonseca-Machado Mde O, Alves LC, Monteiro JC, Stefanello J, Nakano AM, Haas VJ & Gomes-Sponholz F (2015) Depressive disorder in pregnant Latin women: does intimate partner violence matter? *J Clin Nurs* 24(9-10):1289-1299.

Doi: 10.1111/jocn.12728

Forlani C, Morri M, Ferrari B, Dalmonte E, Menchetti M, De Ronchi D & Atti AR (2014) Prevalence and Gender Differences in Late-Life Depression: A Population-Based Study. *The American Journal of Geriatric Psychiatry* 22(4):370-380.

Doi: https://doi.org/10.1016/j.jagp.2012.08.015

Fournie A (2012) Epidémiologie et physiopathologie des prééclampsies. *Revues générales Obstétrique* 164.

Doi:

Fournier T, Handschuh K, Tsatsaris V & Evain-Brion D (2007) Involvement of PPARgamma in human trophoblast invasion. *Placenta* 28 Suppl A:S76-81.

Doi: 10.1016/j.placenta.2006.12.006

Fournier T & Tsatsaris V (2008) Développement du placenta humain et physiopathologie de la prééclampsie. *La lettre du gynécologue* Doi:

Freeman EW (2010) Associations of depression with the transition to menopause. *Menopause* 17(4):823-827.

Doi: 10.1097/gme.0b013e3181db9f8b

Freeman EW, Sammel MD, Liu L, Gracia CR, Nelson DB & Hollander L (2004) Hormones and menopausal status as predictors of depression in women in transition to menopause. *Arch Gen Psychiatry* 61(1):62-70.

Doi: 10.1001/archpsyc.61.1.62

Frendo, Cronier L, Bertin G, Guibourdenche J, Vidaud M, Evain-Brion D & Malassine A (2003a) Involvement of connexin 43 in human trophoblast cell fusion and differentiation. *J Cell Sci* 116(Pt 16):3413-3421.

Doi: 10.1242/jcs.00648

Frendo J-L, Olivier D, Cheynet V, Blond J-L, Bouton O, Vidaud M, Rabreau M, Evain-Brion D & Mallet F (2003b) Direct Involvement of HERV-W Env Glycoprotein in Human Trophoblast Cell Fusion and Differentiation. *Molecular and Cellular Biology* 23(10):3566-3574.

Doi: 10.1128/mcb.23.10.3566-3574.2003

Fujiwara H, Higuchi T, Sato Y, Nishioka Y, Zeng BX, Yoshioka S, Tatsumi K, Ueda M & Maeda M (2005) Regulation of human extravillous trophoblast function by membrane-bound peptidases. *Biochim Biophys Acta* 1751(1):26-32.

Doi: 10.1016/j.bbapap.2005.04.007

Garcia-Lloret MI, Morrish DW, Wegmann TG, Honore L, Turner AR & Guilbert LJ (1994) Demonstration of functional cytokine-placental interactions: CSF-1 and GM-CSF stimulate human cytotrophoblast differentiation and peptide hormone secretion. *Exp Cell Res* 214(1):46-54.

Doi: 10.1006/excr.1994.1232

Gauster M, Moser G, Orendi K & Huppertz B (2009) Factors Involved in Regulating Trophoblast Fusion: Potential Role in the Development of Preeclampsia. *Placenta* 30:49-54.

Doi:

Gavin NI, Gaynes BN, Lohr KN, Meltzer-Brody S, Gartlehner G & Swinson T (2005) Perinatal depression: a systematic review of prevalence and incidence. *Obstet Gynecol* 106(5 Pt 1):1071-1083.

Doi: 10.1097/01.AOG.0000183597.31630.db

Gellersen B, Reimann K, Samalecos A, Aupers S & Bamberger AM (2010) Invasiveness of human endometrial stromal cells is promoted by decidualization and by trophoblast-derived signals. *Hum Reprod* 25(4):862-873.

Doi: 10.1093/humrep/dep468

Genbacev O, Joslin R, Damsky CH, Polliotti BM & Fisher SJ (1996) Hypoxia alters early gestation human cytotrophoblast differentiation/invasion in vitro and models the placental defects that occur in preeclampsia. *J Clin Invest* 97(2):540-550.

Doi: 10.1172/jci118447

Gentile S (2015) Untreated depression during pregnancy: Short- and long-term effects in offspring. A systematic review. *Neuroscience*.

Doi: 10.1016/j.neuroscience.2015.09.001

Gentile S & Fusco ML (2017) Placental and fetal effects of antenatal exposure to antidepressants or untreated maternal depression. *J Matern Fetal Neonatal Med* 30(10):1189-1199.

Doi: 10.1080/14767058.2016.1209184

Gerbaud P, Pidoux G, Guibourdenche J, Pathirage N, Costa JM, Badet J, Frendo JL, Murthi P & Evain-Brion D (2011) Mesenchymal activin-A overcomes defective human trisomy 21 trophoblast fusion. *Endocrinology* 152(12):5017-5028.

Doi: 10.1210/en.2011-1193

Godbole G, Suman P, Gupta SK & Modi D (2011) Decidualized endometrial stromal cell derived factors promote trophoblast invasion. *Fertil Steril* 95(4):1278-1283.

Doi: 10.1016/j.fertnstert.2010.09.045

Gong X, Hao J, Tao F, Zhang J, Wang H & Xu R (2013) Pregnancy loss and anxiety and depression during subsequent pregnancies: data from the C-ABC study. *Eur J Obstet Gynecol Reprod Biol* 166(1):30-36.

Doi: 10.1016/j.ejogrb.2012.09.024

Gouvernement_du_Canada (2006) Aspect humain de la santé mentale et de la maladie mentale au Canada.

Doi:

Grigsby PL (2016) Animal Models to Study Placental Development and Function throughout Normal and Dysfunctional Human Pregnancy. *Semin Reprod Med* 34(1):11-16.

Doi: 10.1055/s-0035-1570031

Grote NK, Bridge JA, Gavin AR, Melville JL, Iyengar S & Katon WJ (2010) A meta-analysis of depression during pregnancy and the risk of preterm birth, low birth weight, and intrauterine growth restriction. *Arch Gen Psychiatry* 67(10):1012-1024.

Doi: 10.1001/archgenpsychiatry.2010.111

Groves AK, Kagee A, Maman S, Moodley D & Rouse P (2012) Associations between intimate partner violence and emotional distress among pregnant women in Durban, South Africa. *J Interpers Violence* 27(7):1341-1356.

Doi: 10.1177/0886260511425247

Guillet-Deniau I, Burnol AF & Girard J (1997) Identification and localization of a skeletal muscle secrotonin 5-HT2A receptor coupled to the Jak/STAT pathway. *J Biol Chem* 272(23):14825-14829.

Doi:

Gundacker C, Neesen J, Straka E, Ellinger I, Dolznig H & Hengstschlager M (2016) Genetics of the human placenta: implications for toxicokinetics. *Arch Toxicol* 90(11):2563-2581.

Doi: 10.1007/s00204-016-1816-6

Gustafsson BI, Thommesen L, Stunes AK, Tommeras K, Westbroek I, Waldum HL, Slordahl K, Tamburstuen MV, Reseland JE & Syversen U (2006) Serotonin and fluoxetine modulate bone cell function in vitro. *J Cell Biochem* 98(1):139-151.

Doi: 10.1002/jcb.20734

Hadden C, Fahmi T, Cooper A, Savenka AV, Lupashin VV, Roberts DJ, Maroteaux L, Hauguel-de Mouzon S & Kilic F (2017) Serotonin Transporter Protects the Placental Cells Against Apoptosis in Caspase 3-Independent Pathway. *J Cell Physiol*.

Doi: 10.1002/jcp.25812

Hakkola J, Pasanen M, Hukkanen J, Pelkonen O, Maenpaa J, Edwards RJ, Boobis AR & Raunio H (1996a) Expression of xenobiotic-metabolizing cytochrome P450 forms in human full-term placenta. *Biochem Pharmacol* 51(4):403-411.

Doi:

Hakkola J, Raunio H, Purkunen R, Pelkonen O, Saarikoski S, Cresteil T & Pasanen M (1996b) Detection of cytochrome P450 gene expression in human placenta in first trimester of pregnancy. *Biochem Pharmacol* 52(2):379-383.

Doi:

Halperin R, Peller S, Rotschild M, Bukovsky I & Schneider D (2000) Placental apoptosis in normal and abnormal pregnancies. *Gynecol Obstet Invest* 50(2):84-87.

Doi: 10287

Hamilton GS, Lysiak JJ, Han VK & Lala PK (1998) Autocrine-paracrine regulation of human trophoblast invasiveness by insulin-like growth factor (IGF)-II and IGF-binding protein (IGFBP)-1. *Exp Cell Res* 244(1):147-156.

Doi: 10.1006/excr.1998.4195

Hammen C & Brennan PA (2003) Severity, chronicity, and timing of maternal depression and risk for adolescent offspring diagnoses in a community sample. *Arch Gen Psychiatry* 60(3):253-258.

Doi:

Hamon M (2010) La sérotonine : un rôle complexe dans la dépression et le remodelage osseux. *Médecine/sciences* 26:671-672.

Doi:

Handley SL (1995) 5-Hydroxytryptamine pathways in anxiety and its treatment. *Pharmacol Ther* 66(1):103-148.

Doi:

Handschuh K, Guibourdenche J, Guesnon M, Laurendeau I, Evain-Brion D & Fournier T (2006) Modulation of PAPP-A expression by PPARgamma in human first trimester trophoblast. *Placenta* 27 Suppl A:S127-134.

Doi: 10.1016/j.placenta.2005.10.012

Handschuh K, Guibourdenche J, Tsatsaris V, Guesnon M, Laurendeau I, Evain-Brion D & Fournier T (2007a) Human chorionic gonadotropin expression in human trophoblasts from early placenta: comparative study between villous and extravillous trophoblastic cells. *Placenta* 28(2-3):175-184.

Doi: 10.1016/j.placenta.2006.01.019

Handschuh K, Guibourdenche J, Tsatsaris V, Guesnon M, Laurendeau I, Evain-Brion D & Fournier T (2007b) Human chorionic gonadotropin produced by the invasive trophoblast but not the villous trophoblast promotes cell invasion and is down-regulated by peroxisome proliferator-activated receptor-gamma. *Endocrinology* 148(10):5011-5019.

Doi: 10.1210/en.2007-0286

Hanna J, Goldman-Wohl D, Hamani Y, Avraham I, Greenfield C, Natanson-Yaron S, Prus D, Cohen-Daniel L, Arnon TI, Manaster I, Gazit R, Yutkin V, Benharroch D, Porgador A, Keshet E, Yagel S & Mandelboim O (2006) Decidual NK cells regulate key developmental processes at the human fetal-maternal interface. *Nat Med* 12(9):1065-1074.

Doi: 10.1038/nm1452

Heikkinen T, Ekblad U, Kero P, Ekblad S & Laine K (2002) Citalopram in pregnancy and lactation. *Clin Pharmacol Ther* 72(2):184-191.

Doi: 10.1067/mcp.2002.126181

Heikkinen T, Ekblad U, Palo P & Laine K (2003) Pharmacokinetics of fluoxetine and norfluoxetine in pregnancy and lactation. *Clin Pharmacol Ther* 73(4):330-337.

Doi:

Hendrick V, Smith LM, Suri R, Hwang S, Haynes D & Altshuler L (2003a) Birth outcomes after prenatal exposure to antidepressant medication. *Am J Obstet Gynecol* 188(3):812-815.

Doi:

Hendrick V, Stowe ZN, Altshuler LL, Hwang S, Lee E & Haynes D (2003b) Placental passage of antidepressant medications. *Am J Psychiatry* 160(5):993-996.

Doi:

Henry LK, Field JR, Adkins EM, Parnas ML, Vaughan RA, Zou MF, Newman AH & Blakely RD (2006) Tyr-95 and Ile-172 in transmembrane segments 1 and 3 of human serotonin transporters interact to establish high affinity recognition of antidepressants. *J Biol Chem* 281(4):2012-2023.

Doi: 10.1074/jbc.M505055200

Hertz R (1959) Choriocarcinoma of women maintained in serial passage in hamster and rat. *Proc Soc Exp Biol Med* 102:77-81.

Doi:

Hiemke C & Hartter S (2000) Pharmacokinetics of selective serotonin reuptake inhibitors. *Pharmacol Ther* 85(1):11-28.

Doi:

Hodge JM, Wang Y, Berk M, Collier FM, Fernandes TJ, Constable MJ, Pasco JA, Dodd S, Nicholson GC, Kennedy RL & Williams LJ (2013) Selective serotonin reuptake inhibitors inhibit human osteoclast and osteoblast formation and function. *Biol Psychiatry* 74(1):32-39.

Doi: 10.1016/j.biopsych.2012.11.003

Hofmann GE, Glatstein I, Schatz F, Heller D & Deligdisch L (1994) Immunohistochemical localization of urokinase-type plasminogen activator and the plasminogen activator inhibitors 1 and 2 in early human implantation sites. *Am J Obstet Gynecol* 170(2):671-676.

Doi:

Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin GR, Mylecharane EJ, Saxena PR & Humphrey PP (1994) International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (Serotonin). *Pharmacol Rev* 46(2):157-203.

Doi:

Hrdina PD, Bakish D, Chudzik J, Ravindran A & Lapierre YD (1995) Serotonergic markers in platelets of patients with major depression: upregulation of 5-HT2 receptors. *J Psychiatry Neurosci* 20(1):11-19.

Doi:

Hu, Jin H, Zhou S, Yang P & Li X (2007) Proteomic analysis of hypoxia-induced responses in the syncytialization of human placental cell line BeWo. *Placenta* 28(5-6):399-407.

Doi: 10.1016/j.placenta.2006.07.005

Hu X, Li Y, Hu Z, Rudd JA, Ling S, Jiang F, Davies H & Fang M (2012) The alteration of 5-HT(2A) and 5-HT(2C) receptors is involved in neuronal apoptosis of goldfish cerebellum following traumatic experience. *Neurochem Int* 61(2):207-218.

Doi: 10.1016/j.neuint.2012.04.022

Huang, Zhang CL, Di XY & Zhang RQ (1998) Studies on the localization of 5-hydroxytryptamine and its receptors in human placenta. *Placenta* 19(8):655-661.

Doi:

Huang H, Coleman S, Bridge JA, Yonkers K & Katon W (2014) A meta-analysis of the relationship between antidepressant use in pregnancy and the risk of preterm birth and low birth weight. *Gen Hosp Psychiatry* 36(1):13-18.

Doi: 10.1016/j.genhosppsych.2013.08.002

Huang Y, Zhu XY, Du MR, Wu X, Wang MY & Li DJ (2006) Chemokine CXCL16, a scavenger receptor, induces proliferation and invasion of first-trimester human trophoblast cells in an autocrine manner. *Hum Reprod* 21(4):1083-1091.

Doi: 10.1093/humrep/dei436

Huber AV, Saleh L, Bauer S, Husslein P & Knofler M (2006) TNFalpha-mediated induction of PAI-1 restricts invasion of HTR-8/SVneo trophoblast cells. *Placenta* 27(2-3):127-136.

Doi: 10.1016/j.placenta.2005.02.012

Hudon Thibeault AA, Laurent L, Duy SV, Sauve S, Caron P, Guillemette C, Sanderson JT & Vaillancourt C (2016) Fluoxetine and its active metabolite norfluoxetine disrupt estrogen synthesis in a co-culture model of the feto-placental unit. *Mol Cell Endocrinol*.

Doi: 10.1016/j.mce.2016.11.021

Hughes DA, Kircher M, He Z, Guo S, Fairbrother GL, Moreno CS, Khaitovich P & Stoneking M (2015) Evaluating intra- and inter-individual variation in the human placental transcriptome. *Genome Biol* 16:54.

Doi: 10.1186/s13059-015-0627-z

Humphrey RG, Sonnenberg-Hirche C, Smith SD, Hu C, Barton A, Sadovsky Y & Nelson DM (2008) Epidermal growth factor abrogates hypoxia-induced apoptosis in cultured human trophoblasts through phosphorylation of BAD Serine 112. *Endocrinology* 149(5):2131-2137.

Doi: 10.1210/en.2007-1253

Hung TH & Burton GJ (2006) Hypoxia and reoxygenation: a possible mechanism for placental oxidative stress in preeclampsia. *Taiwan J Obstet Gynecol* 45(3):189-200.

Doi: 10.1016/s1028-4559(09)60224-2

Huppertz B, Frank HG, Kingdom JC, Reister F & Kaufmann P (1998a) Villous cytotrophoblast regulation of the syncytial apoptotic cascade in the human placenta. *Histochem Cell Biol* 110(5):495-508.

Doi:

Huppertz B, Frank HG, Reister F, Kingdom J, Korr H & Kaufmann P (1999) Apoptosis cascade progresses during turnover of human trophoblast: analysis of villous cytotrophoblast and syncytial fragments in vitro. *Lab Invest* 79(12):1687-1702.

Doi:

Huppertz B & Gauster M (2011) Trophoblast fusion. *Adv Exp Med Biol* 713:81-95.

Doi: 10.1007/978-94-007-0763-4_6

Huppertz B, Gauster M, Orendi K, Konig J & Moser G (2009) Oxygen as modulator of trophoblast invasion. *J Anat* 215(1):14-20.

Doi: 10.1111/j.1469-7580.2008.01036.x

Huppertz B, Kertschanska S, Demir AY, Frank HG & Kaufmann P (1998b) Immunohistochemistry of matrix metalloproteinases (MMP), their substrates, and their inhibitors (TIMP) during trophoblast invasion in the human placenta. *Cell Tissue Res* 291(1):133-148.

Doi:

Huppertz B, Tews DS & Kaufmann P (2001) Apoptosis and syncytial fusion in human placental trophoblast and skeletal muscle. *Int Rev Cytol* 205:215-253.

Doi:

Hurskainen T, Seiki M, Apte SS, Syrjakallio-Ylitalo M, Sorsa T, Oikarinen A & Autio-Harmainen H (1998) Production of membrane-type matrix metalloproteinase-1 (MT-MMP-1) in early human placenta. A possible role in placental implantation? *J Histochem Cytochem* 46(2):221-229.

Doi:

Husain N, Cruickshank K, Husain M, Khan S, Tomenson B & Rahman A (2012) Social stress and depression during pregnancy and in the postnatal period in British Pakistani mothers: a cohort study. *J Affect Disord* 140(3):268-276.

Doi: 10.1016/j.jad.2012.02.009

Hutson JR, Garcia-Bournissen F, Davis A & Koren G (2011) The human placental perfusion model: a systematic review and development of a model to predict in vivo transfer of therapeutic drugs. *Clin Pharmacol Ther* 90(1):67-76.

Doi: 10.1038/clpt.2011.66

Huybrechts, Palmsten K, Avorn J, Cohen LS, Holmes LB, Franklin JM, Mogun H, Levin R, Kowal M, Setoguchi S & Hernandez-Diaz S (2014) Antidepressant Use in Pregnancy and the Risk of Cardiac Defects. *The New England journal of medicine* 370(25):2397-2407.

Doi: 10.1056/NEJMoa1312828

Huybrechts, Palmsten K, Mogun H, Kowal M, Avorn J, Setoguchi-Iwata S & Hernandez-Diaz S (2013) National trends in antidepressant medication treatment among publicly insured pregnant women. *Gen Hosp Psychiatry* 35(3):265-271.

Doi: 10.1016/j.genhosppsych.2012.12.010

Hyttel J (1993) Comparative pharmacological studies of selective serotonin reuptake inhibitors (SSRIs). *Nord J Psychiatry* 47(30):5-12.

Doi:

Ilyas S & Moncrieff J (2012) Trends in prescriptions and costs of drugs for mental disorders in England, 1998-2010. *Br J Psychiatry* 200(5):393-398.

Doi: 10.1192/bjp.bp.111.104257

Isaka K, Usuda S, Ito H, Sagawa Y, Nakamura H, Nishi H, Suzuki Y, Li YF & Takayama M (2003) Expression and activity of matrix metalloproteinase 2 and 9 in human trophoblasts. *Placenta* 24(1):53-64.

Doi:

Islami D, Mock P & Bischof P (2001) Effects of human chorionic gonadotropin on trophoblast invasion. *Semin Reprod Med* 19(1):49-53.

Doi:

Jacobs BL & Azmitia EC (1992) Structure and function of the brain serotonin system. *Physiol Rev* 72(1):165-229.

Doi:

Jacobsen NW, Hansen CH, Nellemann C, Styrishave B & Halling-Sorensen B (2015) Effects of selective serotonin reuptake inhibitors on three sex steroids in two versions of the aromatase enzyme inhibition assay and in the H295R cell assay. *Toxicol In Vitro* 29(7):1729-1735.

Doi: 10.1016/j.tiv.2015.07.005

Jacquemin P, Alsat E, Oury C, Belayew A, Muller M, Evain-Brion D & Martial JA (1996) The enhancers of the human placental lactogen B, A, and L genes: progressive activation during in vitro trophoblast differentiation and importance of the DF-3 element in determining their respective activities. *DNA Cell Biol* 15(10):845-854.

Doi:

Jansson T (2001) Amino acid transporters in the human placenta. *Pediatr Res* 49(2):141-147.

Doi: 10.1203/00006450-200102000-00003

Jensen HM, Gron R, Lidegaard O, Pedersen LH, Andersen PK & Kessing LV (2013) Maternal depression, antidepressant use in pregnancy and Apgar scores in infants. *Br J Psychiatry* 202(5):347-351.

Doi: 10.1192/bjp.bp.112.115931

Jeong HG, Lim JS, Lee MS, Kim SH, Jung IK & Joe SH (2013) The association of psychosocial factors and obstetric history with depression in pregnant women: focus on the role of emotional support. *Gen Hosp Psychiatry* 35(4):354-358.

Doi: 10.1016/j.genhosppsych.2013.02.009

Ji L, Brkic J, Liu M, Fu G, Peng C & Wang YL (2012) Placental trophoblast cell differentiation: Physiological regulation and pathological relevance to preeclampsia. *Mol Aspects Med*.

Doi: 10.1016/j.mam.2012.12.008

Jiang B, Kamat A & Mendelson CR (2000) Hypoxia prevents induction of aromatase expression in human trophoblast cells in culture: potential inhibitory role of the hypoxia-inducible transcription factor Mash-2 (mammalian achaete-scute homologous protein-2). *Mol Endocrinol* 14(10):1661-1673.

Doi:

Jin X, Potter B, Luong TL, Nelson J, Vuong C, Potter C, Xie L, Zhang J, Zhang P, Sousa J, Li Q, Pybus BS, Kreishman-Deitrick M, Hickman M, Smith PL, Paris R, Reichard G & Marcsisin SR (2016) Pre-clinical evaluation of CYP 2D6 dependent drug-drug interactions between primaquine and SSRI/SNRI antidepressants. *Malar J* 15(1):280.

Doi: 10.1186/s12936-016-1329-z

John R & Hemberger M (2012) A placenta for life. *Reprod Biomed Online* 25(1):5-11.

Doi: 10.1016/j.rbmo.2012.03.018

Jonnakuty C & Gragnoli C (2008) What do we know about serotonin? *J Cell Physiol* 217(2):301-306.

Doi: 10.1002/jcp.21533

Jordan S, Morris JK, Davies GI, Tucker D, Thayer DS, Luteijn JM, Morgan M, Garne E, Hansen AV, Klungsoyr K, Engeland A, Boyle B & Dolk H (2016) Selective Serotonin Reuptake Inhibitor (SSRI) Antidepressants in Pregnancy and Congenital Anomalies: Analysis of Linked Databases in Wales, Norway and Funen, Denmark. *PLoS One* 11(12):e0165122.

Doi: 10.1371/journal.pone.0165122

Jovanovic M, Stefanoska I, Radojcic L & Vicovac L (2010) Interleukin-8 (CXCL8) stimulates trophoblast cell migration and invasion by increasing levels of matrix metalloproteinase (MMP)2 and MMP9 and integrins alpha5 and beta1. *Reproduction* 139(4):789-798.

Doi: 10.1530/rep-09-0341

Kalueff AV & Tuohimaa P (2004) Experimental modeling of anxiety and depression. *Acta Neurobiol Exp (Wars)* 64(4):439-448.

Doi:

Kannen V, Garcia SB, Silva WA, Jr., Gasser M, Monch R, Alho EJ, Heinsen H, Scholz CJ, Friedrich M, Heinze KG, Waaga-Gasser AM & Stopper H (2015) Oncostatic effects of fluoxetine in experimental colon cancer models. *Cell Signal* 27(9):1781-1788.

Doi: 10.1016/j.cellsig.2015.05.008

Kaplan YC, Keskin-Arslan E, Acar S & Sozmen K (2017) Maternal SSRI discontinuation, use, psychiatric disorder and the risk of autism in children: A meta-analysis of cohort studies. *Br J Clin Pharmacol*.

Doi: 10.1111/bcp.13382

Karkare SU, Bhattacharjee S, Kamble P & Aparasu R (2011) Prevalence and predictors of antidepressant prescribing in nursing home residents in the United States. *Am J Geriatr Pharmacother* 9(2):109-119.

Doi: 10.1016/j.amjopharm.2011.03.001

Kaufmann, Black S & Huppertz B (2003) Endovascular trophoblast invasion: implications for the pathogenesis of intrauterine growth retardation and preeclampsia. *Biol Reprod* 69(1):1-7.

Doi: 10.1095/biolreprod.102.014977

biolreprod.102.014977 [pii]

Kaufmann P & Castellucci M (1997) Extravillous trophoblast in the human placenta: A review. *Placenta* 18, Supplement 2(0):21-65.

Doi: http://dx.doi.org/10.1016/S0143-4004(97)80079-3

Keating E, Lemos C, Azevedo I & Martel F (2006) Characteristics of thiamine uptake by the BeWo human trophoblast cell line. *J Biochem Mol Biol* 39(4):383-393.

Doi:

Keryer G, Alsat E, Taskén K & Evain-Brion D (1998) Role of cyclic AMP-dependent protein kinases in human villous cytotrophoblast differentiation: A review. *Placenta* 19, Supplement 2(0):295-314.

Doi: http://dx.doi.org/10.1016/S0143-4004(98)80050-7

Ketelhuth DF & Back M (2011) The role of matrix metalloproteinases in atherothrombosis. *Curr Atheroscler Rep* 13(2):162-169.

Doi: 10.1007/s11883-010-0159-7

Kim SW, Park SY & Hwang O (2002) Up-regulation of tryptophan hydroxylase expression and serotonin synthesis by sertraline. *Mol Pharmacol* 61(4):778-785.

Doi:

Kingback M, Karlsson L, Zackrisson AL, Carlsson B, Josefsson M, Bengtsson F, Ahlner J & Kugelberg FC (2012) Influence of CYP2D6 genotype on the disposition of the enantiomers of venlafaxine and its major metabolites in postmortem femoral blood. *Forensic Sci Int* 214(1-3):124-134.

Doi: 10.1016/j.forsciint.2011.07.034

Kliman HJ, Nestler JE, Sermasi E, Sanger JM & Strauss JF, 3rd (1986) Purification, characterization, and in vitro differentiation of cytotrophoblasts from human term placentae. *Endocrinology* 118(4):1567-1582.

Doi:

Knerr I, Schubert SW, Wich C, Amann K, Aigner T, Vogler T, Jung R, Dotsch J, Rascher W & Hashemolhosseini S (2005) Stimulation of GCMa and syncytin via cAMP mediated PKA signaling in human trophoblastic cells under normoxic and hypoxic conditions. *FEBS Lett* 579(18):3991-3998.

Doi: 10.1016/j.febslet.2005.06.029

Knofler M, Stenzel M & Husslein P (1998) Shedding of tumour necrosis factor receptors from purified villous term trophoblasts and cytotrophoblastic BeWo cells. *Hum Reprod* 13(8):2308-2316.

Doi:

Kobayashi T, Washiyama K & Ikeda K (2006) Inhibition of G protein-activated inwardly rectifying K+ channels by the antidepressant paroxetine. *J Pharmacol Sci* 102(3):278-287.

Doi:

Kobayashi T, Washiyama K & Ikeda K (2011) Inhibition of G protein-activated inwardly rectifying K+ channels by different classes of antidepressants. *PLoS One* 6(12):e28208.

Doi: 10.1371/journal.pone.0028208

Koch S, Perry KW, Nelson DL, Conway RG, Threlkeld PG & Bymaster FP (2002) R-fluoxetine increases extracellular DA, NE, as well as 5-HT in rat prefrontal cortex and hypothalamus: an in vivo microdialysis and receptor binding study. *Neuropsychopharmacology* 27(6):949-959.

Doi: 10.1016/s0893-133x(02)00377-9

Kohler PO & Bridson WE (1971) Isolation of hormone-producing clonal lines of human choriocarcinoma. *J Clin Endocrinol Metab* 32(5):683-687.

Doi: 10.1210/jcem-32-5-683

Kohli S, Hoffmann J, Lochmann F, Markmeyer P, Huebner H, Fahlbusch FB, Al-Dabet MM, Gadi I, Manoharan J, Lottge M, Zenclussen AC, Aharon A, Brenner B, Shahzad K, Ruebner M & Isermann B (2017) p45 NF-E2 regulates syncytiotrophoblast differentiation by post-translational GCM1 modifications in human intrauterine growth restriction. *Cell Death Dis* 8(4):e2730.

Doi: 10.1038/cddis.2017.127

Krishnan A, Hariharan R, Nair SA & Pillai MR (2008) Fluoxetine mediates G0/G1 arrest by inducing functional inhibition of cyclin dependent kinase subunit (CKS)1. *Biochem Pharmacol* 75(10):1924-1934.

Doi: 10.1016/j.bcp.2008.02.013

Kudo Y & Boyd CA (2002) Human placental amino acid transporter genes: expression and function. *Reproduction* 124(5):593-600.

Doi:

Kudo Y, Boyd CA, Millo J, Sargent IL & Redman CW (2003a) Manipulation of CD98 expression affects both trophoblast cell fusion and amino acid transport activity during syncytialization of human placental BeWo cells. *J Physiol* 550(Pt 1):3-9.

Doi: 10.1113/jphysiol.2003.040550

Kudo Y, Boyd CA, Sargent IL & Redman CW (2003b) Hypoxia alters expression and function of syncytin and its receptor during trophoblast cell fusion of human placental BeWo cells: implications for impaired trophoblast syncytialisation in pre-eclampsia. *Biochim Biophys Acta* 1638(1):63-71.

Doi:

Kumar S, Qiu H, Oezguen N, Herlyn H, Halpert JR & Wojnowski L (2009) Ligand diversity of human and chimpanzee CYP3A4: activation of human CYP3A4 by lithocholic acid results from positive selection. *Drug Metab Dispos* 37(6):1328-1333.

Doi: 10.1124/dmd.108.024372

Kurki T, Hiilesmaa V, Raitasalo R, Mattila H & Ylikorkala O (2000) Depression and anxiety in early pregnancy and risk for preeclampsia. *Obstet Gynecol* 95(4):487-490.

Doi: S0029-7844(99)00602-X [pii]

Lahti M, Savolainen K, Tuovinen S, Pesonen AK, Lahti J, Heinonen K, Hamalainen E, Laivuori H, Villa PM, Reynolds RM, Kajantie E & Raikkonen K (2017) Maternal Depressive Symptoms During and After Pregnancy and Psychiatric Problems in Children. *J Am Acad Child Adolesc Psychiatry* 56(1):30-39 e37.

Doi: 10.1016/j.jaac.2016.10.007

Lam YW, Gaedigk A, Ereshefsky L, Alfaro CL & Simpson J (2002) CYP2D6 inhibition by selective serotonin reuptake inhibitors: analysis of achievable steady-state plasma concentrations and the effect of ultrarapid metabolism at CYP2D6. *Pharmacotherapy* 22(8):1001-1006.

Doi:

Lanoix D, Beghdadi H, Lafond J & Vaillancourt C (2008) Human placental trophoblasts synthesize melatonin and express its receptors. *J Pineal Res* 45(1):50-60.

Doi: JPI555 [pii]

10.1111/j.1600-079X.2008.00555.x

Lanoix D, Lacasse AA, Reiter RJ & Vaillancourt C (2012a) Melatonin: the smart killer: the human trophoblast as a model. *Mol Cell Endocrinol* 348(1):1-11.

Doi: 10.1016/j.mce.2011.08.025

Lanoix D, Lacasse AA, St-Pierre J, Taylor SC, Ethier-Chiasson M, Lafond J & Vaillancourt C (2012b) Quantitative PCR Pitfalls: The Case of the Human Placenta. *Mol Biotechnol*.

Doi: 10.1007/s12033-012-9539-2

Larsson LI, Bjerregaard B & Talts JF (2008) Cell fusions in mammals. *Histochem Cell Biol* 129(5):551-561.

Doi: 10.1007/s00418-008-0411-1

Lash GE, Hornbuckle J, Brunt A, Kirkley M, Searle RF, Robson SC & Bulmer JN (2007) Effect of low oxygen concentrations on trophoblast-like cell line invasion. *Placenta* 28(5-6):390-398.

Doi: 10.1016/j.placenta.2006.06.001

Lash GE, Otun HA, Innes BA, Bulmer JN, Searle RF & Robson SC (2005) Inhibition of trophoblast cell invasion by TGFB1, 2, and 3 is associated with a decrease in active proteases. *Biol Reprod* 73(2):374-381.

Doi: 10.1095/biolreprod.105.040337

Laurent L, Deroy K, St-Pierre J, Cote F, Sanderson JT & Vaillancourt C (2017) Human placenta expresses both peripheral and neuronal isoform of tryptophan hydroxylase. *Biochimie*.

Doi: 10.1016/j.biochi.2017.07.008

Laurent L, Huang C, Ernest SR, Berard A, Vaillancourt C & Hales BF (2016) In utero exposure to venlafaxine, a serotonin-norepinephrine reuptake inhibitor, increases cardiac anomalies and alters placental and heart serotonin signaling in the rat. *Birth Defects Res A Clin Mol Teratol*.

Doi: 10.1002/bdra.23537

Le Bellego F, Vaillancourt C & Lafond J (2009) Isolation and culture of term human cytotrophoblast cells and in vitro methods for studying human cytotrophoblast cells' calcium uptake. *Methods Mol Biol* 550:73-87.

Doi: 10.1007/978-1-60327-009-0_4

Le Bouteiller P (2001) [HLA molecules, immunity and gestation]. *Gynecol Obstet Fertil* 29(7-8):523-531.

Doi:

Le Poul E, Boni C, Hanoun N, Laporte AM, Laaris N, Chauveau J, Hamon M & Lanfumey L (2000) Differential adaptation of brain 5-HT1A and 5-HT1B receptors and 5-HT transporter in rats treated chronically with fluoxetine. *Neuropharmacology* 39(1):110-122.

Doi:

Leduc K, Bourassa V, Asselin E, Leclerc P, Lafond J & Reyes-Moreno C (2012) Leukemia inhibitory factor regulates differentiation of trophoblastlike BeWo cells through the activation of JAK/STAT and MAPK3/1 MAP kinase-signaling pathways. *Biol Reprod* 86(2):54.

Doi: 10.1095/biolreprod.111.094334

Leisser C, Saleh L, Haider S, Husslein H, Sonderegger S & Knofler M (2006) Tumour necrosis factor-alpha impairs chorionic gonadotrophin beta-subunit expression and cell fusion of human villous cytotrophoblast. *Mol Hum Reprod* 12(10):601-609.

Doi: 10.1093/molehr/gal066

Levinson-Castiel R, Merlob P, Linder N, Sirota L & Klinger G (2006) Neonatal abstinence syndrome after in utero exposure to selective serotonin reuptake inhibitors in term infants. *Arch Pediatr Adolesc Med* 160(2):173-176.

Doi: 10.1001/archpedi.160.2.173

Levkovitz Y, Ben-Shushan G, Hershkovitz A, Isaac R, Gil-Ad I, Shvartsman D, Ronen D, Weizman A & Zick Y (2007) Antidepressants induce cellular insulin resistance by activation of IRS-1 kinases. *Mol Cell Neurosci* 36(3):305-312.

Doi: 10.1016/j.mcn.2007.05.009

Levy J-A (1993) *The Retroviridae, Volume 2*. 446 p

Li B, Zhang S, Li M, Hertz L & Peng L (2009) Chronic treatment of astrocytes with therapeutically relevant fluoxetine concentrations enhances cPLA2 expression secondary to 5-HT2B-induced, transactivation-mediated ERK1/2 phosphorylation. *Psychopharmacology (Berl)* 207(1):1-12.

Doi: 10.1007/s00213-009-1631-3

Li B, Zhang S, Zhang H, Nu W, Cai L, Hertz L & Peng L (2008) Fluoxetine-mediated 5-HT2B receptor stimulation in astrocytes causes EGF receptor transactivation and ERK phosphorylation. *Psychopharmacology (Berl)* 201(3):443-458.

Doi: 10.1007/s00213-008-1306-5

Li H, Dakour J, Guilbert LJ, Winkler-Lowen B, Lyall F & Morrish DW (2005) PL74, a novel member of the transforming growth factor-beta superfamily, is overexpressed in preeclampsia and causes apoptosis in trophoblast cells. *J Clin Endocrinol Metab* 90(5):3045-3053.

Doi: 10.1210/jc.2004-0808

Li Y, Klausen C, Zhu H & Leung PC (2015) Activin A Increases Human Trophoblast Invasion by Inducing SNAIL-Mediated MMP2 Up-Regulation Through ALK4. *J Clin Endocrinol Metab* 100(11):E1415-1427.

Doi: 10.1210/jc.2015-2134

Librach CL, Feigenbaum SL, Bass KE, Cui TY, Verastas N, Sadovsky Y, Quigley JP, French DL & Fisher SJ (1994) Interleukin-1 beta regulates human cytotrophoblast metalloproteinase activity and invasion in vitro. *J Biol Chem* 269(25):17125-17131.

Doi:

Librach CL, Werb Z, Fitzgerald ML, Chiu K, Corwin NM, Esteves RA, Grobelny D, Galardy R, Damsky CH & Fisher SJ (1991) 92-kD type IV collagenase mediates invasion of human cytotrophoblasts. *J Cell Biol* 113(2):437-449.

Doi:

Lidberg L, Belfrage H, Bertilsson L, Evenden MM & Asberg M (2000) Suicide attempts and impulse control disorder are related to low cerebrospinal fluid 5-HIAA in mentally disordered violent offenders. *Acta Psychiatr Scand* 101(5):395-402.

Doi:

Lim HK, Duczak N, Jr., Brougham L, Elliot M, Patel K & Chan K (2005) Automated screening with confirmation of mechanism-based inactivation of CYP3A4, CYP2C9, CYP2C19, CYP2D6, and CYP1A2 in pooled human liver microsomes. *Drug Metab Dispos* 33(8):1211-1219.

Doi: 10.1124/dmd.104.003475

Loebstein R, Lalkin A & Koren G (1997) Pharmacokinetic changes during pregnancy and their clinical relevance. *Clin Pharmacokinet* 33(5):328-343.

Doi: 10.2165/00003088-199733050-00002

Lôo H, Galinowski A, Poirier M-F, Chauchot F, Hartmann F, Krebs M-O & Olié J-P (2004) Antidépresseurs. Classifications. *Psychiatrie* 1-6.

Doi:

Lower R, Lower J & Kurth R (1996) The viruses in all of us: characteristics and biological significance of human endogenous retrovirus sequences. *Proc Natl Acad Sci U S A* 93(11):5177-5184.

Doi:

Lydsdottir LB, Howard LM, Olafsdottir H, Thome M, Tyrfingsson P & Sigurdsson JF (2014) The mental health characteristics of pregnant women with depressive symptoms identified by the Edinburgh Postnatal Depression Scale. *J Clin Psychiatry* 75(4):393-398.

Doi: 10.4088/JCP.13m08646

Ma H, Qi MY, Zhang X, Zhang YL, Wang L, Li ZQ, Fu B, Wang WT & Liu D (2014) HSPC117 is regulated by epigenetic modification and is involved in the migration of JEG-3 cells. *Int J Mol Sci* 15(6):10936-10949.

Doi: 10.3390/ijms150610936

Machado-Vieira R, Henter ID & Zarate Jr CA (2017) New targets for rapid antidepressant action. *Progress in Neurobiology* 152(Supplement C):21-37.

Doi: https://doi.org/10.1016/j.pneurobio.2015.12.001

Mahenge B, Likindikoki S, Stockl H & Mbwambo J (2013) Intimate partner violence during pregnancy and associated mental health symptoms among pregnant women in Tanzania: a cross-sectional study. *BJOG* 120(8):940-946.

Doi: 10.1111/1471-0528.12185

Malassine A (2001) [Morphological variability and placental function]. *Gynecol Obstet Fertil* 29(7-8):489-496.

Doi:

Malm H, Sourander A, Gissler M, Gyllenberg D, Hinkka-Yli-Salomaki S, McKeague IW, Artama M & Brown AS (2015) Pregnancy Complications Following Prenatal Exposure to SSRIs or Maternal Psychiatric Disorders: Results From Population-Based National Register Data. *Am J Psychiatry* 172(12):1224-1232.

Doi: 10.1176/appi.ajp.2015.14121575

Mann JJ (1999) Role of the serotonergic system in the pathogenesis of major depression and suicidal behavior. *Neuropsychopharmacology* 21(2 Suppl):99S-105S.

Doi: 10.1016/s0893-133x(99)00040-8

Marcus SM, Flynn HA, Blow FC & Barry KL (2003) Depressive symptoms among pregnant women screened in obstetrics settings. *J Womens Health (Larchmt)* 12(4):373-380.

Doi: 10.1089/154099903765448880

Marin M, Lavillette D, Kelly SM & Kabat D (2003) N-linked glycosylation and sequence changes in a critical negative control region of the ASCT1 and ASCT2 neutral amino acid transporters determine their retroviral receptor functions. *J Virol* 77(5):2936-2945.

Doi:

Mars B, Heron J, Kessler D, Davies NM, Martin RM, Thomas KH & Gunnell D (2017) Influences on antidepressant prescribing trends in the UK: 1995–2011. *Social Psychiatry and Psychiatric Epidemiology* 52(2):193-200.

Doi: 10.1007/s00127-016-1306-4

Maurer-Spurej E, Pittendreigh C & Misri S (2007) Platelet serotonin levels support depression scores for women with postpartum depression. *Journal of Psychiatry and Neuroscience* 32(1):23-29.

Doi:

May P & May E (1999) Rôles de P53 dans le cycle cellulaire, l'apoptose et le cancer. *Revue Française des Laboratoires* 1999(311):35-42.

Doi: https://doi.org/10.1016/S0338-9898(99)80035-3

Mi S, Lee X, Li X, Veldman GM, Finnerty H, Racie L, LaVallie E, Tang XY, Edouard P, Howes S, Keith JC, Jr. & McCoy JM (2000) Syncytin is a captive retroviral envelope protein involved in human placental morphogenesis. *Nature* 403(6771):785-789.

Doi: 10.1038/35001608

Michopoulos V, Berga SL & Wilson ME (2011) Estradiol and progesterone modify the effects of the serotonin reuptake transporter polymorphism on serotonergic responsivity to citalopram. *Exp Clin Psychopharmacol* 19(6):401-408.

Doi: 10.1037/a0025008

Millard SJ, Weston-Green K & Newell KA (2017) The effects of maternal antidepressant use on offspring behaviour and brain development: implications for risk of neurodevelopmental disorders. *Neurosci Biobehav Rev*.

Doi: 10.1016/j.neubiorev.2017.06.008

Miyata S, Hamamura T, Lee Y, Miki M, Habara T, Oka T, Endo S, Taoka H & Kuroda S (2005) Contrasting Fos expression induced by acute reboxetine and fluoxetine in the rat forebrain: neuroanatomical substrates for the antidepressant effect. *Psychopharmacology (Berl)* 177(3):289-295.

Doi: 10.1007/s00213-004-2072-7

Molteni R, Calabrese F, Bedogni F, Tongiorgi E, Fumagalli F, Racagni G & Riva MA (2006) Chronic treatment with fluoxetine up-regulates cellular BDNF mRNA expression in rat dopaminergic regions. *Int J Neuropsychopharmacol* 9(3):307-317.

Doi: 10.1017/s1461145705005766

Montgomery SA (2008) Tolerability of serotonin norepinephrine reuptake inhibitor antidepressants. *CNS Spectr* 13(7 Suppl 11):27-33.

Doi:

Morikawa O, Sakai N, Obara H & Saito N (1998) Effects of interferon-alpha, interferon-gamma and cAMP on the transcriptional regulation of the serotonin transporter. *Eur J Pharmacol* 349(2-3):317-324.

Doi:

Morrish DW, Bhardwaj D, Dabbagh LK, Marusyk H & Siy O (1987) Epidermal growth factor induces differentiation and secretion of human chorionic gonadotropin and placental lactogen in normal human placenta. *J Clin Endocrinol Metab* 65(6):1282-1290.

Doi:

Morrish DW, Bhardwaj D & Paras MT (1991) Transforming growth factor beta 1 inhibits placental differentiation and human chorionic gonadotropin and human placental lactogen secretion. *Endocrinology* 129(1):22-26.

Doi:

Muller-Oerlinghausen B, Roggenbach J & Franke L (2004) Serotonergic platelet markers of suicidal behavior--do they really exist? *J Affect Disord* 79(1-3):13-24.

Doi: 10.1016/s0165-0327(02)00367-1

Nachtigall MJ, Kliman HJ, Feinberg RF, Olive DL, Engin O & Arici A (1996) The effect of leukemia inhibitory factor (LIF) on trophoblast differentiation: a potential role in human implantation. *J Clin Endocrinol Metab* 81(2):801-806.

Doi:

Nebigil CG, Garnovskaya MN, Spurney RF & Raymond JR (1995) Identification of a rat glomerular mesangial cell mitogenic 5-HT2A receptor. *Am J Physiol* 268(1 Pt 2):F122-127.

Doi:

Nembhard WN, Tang X, Hu Z, MacLeod S, Stowe Z & Webber D (2017) Maternal and infant genetic variants, maternal periconceptional use of selective serotonin reuptake inhibitors, and risk of congenital heart defects in offspring: population based study. *BMJ* 356:j832.

Doi: 10.1136/bmj.j832

Neumaier JF, Root DC & Hamblin MW (1996) Chronic fluoxetine reduces serotonin transporter mRNA and 5-HT1B mRNA in a sequential manner in the rat dorsal raphe nucleus. *Neuropsychopharmacology* 15(5):515-522.

Doi: 10.1016/s0893-133x(96)00095-4

Nevels RM, Gontkovsky ST & Williams BE (2016) Paroxetine-The Antidepressant from Hell? Probably Not, But Caution Required. *Psychopharmacol Bull* 46(1):77-104.

Doi:

Newby D, Dalgliesh GL, Aitken DA & Lyall F (2007) Effect of cryopreservation on human cytotrophoblast cells in culture: hCG and PALP production. *Placenta* 28(4):350-352.

Doi: 10.1016/j.placenta.2006.04.012

Ni YG & Miledi R (1997) Blockage of 5HT2C serotonin receptors by fluoxetine (Prozac). *Proc Natl Acad Sci U S A* 94(5):2036-2040.

Doi:

Niederhoffer N, Etienne-Selloum N & Faure S (2016) Les différentes familles d'antidépresseurs. *Actualités Pharmaceutiques* 55(561, Supplement):1-10.

Doi: http://dx.doi.org/10.1016/j.actpha.2016.09.018

Norby U, Forsberg L, Wide K, Sjors G, Winbladh B & Kallen K (2016) Neonatal Morbidity After Maternal Use of Antidepressant Drugs During Pregnancy. *Pediatrics* 138(5).

Doi: 10.1542/peds.2016-0181

Nulman I, Koren G, Rovet J, Barrera M, Pulver A, Streiner D & Feldman B (2012) Neurodevelopment of children following prenatal exposure to venlafaxine, selective serotonin reuptake inhibitors, or untreated maternal depression. *Am J Psychiatry* 169(11):1165-1174.

Doi: 10.1176/appi.ajp.2012.11111721

O'Tierney-Ginn PF & Lash GE (2014) Beyond pregnancy: modulation of trophoblast invasion and its consequences for fetal growth and long-term children's health. *J Reprod Immunol* 104-105:37-42.

Doi: 10.1016/j.jri.2014.04.002

Oates M (2003) Perinatal psychiatric disorders: a leading cause of maternal morbidity and mortality. *Br Med Bull* 67:219-229.

Doi:

Obach RS, Cox LM & Tremaine LM (2005) Sertraline is metabolized by multiple cytochrome P450 enzymes, monoamine oxidases, and glucuronyl transferases in human: an in vitro study. *Drug Metab Dispos* 33(2):262-270.

Doi: 10.1124/dmd.104.002428

Oberlander TF, Warburton W, Misri S, Aghajanian J & Hertzman C (2006) Neonatal outcomes after prenatal exposure to selective serotonin reuptake inhibitor antidepressants and maternal depression using population-based linked health data. *Arch Gen Psychiatry* 63(8):898-906.

Doi: 10.1001/archpsyc.63.8.898

Odile S-V (2012) *Sérotonine : Aspects biologiques et cliniques.* Médecine Sciences Publications,

Olivier JD, Akerud H, Skalkidou A, Kaihola H & Sundstrom-Poromaa I (2014) The effects of antenatal depression and antidepressant treatment on placental gene expression. *Front Cell Neurosci* 8:465.

Doi: 10.3389/fncel.2014.00465

OMS (2013) *Gender and women's mental health.*), http://www.who.int/mental_health/prevention/genderwomen/en/

Orendi K, Gauster M, Moser G, Meiri H & Huppertz B (2010) The choriocarcinoma cell line BeWo: syncytial fusion and expression of syncytium-specific proteins. *Reproduction* 140(5):759-766.

Doi: 10.1530/REP-10-0221

Ornoy A & Koren G (2017) Selective Serotonin Reuptake Inhibitors during Pregnancy: Do We Have Now More Definite Answers Related to Prenatal Exposure? *Birth Defects Res* 109(12):898-908.

Doi: 10.1002/bdr2.1078

Oufkir T, Arseneault M, Sanderson JT & Vaillancourt C (2010) The 5-HT 2A serotonin receptor enhances cell viability, affects cell cycle progression and activates MEK-ERK1/2 and JAK2-STAT3 signalling pathways in human choriocarcinoma cell lines. *Placenta* 31(5):439-447.

Doi: S0143-4004(10)00098-6 [pii]

10.1016/j.placenta.2010.02.019

Oufkir T & Vaillancourt C (2011) Phosphorylation of JAK2 by serotonin 5-HT (2A) receptor activates both STAT3 and ERK1/2 pathways and increases growth of JEG-3 human placental choriocarcinoma cell. *Placenta* 32(12):1033-1040.

Doi: S0143-4004(11)00450-4 [pii]

10.1016/j.placenta.2011.09.005

Paiva P, Salamonsen LA, Manuelpillai U & Dimitriadis E (2009) Interleukin 11 inhibits human trophoblast invasion indicating a likely role in the decidual restraint of trophoblast invasion during placentation. *Biol Reprod* 80(2):302-310.

Doi: 10.1095/biolreprod.108.071415

Palvimaki EP, Roth BL, Majasuo H, Laakso A, Kuoppamaki M, Syvalahti E & Hietala J (1996) Interactions of selective serotonin reuptake inhibitors with the serotonin 5-HT2c receptor. *Psychopharmacology (Berl)* 126(3):234-240.

Doi:

Pandey GN, Pandey SC, Dwivedi Y, Sharma RP, Janicak PG & Davis JM (1995) Platelet serotonin-2A receptors: a potential biological marker for suicidal behavior. *Am J Psychiatry* 152(6):850-855.

Doi: 10.1176/ajp.152.6.850

Pasanen M (1999) The expression and regulation of drug metabolism in human placenta. *Adv Drug Deliv Rev* 38(1):81-97.

Doi:

Paskova A, Jirak R, Mikesova M, Adamcova K, Fartakova Z, Horakova V, Koucky M, Hill M, Hruskovicova H, Starka L, Duskova M & Parizek A (2014) The role of steroids in the development of post-partum mental disorders. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub* 158(3):361-364.

Doi: 10.5507/bp.2012.098

Pavan L, Tarrade A, Hermouet A, Delouis C, Titeux M, Vidaud M, Therond P, Evain-Brion D & Fournier T (2003) Human invasive trophoblasts transformed with simian virus 40 provide a new tool to study the role of PPARgamma in cell invasion process. *Carcinogenesis* 24(8):1325-1336.

Doi: 10.1093/carcin/bgg074

Peacock BN, Scheiderer DJ & Kellermann GH (2016) Biomolecular aspects of depression: A retrospective analysis. *Compr Psychiatry* 73:168-180.

Doi: 10.1016/j.comppsych.2016.11.002

Pei Q, Sprakes M, Millan MJ, Rochat C & Sharp T (2004) The novel monoamine reuptake inhibitor and potential antidepressant, S33005, induces Arc gene expression in cerebral cortex. *Eur J Pharmacol* 495(2-3):227-233.

Doi:

Pei Q, Zetterstrom TS, Sprakes M, Tordera R & Sharp T (2003) Antidepressant drug treatment induces Arc gene expression in the rat brain. *Neuroscience* 121(4):975-982.

Doi:

Peng ZW, Xue YY, Wang HN, Wang HH, Xue F, Kuang F, Wang BR, Chen YC, Zhang LY & Tan QR (2012) Sertraline promotes hippocampus-derived neural stem cells differentiating into neurons but not glia and attenuates LPS-induced cellular damage. *Prog Neuropsychopharmacol Biol Psychiatry* 36(1):183-188.

Doi: 10.1016/j.pnpbp.2011.08.014

Perry LM, Goldstein-Piekarski AN & Williams LM (2017) Sex differences modulating serotonergic polymorphisms implicated in the mechanistic pathways of risk for depression and related disorders. *J Neurosci Res* 95(1-2):737-762.

Doi: 10.1002/jnr.23877

Petroff MG, Phillips TA, Ka H, Pace JL & Hunt JS (2006) Isolation and culture of term human trophoblast cells. *Methods Mol Med* 121:203-217.

Doi: 1-59259-983-4:201 [pii]

Pharmacorama (2017) *Sérotonine.*), http://www.pharmacorama.com/Rubriques/Output/Serotoninea2.php (Consulté le Consulté le 2 janvier 2017)

Philippe E & Dreyfus M (1998) Maladies trophoblastiques gestationnelles. *Encycl Méd Chir, Obstétrique*, Elsevier (Édit.). p 12p.

Pidoux G, Gerbaud P, Laurendeau I, Guibourdenche J, Bertin G, Vidaud M, Evain-Brion D & Frendo JL (2004) Large variability of trophoblast gene expression within and between human normal term placentae. *Placenta* 25(5):469-473.

Doi: 10.1016/j.placenta.2003.10.016

Pitt BR, Weng W, Steve AR, Blakely RD, Reynolds I & Davies P (1994) Serotonin increases DNA synthesis in rat proximal and distal pulmonary vascular smooth muscle cells in culture. *Am J Physiol* 266(2 Pt 1):L178-186.

Doi:

Poehlmann TG, Fitzgerald JS, Meissner A, Wengenmayer T, Schleussner E, Friedrich K & Markert UR (2005) Trophoblast invasion: tuning through LIF, signalling via Stat3. *Placenta* 26 Suppl A:S37-41.

Doi: 10.1016/j.placenta.2005.01.007

Polette M, Nawrocki B, Pintiaux A, Massenat C, Maquoi E, Volders L, Schaaps JP, Birembaut P & Foidart JM (1994) Expression of gelatinases A and B and their tissue inhibitors by cells of early and term human placenta and gestational endometrium. *Lab Invest* 71(6):838-846.

Doi:

Pollheimer J & Knofler M (2005) Signalling pathways regulating the invasive differentiation of human trophoblasts: a review. *Placenta* 26 Suppl A:S21-30.

Doi: 10.1016/j.placenta.2004.11.013

Pollheimer J & Knofler M (2012) The role of the invasive, placental trophoblast in human pregnancy. *Wien Med Wochenschr* 162(9-10):187-190.

Doi: 10.1007/s10354-012-0071-6

Ponder KL, Salisbury A, McGonnigal B, Laliberte A, Lester B & Padbury JF (2011) Maternal depression and anxiety are associated with altered gene expression in the human placenta without modification by antidepressant use: implications for fetal programming. *Dev Psychobiol* 53(7):711-723.

Doi: 10.1002/dev.20549

Potgens AJ, Kataoka H, Ferstl S, Frank HG & Kaufmann P (2003) A positive immunoselection method to isolate villous cytotrophoblast cells from first trimester and term placenta to high purity. *Placenta* 24(4):412-423.

Doi:

Prasad PD, Hoffmans BJ, Moe AJ, Smith CH, Leibach FH & Ganapathy V (1996) Functional expression of the plasma membrane serotonin transporter but not the vesicular monoamine transporter in human placental trophoblasts and choriocarcinoma cells. *Placenta* 17(4):201-207.

Doi:

Preskorn SH & Flockhart D (2009) 2010 guide to psychiatric drug interactions. *Primary Psychiatry* 16(12):45-74.

Doi:

Prouillac C & Lecoeur S (2010) The role of the placenta in fetal exposure to xenobiotics: importance of membrane transporters and human models for transfer studies. *Drug Metab Dispos* 38(10):1623-1635.

Doi: 10.1124/dmd.110.033571

Qiu H, Tang X, Ma J, Shaverdashvili K, Zhang K & Bedogni B (2015) Notch1 Autoactivation via Transcriptional Regulation of Furin, Which Sustains Notch1 Signaling by Processing Notch1-Activating Proteases ADAM10 and Membrane Type 1 Matrix Metalloproteinase. *Mol Cell Biol* 35(21):3622-3632.

Doi: 10.1128/mcb.00116-15

Qiu W, Bai SX, Zhao MR, Wu XQ, Zhao YG, Sang QX & Wang YL (2005) Spatio-temporal expression of matrix metalloproteinase-26 in human placental trophoblasts and fetal red cells during normal placentation. *Biol Reprod* 72(4):954-959.

Doi: 10.1095/biolreprod.104.036772

Racca AC, Ridano ME, Camolotto S, Genti-Raimondi S & Panzetta-Dutari GM (2015) A novel regulator of human villous trophoblast fusion: the Kruppel-like factor 6. *Mol Hum Reprod* 21(4):347-358.

Doi: 10.1093/molehr/gau113

Raisanen S, Lehto SM, Nielsen HS, Gissler M, Kramer MR & Heinonen S (2014) Risk factors for and perinatal outcomes of major depression during pregnancy: a population-based analysis during 2002-2010 in Finland. *BMJ Open* 4(11):e004883.

Doi: 10.1136/bmjopen-2014-004883

Ram D & Gandotra S (2015) Antidepressants, anxiolytics, and hypnotics in pregnancy and lactation. *Indian J Psychiatry* 57(Suppl 2):S354-371.

Doi: 10.4103/0019-5545.161504

Rampono J, Simmer K, Ilett KF, Hackett LP, Doherty DA, Elliot R, Kok CH, Coenen A & Forman T (2009) Placental transfer of SSRI and SNRI antidepressants and effects on the neonate. *Pharmacopsychiatry* 42(3):95-100.

Doi: 10.1055/s-0028-1103296

Rao JS, Ertley RN, Lee HJ, Rapoport SI & Bazinet RP (2006) Chronic fluoxetine upregulates activity, protein and mRNA levels of cytosolic phospholipase A2 in rat frontal cortex. *Pharmacogenomics J* 6(6):413-420.

Doi: 10.1038/sj.tpj.6500391

Rasstrigina IM, Milovanov AP, Fokina TV & Kadyrov M (2014) [The intensity of expression of matrix metalloproteinases type 2 and type 9 by invasive trophoblast cells in uncomplicated pregnancy and preeclampsia]. *Arkh Patol* 76(3):24-29.

Doi:

Redman CW (2011) Preeclampsia: a multi-stress disorder. *Rev Med Interne* 32 Suppl 1:S41-44.

Doi: 10.1016/j.revmed.2011.03.331

Reefhuis J, Devine O, Friedman JM, Louik C & Honein MA (2015) Specific SSRIs and birth defects: Bayesian analysis to interpret new data in the context of previous reports. *BMJ* 351:h3190.

Doi: 10.1136/bmj.h3190

Richart R (1961a) Studies of placental morphogenesis. I. Radioautographic studies of human placenta utilizing tritiated thymidine. *Proceedings of the Society for Experimental Biology and Medicine* 106:829-831.

Doi:

Richart R (1961b) Studies of placental morphogenesis. I. Radioautographic studies of human placenta utilizing tritiated thymidine. *Proc Soc Exp Biol Med* 106:829-831.

Doi:

Robertson-Blackmore E, Putnam FW, Rubinow DR, Matthieu M, Hunn JE, Putnam KT, Moynihan JA & O'Connor TG (2013) Antecedent trauma exposure and risk of depression in the perinatal period. *J Clin Psychiatry* 74(10):e942-948.

Doi: 10.4088/JCP.13m08364

Rochat B, Amey M, Gillet M, Meyer UA & Baumann P (1997) Identification of three cytochrome P450 isozymes involved in N-demethylation of citalopram enantiomers in human liver microsomes. *Pharmacogenetics* 7(1):1-10.

Doi:

Rodriguez A & Bohlin G (2005) Are maternal smoking and stress during pregnancy related to ADHD symptoms in children? *J Child Psychol Psychiatry* 46(3):246-254.

Doi: 10.1111/j.1469-7610.2004.00359.x

Rote NS (2005) Intercellular fusion of BeWo. *Placenta* 26(8-9):686; author reply 687.

Doi: 10.1016/j.placenta.2004.09.003

Roth I & Fisher SJ (1999) IL-10 is an autocrine inhibitor of human placental cytotrophoblast MMP-9 production and invasion. *Dev Biol* 205(1):194-204.

Doi: 10.1006/dbio.1998.9122

Roumestan C, Michel A, Bichon F, Portet K, Detoc M, Henriquet C, Jaffuel D & Mathieu M (2007) Anti-inflammatory properties of desipramine and fluoxetine. *Respir Res* 8:35.

Doi: 10.1186/1465-9921-8-35

Roux PP & Blenis J (2004) ERK and p38 MAPK-activated protein kinases: a family of protein kinases with diverse biological functions. *Microbiol Mol Biol Rev* 68(2):320-344.

Doi: 10.1128/MMBR.68.2.320-344.2004

Rubertsson C, Hellstrom J, Cross M & Sydsjo G (2014) Anxiety in early pregnancy: prevalence and contributing factors. *Arch Womens Ment Health* 17(3):221-228.

Doi: 10.1007/s00737-013-0409-0

Sabolovic Rudman S, Mustapic M, Kosec V, Pivac N, Rudman F & Muck-Seler D (2015) Serotonin risk factors for the development of hypertension in pregnancy. *Arch Gynecol Obstet* 291(4):779-785.

Doi: 10.1007/s00404-014-3461-8

Sadler TW (2012) *Langman's medical embryology*. Wolters Kluwer Health/Lippincott Philadelphia, 12th ed. 384 p

Sagrillo-Fagundes L, Clabault H, Laurent L, Hudon-Thibeault AA, Salustiano EM, Fortier M, Bienvenue-Pariseault J, Wong Yen P, Sanderson JT & Vaillancourt C (2016) Human Primary Trophoblast Cell Culture Model to Study the Protective Effects of Melatonin Against Hypoxia/reoxygenation-induced Disruption. *J Vis Exp* (113).

Doi: 10.3791/54228

Sahingoz M, Yuksel G, Karsidag C, Uguz F, Sonmez EO, Annagur BB & Annagur A (2014) Birth weight and preterm birth in babies of pregnant women with major depression in relation to treatment with antidepressants. *J Clin Psychopharmacol* 34(2):226-229.

Doi: 10.1097/jcp.0000000000000077

Salchner P & Singewald N (2006) 5-HT receptor subtypes involved in the anxiogenic-like action and associated Fos response of acute fluoxetine treatment in rats. *Psychopharmacology (Berl)* 185(3):282-288.

Doi: 10.1007/s00213-005-0247-5

Saleh L, Prast J, Haslinger P, Husslein P, Helmer H & Knofler M (2007) Effects of different human chorionic gonadotrophin preparations on trophoblast differentiation. *Placenta* 28(2-3):199-203.

Doi: 10.1016/j.placenta.2006.02.008

Sato Y, Fujiwara H, Higuchi T, Yoshioka S, Tatsumi K, Maeda M & Fujii S (2002) Involvement of dipeptidyl peptidase IV in extravillous trophoblast invasion and differentiation. *J Clin Endocrinol Metab* 87(9):4287-4296.

Doi:

Schaaps J, Thoumsin H, Foidart J & Hustin J (1998) Physiologie placentaire. *Encycl Méd Chir*, Elsevier (Édit.). p 20.

Schaz U, Fohr KJ, Liebau S, Fulda S, Koelch M, Fegert JM, Boeckers TM & Ludolph AG (2011) Dose-dependent modulation of apoptotic processes by fluoxetine in maturing neuronal cells: an in vitro study. *World J Biol Psychiatry* 12(2):89-98.

Doi: 10.3109/15622975.2010.506927

Scifres CM & Nelson DM (2009) Intrauterine growth restriction, human placental development and trophoblast cell death. *J Physiol* 587(Pt 14):3453-3458.

Doi: 10.1113/jphysiol.2009.173252

Scordo MG, Spina E, Dahl ML, Gatti G & Perucca E (2005) Influence of CYP2C9, 2C19 and 2D6 genetic polymorphisms on the steady-state plasma concentrations of the enantiomers of fluoxetine and norfluoxetine. *Basic Clin Pharmacol Toxicol* 97(5):296-301.

Doi: 10.1111/j.1742-7843.2005.pto_194.x

Sell SL, Craft RM, Seitz PK, Stutz SJ, Cunningham KA & Thomas ML (2008) Estradiol-sertraline synergy in ovariectomized rats. *Psychoneuroendocrinology* 33(8):1051-1060.

Doi: 10.1016/j.psyneuen.2008.05.006

Senaris R, Garcia-Caballero T, Casabiell X, Gallego R, Castro R, Considine RV, Dieguez C & Casanueva FF (1997) Synthesis of leptin in human placenta. *Endocrinology* 138(10):4501-4504.

Doi:

Seng JS, D'Andrea W & Ford JD (2014) Complex Mental Health Sequelae of Psychological Trauma Among Women in Prenatal Care. *Psychol Trauma* 6(1):41-49.

Doi: 10.1037/a0031467

Serafeim A, Holder MJ, Grafton G, Chamba A, Drayson MT, Luong QT, Bunce CM, Gregory CD, Barnes NM & Gordon J (2003) Selective serotonin reuptake inhibitors directly signal for apoptosis in biopsy-like Burkitt lymphoma cells. *Blood* 101(8):3212-3219.

Doi: 10.1182/blood-2002-07-2044

Shakeel N, Eberhard-Gran M, Sletner L, Slinning K, Martinsen EW, Holme I & Jenum AK (2015) A prospective cohort study of depression in pregnancy, prevalence and risk factors in a multi-ethnic population. *BMC Pregnancy Childbirth* 15:5.

Doi: 10.1186/s12884-014-0420-0

Shams ME, Arneth B, Hiemke C, Dragicevic A, Muller MJ, Kaiser R, Lackner K & Hartter S (2006) CYP2D6 polymorphism and clinical effect of the antidepressant venlafaxine. *J Clin Pharm Ther* 31(5):493-502.

Doi: 10.1111/j.1365-2710.2006.00763.x

Shea AK, Oberlander TF & Rurak D (2012) Fetal serotonin reuptake inhibitor antidepressant exposure: maternal and fetal factors. *Can J Psychiatry* 57(9):523-529.

Doi: 10.1177/070674371205700902

Shi, Fu L, Hasebe T & Ishizuya-Oka A (2007) Regulation of extracellular matrix remodeling and cell fate determination by matrix metalloproteinase stromelysin-3 during thyroid hormone-dependent post-embryonic development. *Pharmacol Ther* 116(3):391-400.

Doi: 10.1016/j.pharmthera.2007.07.005

Shi, Lei ZM, Rao CV & Lin J (1993) Novel role of human chorionic gonadotropin in differentiation of human cytotrophoblasts. *Endocrinology* 132(3):1387-1395.

Doi:

Shimonovitz S, Hurwitz A, Dushnik M, Anteby E, Geva-Eldar T & Yagel S (1994) Developmental regulation of the expression of 72 and 92 kd type IV collagenases in human trophoblasts: a possible mechanism for control of trophoblast invasion. *Am J Obstet Gynecol* 171(3):832-838.

Doi:

Shishkina GT, Kalinina TS, Berezova IV & Dygalo NN (2012) Stress-induced activation of the brainstem Bcl-xL gene expression in rats treated with fluoxetine: correlations with serotonin metabolism and depressive-like behavior. *Neuropharmacology* 62(1):177-183.

Doi: 10.1016/j.neuropharm.2011.06.016

Shors TJ & Leuner B (2003) Estrogen-mediated effects on depression and memory formation in females. *J Affect Disord* 74(1):85-96.

Doi:

Sit, Perel JM, Helsel JC & Wisner KL (2008) Changes in antidepressant metabolism and dosing across pregnancy and early postpartum. *J Clin Psychiatry* 69(4):652-658.

Doi:

Sit D, Perel JM, Wisniewski SR, Helsel JC, Luther JF & Wisner KL (2011) Mother-infant antidepressant concentrations, maternal depression, and perinatal events. *J Clin Psychiatry* 72(7):994-1001.

Doi: 10.4088/JCP.10m06461

Smedberg J, Lupattelli A, Mardby AC, Overland S & Nordeng H (2015) The relationship between maternal depression and smoking cessation during pregnancy--a cross-sectional study of pregnant women from 15 European countries. *Arch Womens Ment Health* 18(1):73-84.

Doi: 10.1007/s00737-014-0470-3

Smith GS, Lotrich FE, Malhotra AK, Lee AT, Ma Y, Kramer E, Gregersen PK, Eidelberg D & Pollock BG (2004) Effects of serotonin transporter promoter polymorphisms on serotonin function. *Neuropsychopharmacology* 29(12):2226-2234.

Doi: 10.1038/sj.npp.1300552

Song J, Hou X, Hu X, Lu C, Liu C, Wang J, Liu W, Teng L & Wang D (2015) Not only serotonergic system, but also dopaminergic system involved in albiflorin against chronic unpredictable mild stress-induced depression-like behavior in rats. *Chem Biol Interact* 242:211-217.

Doi: 10.1016/j.cbi.2015.10.001

Sonier B, Lavigne C, Arseneault M, Ouellette R & Vaillancourt C (2005) Expression of the 5-HT2A serotoninergic receptor in human placenta and choriocarcinoma cells: mitogenic implications of serotonin. *Placenta* 26(6):484-490.

Doi: 10.1016/j.placenta.2004.08.003

Spinelli MG, Endicott J, Goetz RR & Segre LS (2016) Reanalysis of efficacy of interpersonal psychotherapy for antepartum depression versus parenting education program: initial severity of depression as a predictor of treatment outcome. *J Clin Psychiatry* 77(4):535-540.

Doi: 10.4088/JCP.15m09787

St-Pierre J, Laurent L, King S & Vaillancourt C (2016) Effects of prenatal maternal stress on serotonin and fetal development. *Placenta* 48 Suppl 1:S66-S71.

Doi: 10.1016/j.placenta.2015.11.013

Statistique.Canada (2017a) *Trouble de l'humeur, selon le groupe d'âge et le sexe (nombre).*), http://www.statcan.gc.ca/tables-tableaux/sum-som/l02/cst01/health113a-fra.htm (Consulté le Consulté le 6 janvier 2017)

Statistique.Canada (2017b) *Trouble de l'humeur, selon le groupe d'âge et le sexe (pourcentage).*), http://www.statcan.gc.ca/tables-tableaux/sum-som/l02/cst01/health113b-fra.htm (Consulté le Consulté le 6 janvier 2017)

Staun-Ram E, Goldman S, Gabarin D & Shalev E (2004) Expression and importance of matrix metalloproteinase 2 and 9 (MMP-2 and -9) in human trophoblast invasion. *Reprod Biol Endocrinol* 2:59.

Doi: 10.1186/1477-7827-2-59

Steinberg ML & Robins JC (2016) Cellular Models of Trophoblast Differentiation. *Semin Reprod Med* 34(1):50-56.

Doi: 10.1055/s-0035-1570026

Syme MR, Paxton JW & Keelan JA (2004) Drug transfer and metabolism by the human placenta. *Clin Pharmacokinet* 43(8):487-514.

Doi: 10.2165/00003088-200443080-00001

Taler M, Gil-Ad I, Lomnitski L, Korov I, Baharav E, Bar M, Zolokov A & Weizman A (2007) Immunomodulatory effect of selective serotonin reuptake inhibitors (SSRIs) on human T lymphocyte function and gene expression. *Eur Neuropsychopharmacol* 17(12):774-780.

Doi: 10.1016/j.euroneuro.2007.03.010

Tapia-Pizarro A, Argandona F, Palomino WA & Devoto L (2013) Human chorionic gonadotropin (hCG) modulation of TIMP1 secretion by human endometrial stromal cells facilitates extravillous trophoblast invasion in vitro. *Hum Reprod*.

Doi: 10.1093/humrep/det136

Torzilli PA, Bourne JW, Cigler T & Vincent CT (2012) A new paradigm for mechanobiological mechanisms in tumor metastasis. *Semin Cancer Biol* 22(5-6):385-395.

Doi: 10.1016/j.semcancer.2012.05.002

Toufaily C, Vargas A, Lemire M, Lafond J, Rassart E & Barbeau B (2013) MFSD2a, the Syncytin-2 receptor, is important for trophoblast fusion. *Placenta* 34(1):85-88.

Doi: 10.1016/j.placenta.2012.10.012

Traskman L, Asberg M, Bertilsson L & Sjostrand L (1981) Monoamine metabolites in CSF and suicidal behavior. *Arch Gen Psychiatry* 38(6):631-636.

Doi:

Tsatsaris V, Malassiné A, Fournier T, Handschuh K, Schaaps J-P, Foidart J-M & Evain-Brion D (2006) Placenta humain. *Obstétrique*:1-22.

Doi:

Tyce GM (1990) Origin and metabolism of serotonin. *J Cardiovasc Pharmacol* 16 Suppl 3:S1-7.

Doi:

Tyler CR, Solomon BR, Ulibarri AL & Allan AM (2014) Fluoxetine treatment ameliorates depression induced by perinatal arsenic exposure via a neurogenic mechanism. *Neurotoxicology* 44:98-109.

Doi: 10.1016/j.neuro.2014.06.006

Uguz F (2017) Is There Any Association Between Use of Antidepressants and Preeclampsia or Gestational Hypertension?: A Systematic Review of Current Studies. *J Clin Psychopharmacol* 37(1):72-77.

Doi: 10.1097/jcp.0000000000000618

Ulrich F & Petermann F (2016) Consequences and Possible Predictors of Health-damaging Behaviors and Mental Health Problems in Pregnancy - A Review. *Geburtshilfe Frauenheilkd* 76(11):1136-1156.

Doi: 10.1055/s-0042-118180

Vaillancourt C, Lanoix D, Le Bellego F, Daoud G & Lafond J (2009) Involvement of MAPK signalling in human villous trophoblast differentiation. *Mini Rev Med Chem* 9(8):962-973.

Doi:

Vaillancourt C, Petit A, Gallo-Payet N, Bellabarba D, Lehoux JG & Belisle S (1994) Labelling of D2-dopaminergic and 5-HT2-serotonergic binding sites in human trophoblastic cells using [3H]-spiperone. *J Recept Res* 14(1):11-22.

Doi:

Vargas A, Moreau J, Landry S, LeBellego F, Toufaily C, Rassart E, Lafond J & Barbeau B (2009) Syncytin-2 plays an important role in the fusion of human trophoblast cells. *J Mol Biol* 392(2):301-318.

Doi: 10.1016/j.jmb.2009.07.025

Velasquez JC, Goeden N, Herod SM & Bonnin A (2016) Maternal Pharmacokinetics and Fetal Disposition of (+/-)-Citalopram during Mouse Pregnancy. *ACS Chem Neurosci* 7(3):327-338.

Doi: 10.1021/acschemneuro.5b00287

Ververs FF, Voorbij HA, Zwarts P, Belitser SV, Egberts TC, Visser GH & Schobben AF (2009) Effect of cytochrome P450 2D6 genotype on maternal paroxetine plasma concentrations during pregnancy. *Clin Pharmacokinet* 48(10):677-683.

Doi: 10.2165/11318050-000000000-00000

Vettraino IM, Roby J, Tolley T & Parks WC (1996) Collagenase-I, stromelysin-I, and matrilysin are expressed within the placenta during multiple stages of human pregnancy. *Placenta* 17(8):557-563.

Doi:

Viau M (2008) *Caractérisation du système sérotoninergique placentaire : implication des contaminants environnementaux et du diabète gestationnel*. Maîtrise en sciences expérimentales de la santé (INRS-Institut Armand Frappier). 103 p

Viau M, Lafond J & Vaillancourt C (2009) Expression of placental serotonin transporter and 5-HT 2A receptor in normal and gestational diabetes mellitus pregnancies. *Reprod Biomed Online* 19(2):207-215.

Doi:

Vidal (2017a) *Citalopram, effets indésirables.*), https://www.univadis.fr/references/drug-database/N06/viewdrug/66320467

Vidal (2017b) *FLUOXETINE : FERTILITÉ/GROSSESSE/ALLAITEMENT.*), https://www.vidal.fr/Medicament/prozac-13927-fertilite_grossesse_allaitement.htm (Consulté le Consulté le 6 août 2017)

Vidal (2017c) *Fluoxétine, effets indérisables.*), https://www.univadis.fr/references/drug-database/N06/viewdrug/63730233

Vidal (2017d) *Paroxétine, effets indésirables.*), https://www.univadis.fr/references/drug-database/N06/viewdrug/66275029

Vidal (2017e) *Sertraline, effets indérisables.*), https://www.univadis.fr/references/drug-database/N06/viewdrug/65155219

Vignery A (2000) Osteoclasts and giant cells: macrophage-macrophage fusion mechanism. *Int J Exp Pathol* 81(5):291-304.

Doi:

Vigod SN & Stewart DE (2009) Emergent research in the cause of mental illness in women across the lifespan. *Curr Opin Psychiatry* 22(4):396-400.

Doi: 10.1097/YCO.0b013e3283297127

Wang, Li KF, Adams E & Van Schepdael A (2011) Matrix metalloproteinase inhibitors: a review on bioanalytical methods, pharmacokinetics and metabolism. *Curr Drug Metab* 12(4):395-410.

Doi:

Wang, Newport DJ, Stowe ZN, Donovan JL, Pennell PB & DeVane CL (2007) The emerging importance of transporter proteins in the psychopharmacological treatment of the pregnant patient. *Drug Metab Rev* 39(4):723-746.

Doi: 10.1080/03602530701690390

Wang J-m, Zhao H-x, Wang L, Gao Z-y & Yao Y-q (2013a) The human leukocyte antigen G promotes trophoblast fusion and β-hCG production through the Erk1/2 pathway in human choriocarcinoma cell lines. *Biochemical and Biophysical Research Communications* 434(3):460-465.

Doi: http://dx.doi.org/10.1016/j.bbrc.2013.04.004

Wang JH, Liu ZQ, Wang W, Chen XP, Shu Y, He N & Zhou HH (2001) Pharmacokinetics of sertraline in relation to genetic polymorphism of CYP2C19. *Clin Pharmacol Ther* 70(1):42-47.

Doi: 10.1067/mcp.2001.116513

Wang Z, Gu J, Wang X, Xie K, Luan Q, Wan N, Zhang Q, Jiang H & Liu D (2013b) Antidepressant-like activity of resveratrol treatment in the forced swim test and tail suspension test in mice: the HPA axis, BDNF expression and phosphorylation of ERK. *Pharmacol Biochem Behav* 112:104-110.

Doi: 10.1016/j.pbb.2013.10.007

Waqas A, Raza N, Lodhi HW, Muhammad Z, Jamal M & Rehman A (2015) Psychosocial factors of antenatal anxiety and depression in Pakistan: is social support a mediator? *PLoS One* 10(1):e0116510.

Doi: 10.1371/journal.pone.0116510

White L, Dharmarajan A & Charles A (2007) Caspase-14: a new player in cytotrophoblast differentiation. *Reprod Biomed Online* 14(3):300-307.

Doi:

Wille SM, Cooreman SG, Neels HM & Lambert WE (2008) Relevant issues in the monitoring and the toxicology of antidepressants. *Crit Rev Clin Lab Sci* 45(1):25-89.

Doi: 10.1080/10408360701713112

Xie Y, Cui D, Sui L, Xu Y, Zhang N, Ma Y, Li Y & Kong Y (2015) Induction of forkhead box M1 (FoxM1) by EGF through ERK signaling pathway promotes trophoblast cell invasion. *Cell Tissue Res* 362(2):421-430.

Doi: 10.1007/s00441-015-2211-y

Xie Z, Westmoreland SV, Bahn ME, Chen GL, Yang H, Vallender EJ, Yao WD, Madras BK & Miller GM (2007) Rhesus monkey trace amine-associated receptor 1 signaling: enhancement by monoamine transporters and attenuation by the D2 autoreceptor in vitro. *J Pharmacol Exp Ther* 321(1):116-127.

Doi: 10.1124/jpet.106.116863

Xu B, Nakhla S, Makris A & Hennessy A (2011) TNF-alpha inhibits trophoblast integration into endothelial cellular networks. *Placenta* 32(3):241-246.

Doi: 10.1016/j.placenta.2010.12.005

Xu P, Wang YL, Zhu SJ, Luo SY, Piao YS & Zhuang LZ (2000) Expression of matrix metalloproteinase-2, -9, and -14, tissue inhibitors of metalloproteinase-1, and matrix proteins in human placenta during the first trimester. *Biol Reprod* 62(4):988-994.

Doi:

Yamada M, Yamada M, Kiuchi Y, Nara K, Kanda Y, Morinobu S, Momose K, Oguchi K, Kamijima K & Higuchi T (1999) Identification of a novel splice variant of heat shock cognate protein 70 after chronic antidepressant treatment in rat frontal cortex. *Biochem Biophys Res Commun* 261(2):541-545.

Doi: 10.1006/bbrc.1999.1067

Yang M, Lei ZM & Rao Ch V (2003) The central role of human chorionic gonadotropin in the formation of human placental syncytium. *Endocrinology* 144(3):1108-1120.

Doi:

Yavarone MS, Shuey DL, Tamir H, Sadler TW & Lauder JM (1993) Serotonin and cardiac morphogenesis in the mouse embryo. *Teratology* 47(6):573-584.

Doi: 10.1002/tera.1420470609

Yu BN, Chen GL, He N, Ouyang DS, Chen XP, Liu ZQ & Zhou HH (2003) Pharmacokinetics of citalopram in relation to genetic polymorphism of CYP2C19. *Drug Metab Dispos* 31(10):1255-1259.

Doi: 10.1124/dmd.31.10.1255

Yu C, Shen K, Lin M, Chen P, Lin C, Chang GD & Chen H (2002) GCMa regulates the syncytin-mediated trophoblastic fusion. *J Biol Chem* 277(51):50062-50068.

Doi: 10.1074/jbc.M209316200

Yui J, Garcia-Lloret M, Brown AJ, Berdan RC, Morrish DW, Wegmann TG & Guilbert LJ (1994) Functional, long-term cultures of human term trophoblasts purified by column-elimination of CD9 expressing cells. *Placenta* 15(3):231-246.

Doi:

Zhao Z, Zhang HT, Bootzin E, Millan MJ & O'Donnell JM (2009) Association of changes in norepinephrine and serotonin transporter expression with the long-term behavioral effects of antidepressant drugs. *Neuropsychopharmacology* 34(6):1467-1481.

Doi: 10.1038/npp.2008.183

Zhou, Damsky CH, Chiu K, Roberts JM & Fisher SJ (1993) Preeclampsia is associated with abnormal expression of adhesion molecules by invasive cytotrophoblasts. *J Clin Invest* 91(3):950-960.

Doi: 10.1172/JCI116316

Zhou, Damsky CH & Fisher SJ (1997a) Preeclampsia is associated with failure of human cytotrophoblasts to mimic a vascular adhesion phenotype. One cause of defective endovascular invasion in this syndrome? *J Clin Invest* 99(9):2152-2164.

Doi: 10.1172/JCI119388

Zhou, Fisher SJ, Janatpour M, Genbacev O, Dejana E, Wheelock M & Damsky CH (1997b) Human cytotrophoblasts adopt a vascular phenotype as they differentiate. A strategy for successful endovascular invasion? *J Clin Invest* 99(9):2139-2151.

Doi: 10.1172/jci119387

Zhou J & Bondy C (1992) Insulin-like growth factor-II and its binding proteins in placental development. *Endocrinology* 131(3):1230-1240.

Doi:

ANNEXE I : RÉSULTATS PRÉLIMINAIRES

I.1 Effets du citalopram et de la paroxétine sur la lignée de trophoblastes villeux BeWo

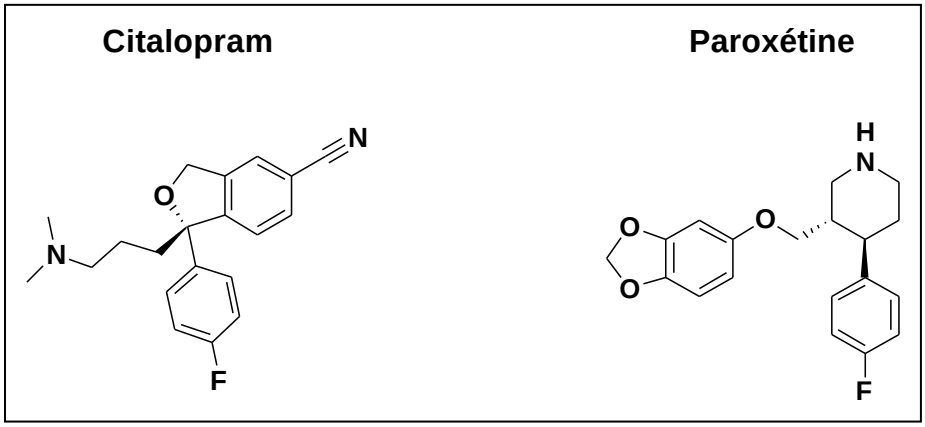


Figure A1.1 Structures moléculaires du citalopram et de la paroxétine.

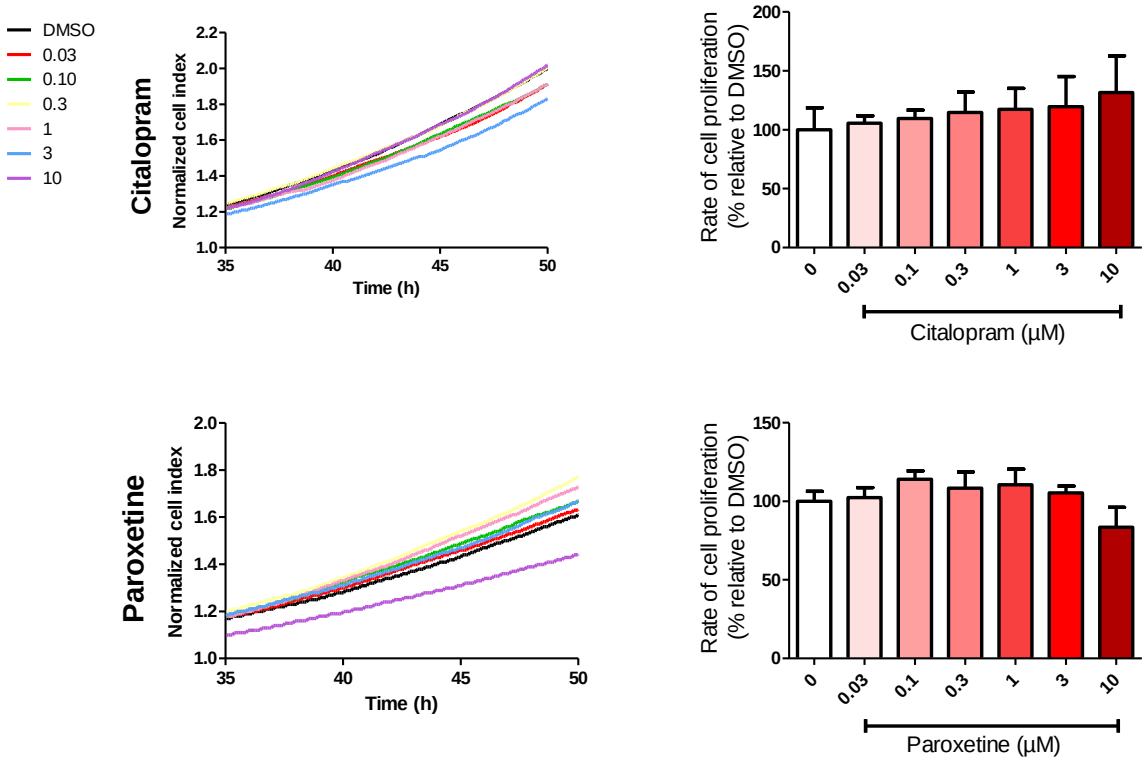


Figure A1.2 **Effects of citalopram and paroxetine on real-time proliferation of BeWo human choriocarcinoma cells.** Relative cell proliferation rates are presented as mean ± standard error of the mean (n=3-5; each sample run in triplicate). No statistically significant difference was detected between SSRIs treatments and vehicle control (0.1% DMSO) (one-way ANOVA and Dunnett posteriori test or Kruskal-Wallis test, P>0.05).

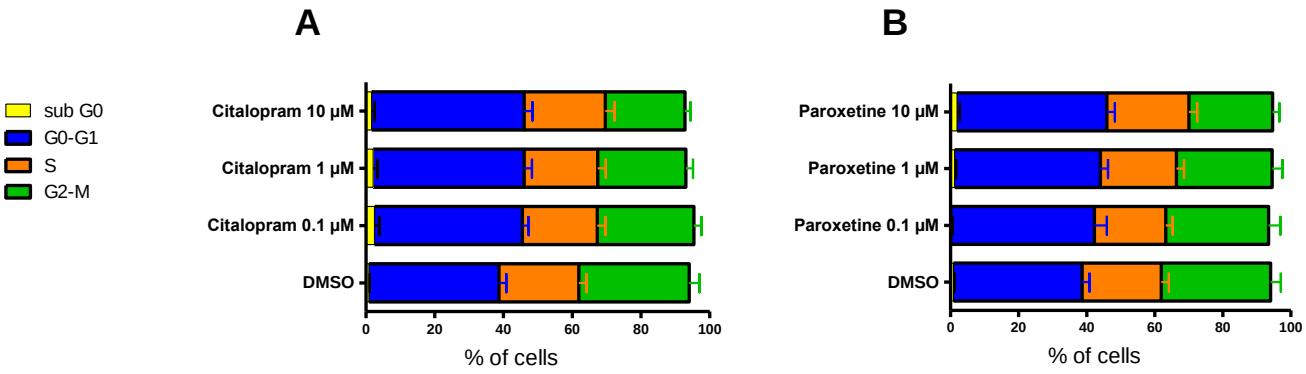


Figure A1.3 **Effects of citalopram and paroxetine on cell cycle of BeWo human choriocarcinoma cells.** Cells (1.10^5 cells/well) were treated with the SSRIs for 24 h, stained using propidium iodide and analyzed by flow cytometry. The percentage of cells in each cell cycle phase is expressed as mean ± standard error of the mean; n=3. No statistically significant difference was detected between SSRIs treatments and vehicle control (0.1% DMSO) (Kruskal-Wallis test, P>0.05).

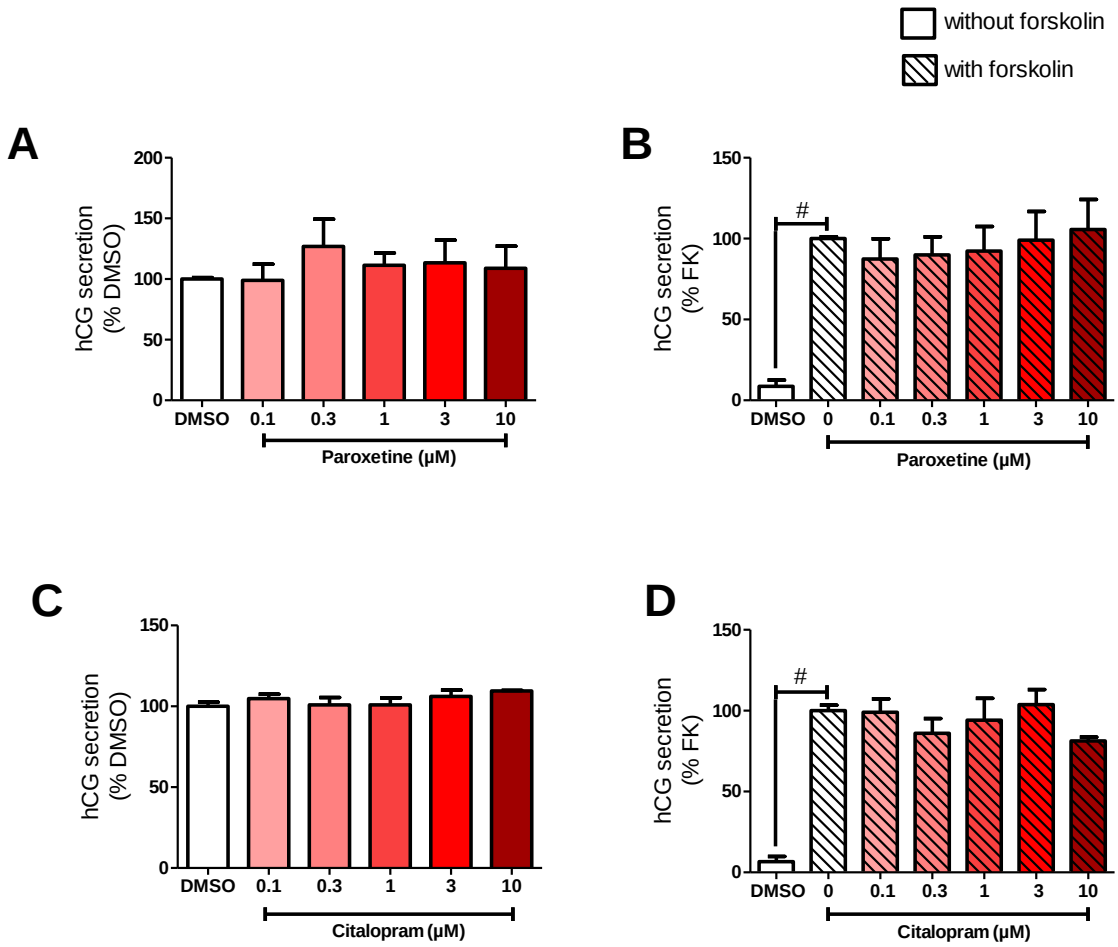


Figure A1.4 **Effects of citalopram and paroxetine on hCG secretion from BeWo human choriocarcinoma cells.** Cells were treated for 48 h with increasing concentrations of citalopram and paroxetine, or vehicle control (0.1% DMSO) combined or not with 20 µM forskolin (FK). The amount of hCG released was expressed are mean ± SEM, control set as 100 % (n=3, each sample in triplicate). (#) Statistically significant difference between DMSO- and forskolin-treated cells (Mann-Whitney; $P<0.05$). No statistically significant difference was detected between SSRI treatments and control (one-way ANOVA, Kruskal-Wallis or Welch test).

I.2 Effets de la paroxétine sur les lignées de trophoblastes extravilleux JEG-3 et HIPEC.

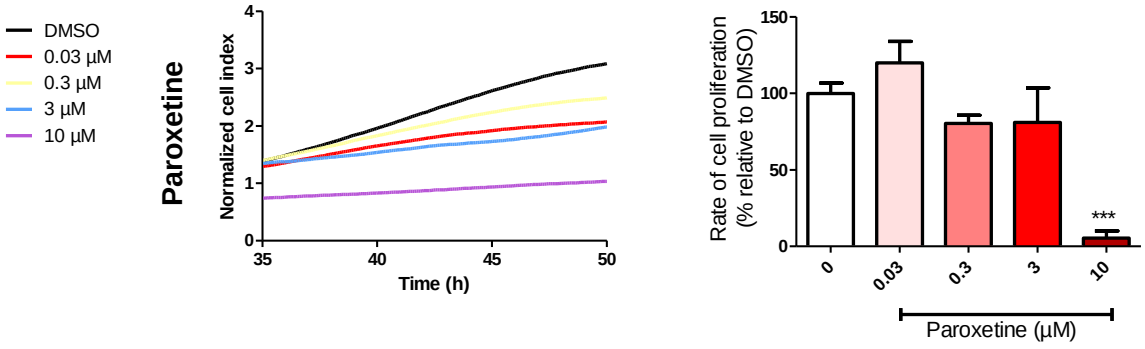


Figure A2.1 **Effects of paroxetine on real-time proliferation of JEG-3 cells.** Relative cell proliferation rates are presented as mean ± standard error of the mean (n=3, each sample run in triplicate). (***) Statistically significant difference between paroxetine treatment and vehicle control (0.1% DMSO) (Welch test; ****P*<0.001).

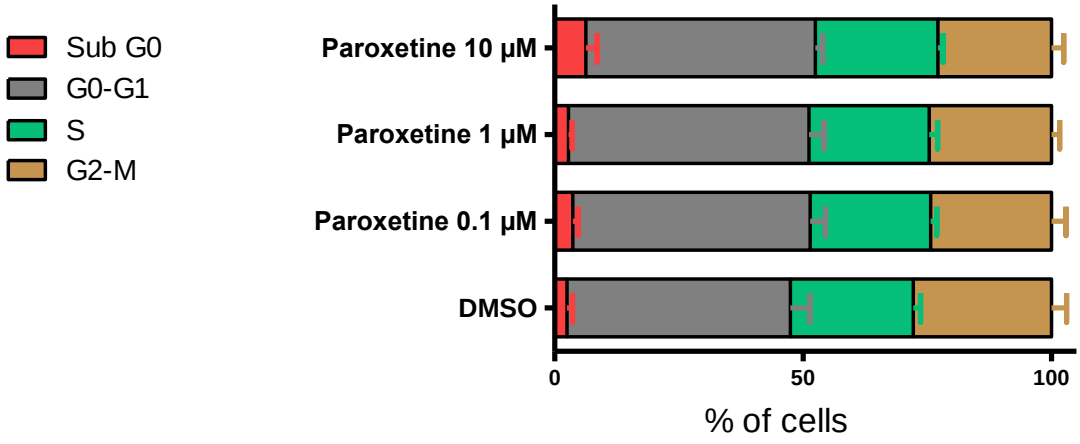


Figure A2.2 **Effects of paroxetine on cell cycle of JEG-3 cells.** After 24 h in culture, cells (1 x 10^5 cells/ml) were treated 24 h with the SSRIs, then stained using propidium iodide and analyzed by flow cytometry. The percentage of cells in each cell cycle phase is expressed as mean ± standard error of the mean (n=3). No statistically significant difference was detected between paroxetine treatment and vehicle control (0.1% DMSO) (two-way ANOVA and Bonferroni posteriori test, *P*>0.05).

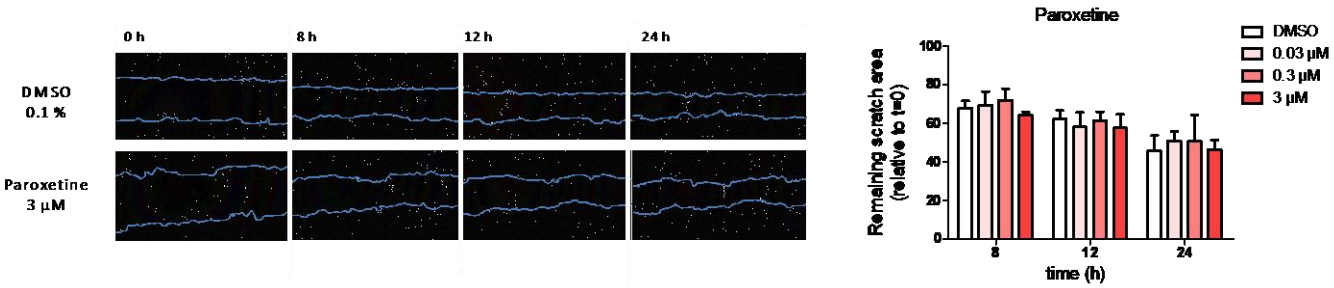


Figure A2.3 **Effects of paroxetine on the migration rate of JEG-3 cells.** Monolayers of JEG-3 treated with paroxetine in medium without FBS were scratched. Photos were taken immediately and also 8 h, 12 h and 24 h after scratching. The percentage of wound closure is compared to the area of initial wound and is represented as a mean ± standard error of the mean (n=3). No statistically significant difference was detected between paroxetine and vehicle control (0.1% DMSO) (one-way ANOVA and Dunnett posteriori test, *P*>0.05).

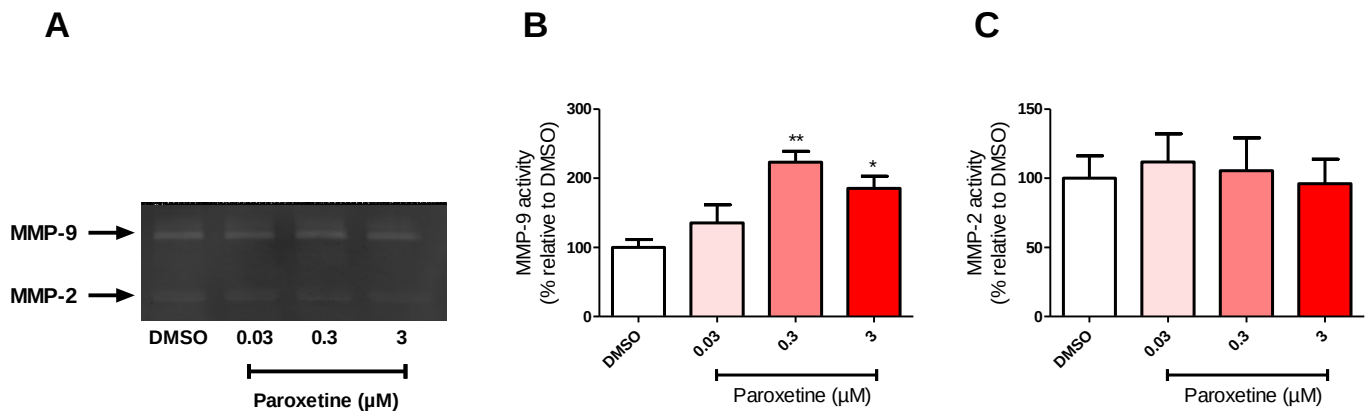


Figure A2.4 **Effects of paroxetine on MMP-2 and MMP-9 activities of JEG-3 cells.** After 24 h in culture, cells (2 x 10^5 cells/ml) were treated for 24 h with paroxetine using a medium with 0.5 % FBS. Activity of gelatinases in supernatants was analyzed by zymography Representative gels (A), and MMP-9 (B) and MMP-2 (C). MMP activity is expressed as a mean ± standard error of the mean (n=4). (*, **) Statistically significant difference between paroxetine treatment and vehicle control (0.1% DMSO) (one-way ANOVA and Dunnett posteriori test; *P<0.05, **P<0.01).

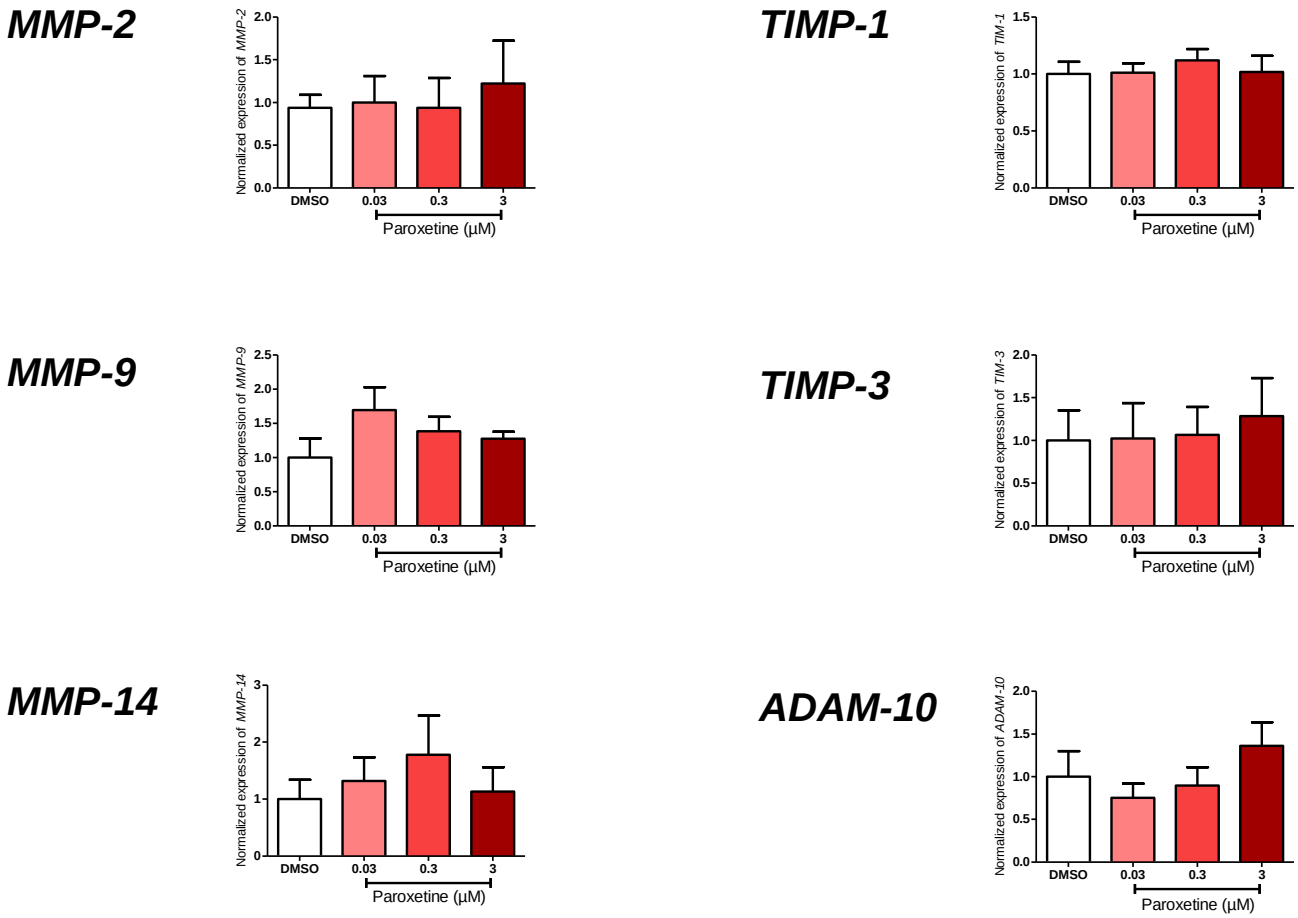


Figure A2.5 **Effects of paroxetine on the expression of *matrix metalloproteinase* (*MMP*)*-2*, *-9* and *-14*, *tissue inhibitor of metalloproteinases* (*TIMP*)*-1* and *-3,* and *a disintegrin and metalloproteinase domain-containing protein* (*ADAM*)*-10* in JEG-3 cells.** After 24 h in culture, cells were treated for 24 h with increasing concentrations of paroxetine, or vehicle control (0.1% DMSO) using a medium with 0.5 % FBS. Levels of mRNA expression were determined by reverse-transcription quantitative polymerase chain reaction (RT-qPCR) using *peptidylprolyl isomerase A* (*PPIA*) and *succinate dehydrogenase complex subunit A* (*SDHA*) as reference genes and presented as mean ± standard error of the mean; n=3-5; each sample run in triplicate. No statistically significant difference was detected between paroxetine and vehicle control (0.1% DMSO) (one-way ANOVA and Dunnett posteriori or Kruskal-Wallis test; *P*>0.05).

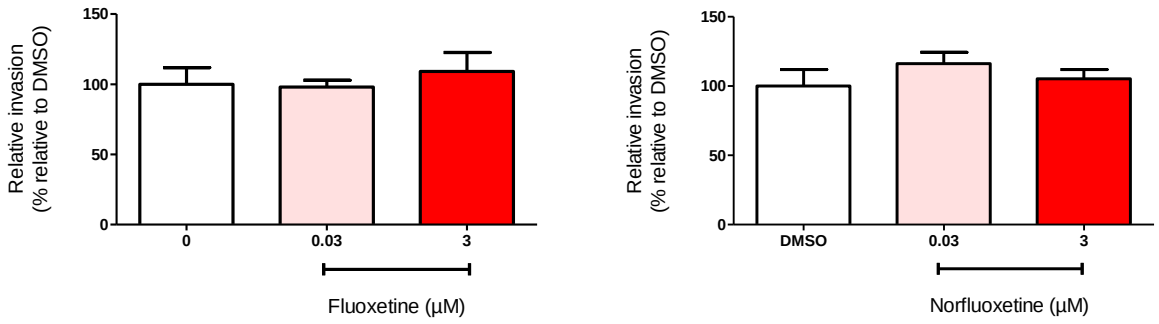


Figure A2.6 **Effects of fluoxetine and norfluoxetine on Jeg-3 cells invasion.** JEG-3 cells in culture in the upper compartment of a transwell chamber were treated with different concentrations of fluoxetine and norfluoxetine in a 0.5% FBS medium. The same medium was added to the lower chamber. After 48 h incubation, cells were stained with crystal violet, then washed. The stain was extracted and the dye mixture absorbance was measured at 560 nm. Relative absorbance to DMSO are presented as mean ± standard error of the mean (n=3, each sample run in duplicate). No statistically significant difference was detected between SSRIs and vehicle control (0.1% DMSO) (Kruskal-Wallis test; *P*>0.05).

ANNEXE II : HUMAN PRIMARY TROPHOBLAST CELL CULTURE MODEL TO STUDY THE PROTECTIVE EFFECTS OF MELATONIN AGAINST HYPOXIA/REOXYGENATION-INDUCED DISRUPTION

II.1 Présentation de l'article

Titre en français : Modèle de primoculture de cellules trophoblastiques humaines pour étudier l'effet protecteur de la mélatonine contre les perturbations induites par l'hypoxie/ réoxygénation.

Lucas Sagrillo-Fagundes[*1], Hélène Clabault[*1], Laetitia Laurent[*1], Andrée-Anne Hudon-Thibeault[1], Eugênia Maria Assunção Salustiano[1], Marlène Fortier[1], Josianne Bienvenue-Pariseault[1], Philippe Wong Yen[1], Thomas Sanderson1, Cathy Vaillancourt[1]

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L'article est publié dans « Journal of visualized experiments » (JOVE). 30 juillet 2016; Exp. (113). doi: 10.3791/54228.

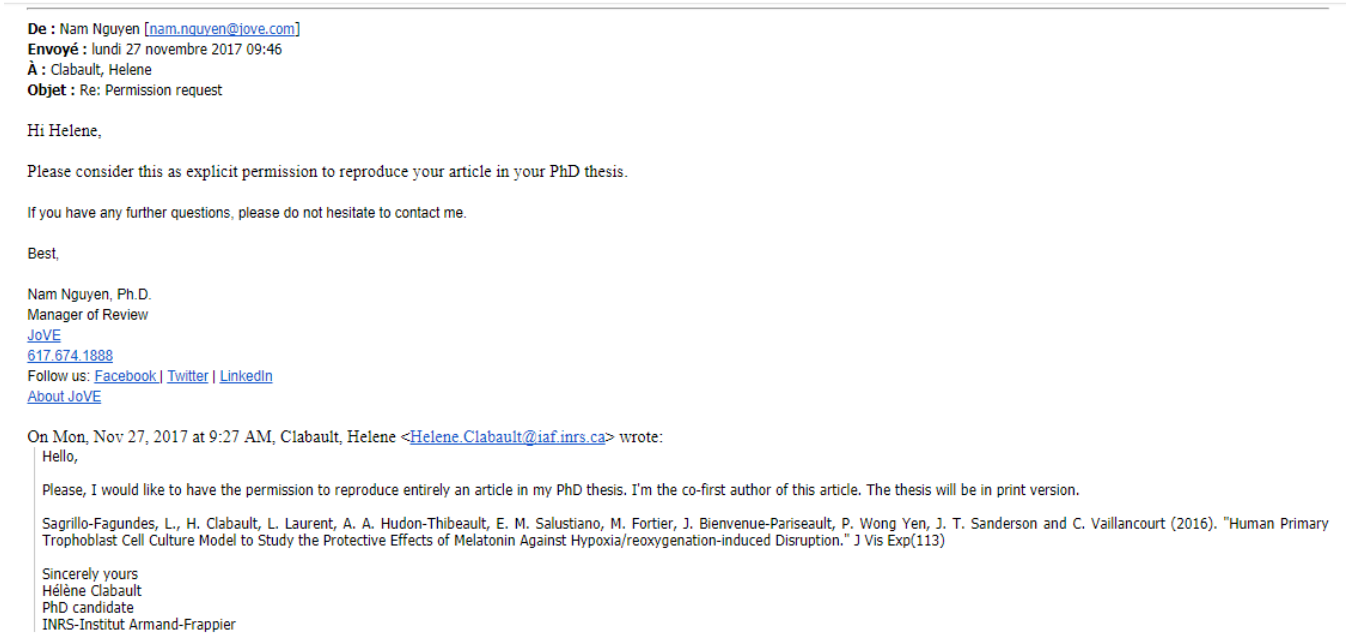
II.2 Résumé de l'article en français

Ce protocole décrit la technique d'isolement et de purification des cytotrophoblastes villeux de placentas à terme : digestions enzymatiques suivies par une centrifugation et un isolement sur un gradient, et une purification immunomagnétique. Tel qu'observé *in vivo*, les cultures primaires de cytotrophoblastes villeux mononucléés se différencient en syncytiotrophoblastes multinucléés après 72 h de culture. Comparés à une culture en normoxie (8% d'O_2), les cytotrophoblastes villeux en condition d'hypoxie/réoxygénation (0,5% / 8% d'O_2) présentent une augmentation du stress oxydant, et de l'apoptose intrinsèque, similaires à l'augmentation observée *in vivo* dans les grossesses pathologiques telles que les prééclampsies, les naissances prématurées et les restrictions de grossesse intra-utérine. Dans ce contexte, les cultures primaires de trophoblastes villeux en condition d'hypoxie/réoxygénation représentent un excellent modèle d'étude pour mieux comprendre les mécanismes et les voies de signalisation altérées dans les placentas humains, ce qui pourrait faciliter la recherche de médicaments efficaces contre les pathologies de grossesse. Le trophoblaste villeux humain produit de la mélatonine et exprime ses enzymes de synthèse, ainsi que ses récepteurs. L'utilisation de la mélatonine comme traitement de la prééclampsie et des retards de croissance intra-utérine a été suggérée, de par ses effets antioxydants. Dans le modèle de primocultures de cytotrophoblastes villeux décrit dans cet article, la mélatonine n'a pas d'effet sur les cellules trophoblastiques en normoxie, tandis qu'elle restaure l'équilibre d'oxydoréduction dans les cellules perturbées par une hypoxie/réoxygénation. Ainsi, les primocultures de trophoblastes villeux humains sont un excellent modèle pour l'étude des mécanismes protecteurs de la mélatonine sur les fonctions placentaires durant l'hypoxie/réoxygénation.

II.3 Contribution de l'étudiante

L'étudiante a contribué à la rédaction de l'article, à la réalisation des figures et tableaux à égalité avec Lucas Sagrillo-Fagundes et Laetitia Laurent. Elle a participé aux corrections nécessaires à la publication de la version finale de l'article, elle a participé à la rédaction et à la correction de script de la vidéo, ainsi qu'au tournage de la vidéo.

II.4 Article



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Video Article

Human Primary Trophoblast Cell Culture Model to Study The Protective Effects of Melatonin Against Hypoxia/Reoxygenation-Induced Disruption

Lucas Sagrillo-Fagundes*[1], Hélène Clabault*[1], Laetitia Laurent*[1], Andrée-Anne Hudon-Thibeault[1], Eugênia Maria Assunção Salustiano[1], Marlène Fortier[1], Josianne Bienvenue-Pariseault[1], Philippe Wong Yen[1], Thomas Sanderson[1], Cathy Vaillancourt[1]

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URL: http://www.jove.com/video/54228
DOI: doi:10.3791/54228

Keywords: Human placenta, hypoxia chamber, immunopurification, melatonin, normoxia, oxidative stress, density gradient, primary cell culture, syncytiotrophoblast, villous cytotrophoblast.

Date Published: 3/24/2016

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Abstract

This protocol describes how villous cytotrophoblast cells are isolated from placentas at term by successive enzymatic digestions, followed by density centrifugation, media gradient isolation and immunomagnetic purification. As observed *in vivo*, mononucleated villous cytotrophoblast cells in primary culture differentiate into multinucleated syncytiotrophoblast cells after 72 hr. Compared to normoxia (8% O_2), villous cytotrophoblast cells that undergo hypoxia/reoxygenation (0.5% / 8% O_2) undergo increased oxidative stress and intrinsic apoptosis, similar to that observed *in vivo* in pregnancy complications such as preeclampsia, preterm birth, and intrauterine growth restriction. In this context, primary villous trophoblasts cultured under hypoxia/reoxygenation conditions represent a unique experimental system to better understand the mechanisms and signalling pathways that are altered in human placenta and facilitate the search for effective drugs that protect against certain pregnancy disorders. Human villous trophoblasts produce melatonin and express its synthesizing enzymes and receptors. Melatonin has been suggested as a treatment for preeclampsia and intrauterine growth restriction because of its protective antioxidant effects. In the primary villous cytotrophoblast cell model described in this paper, melatonin has no effect on trophoblast cells in normoxic state but restores the redox balance of syncytiotrophoblast cells disrupted by hypoxia/reoxygenation. Thus, human villous trophoblast cells in primary culture are an excellent approach to study the mechanisms behind the protective effects of melatonin on placental function during hypoxia/reoxygenation.

Introduction

Throughout human pregnancy, the placental cytotrophoblast cells, which are mononucleated stem cells, rapidly proliferate and differentiate into either villous or extravillous cytotrophoblast cells. Extravillous cytotrophoblasts invade and remodel the spiral arteries of the uterine wall. Villous cytotrophoblasts, on the other hand, continue to proliferate, differentiate and fuse to form multinucleated syncytiotrophoblast (the syncytium)[1]. The maintenance of villous trophoblast homeostasis is essential for fetal well-being and healthy pregnancy. In fact, villous trophoblasts allow maternal-fetal exchange of oxygen and nutrients, and produce essential hormones for pregnancy. Moreover, the syncytiotrophoblast is the only cell-type in direct contact with the maternal blood circulation and provides an essential physical and immunological barrier. Therefore, the syncytiotrophoblast must undergo apoptosis and replacement for homeostatic maintenance and to avoid placental pathologies[2-5].

The technique developed by Kliman *et al.*[6] in 1986 to isolate primary villous cytotrophoblasts from human placentas caused a revolution in placental research by allowing the study of the molecular mechanisms involved in villous trophoblast differentiation. This classical technique, based on sequential enzymatic digestions with trypsin and DNase, followed by isolation in density centrifugation media (colloidal silica particles coated by polyvinylpyrrolidone, or Percoll) is now recognized as the gold standard for isolating villous cytotrophoblast cells. The technique can be optimized by magnetic immunopurification, a procedure that separates villous cytotrophoblasts from non-trophoblastic cells based on the differential expression of specific antigens on the surfaces of these cells. We chose the human leukocyte antigen ABC (HLA-ABC) due to the absence of its expression on the trophoblastic cell membrane[7,8].

The placenta is an organ that undergoes dramatic variations in oxygen levels during pregnancy. In the first trimester, the oxygenation ratio is physiologically very low (2% O_2) but increases to mild levels of oxygenation (8% O_2) in the second and third trimester. Tuuli *et al.*[9] described that the *in vitro* reproduction of the trophoblast environment inside the placental villi is a challenge and variations in oxygenation levels may even lead to phenotypical changes. It is, therefore, suggested to adopt 8% oxygen as normoxia to mimic the oxygen tension found in placental villi during the third trimester of gestation[8,9]. Chen *et al.*[10] extensively studied several variables related to oxygen tension in trophoblast cell culture and demonstrated the importance of determining oxygen levels in a pericellular environment. The levels of oxygen in the villi tend to increase due to vasculogenesis. The blood flow in placental villi increases constantly and the level of hydrogen peroxide (an abundant reactive oxygen species) is an important signal that controls vasculogenesis[11,12]. In pregnancy complications, a lack of vasculogenesis generates hypoxia, and more importantly, intermittent variations of oxygenation (called hypoxia/reoxygenation). These conditions lead to an abnormal increase in oxidative stress, which compromises placental and fetal viability[13,14]. The alterations that trophoblast cells undergo *in vivo* during episodes of hypoxia/reoxygenation can be mimicked *in vitro* as follows: villous cytotrophoblasts are maintained under normoxic conditions (8% O_2) until

they differentiate into syncytiotrophoblast. They are then subjected to hypoxic conditions (0.5% O_2) for 4 hr, followed by an additional 18 hr of normoxia (reoxygenation). Using this hypoxia/reoxygenation approach, trophoblasts exhibit deregulated redox status and increased levels of intrinsic apoptosis[8], as has been observed in certain pregnancy complications. Hence, this is a useful *in vitro* model to evaluate new preventive and therapeutic approaches to combat pregnancy complications associated with placental hypoxia/reoxygenation.

Placental cells produce melatonin, which has several important functions, such as an ability to obviate oxidative stress and placental dysfunction[15]. Here, we present the experimental approach and cell models used to demonstrate the protective effects of melatonin in placental trophoblast cells at the molecular, cellular and functional level[8].

Protocol

Placentas were obtained immediately after spontaneous vaginal deliveries from uncomplicated pregnancies at the CHUM-St-Luc Hospital, Montreal, QC, Canada, with informed patient consent and approval of ethical committees (CHUM-St-Luc Hospital and INRS-Institut Armand-Frappier, Laval, QC, Canada).

1. Isolation and purification of villous cytotrophoblast cells

1. Solutions and media
    1. Prepare transport media by supplementing Dulbecco's Modified Eagle's Medium High-Glucose (DMEM-HG) with 1% *vol/vol* antibiotic (10,000 units/mL penicillin G, 100 mg/mL streptomycin sulphate) and store at 4 °C.
    2. Prepare primary culture media by supplementing DMEM-HG with 10% *vol/vol* fetal bovine serum (FBS), 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 1% *vol/vol* antibiotic (10,000 units/mL penicillin G, 100 mg/mL streptomycin sulphate) and store at 4 °C. Warm media to 37 °C before use.
    3. Prepare 4 L of saline solution (0.9% weight/vol sodium chloride).
    4. Prepare modified Hank's Balanced Salt Solution (HBSS) by adding 25 mM HEPES to 1X HBSS (pH 7.4).
    5. Prepare fresh, four bottles of digestion solution with modified HBSS (prepared in 1.1.4), magnesium sulfate ($MgSO_4$), calcium chloride ($CaCl_2$), trypsin, deoxyribonuclease IV (DNase IV), and 1% *vol/vol* antibiotic (10,000 units/mL penicillin G, 100 mg/mL streptomycin sulfate) as shown in Table 1.
    6. Prepare density centrifugation media gradient
        1. Prepare density centrifugation media solution by supplementing density centrifugation media with 10% *vol/vol* HBSS 10X.
        2. Prepare 14 assay tubes with density centrifugation media solution and modified HBSS, as described in Table 2.
        3. Mix each density solution (step 1.1.6.2) vigorously before adding its content to the gradient. Gently add the density solutions to a 50 mL glass centrifuge tube with a peristaltic pump (1 mL/min), beginning with the highest concentration (70%). Avoid droplets by draining the solutions against the tube wall to maintain the proper separation of each layer of the gradient.
            1. In absence of a peristaltic pump, apply the layers very gently with Pasteur pipettes.
    7. Prepare final running buffer by supplementing the running buffer (see Table of Materials) with 2% *vol/vol* antibiotic/antimycotic (10,000 units/mL penicillin G, 100 mg/mL streptomycin sulfate). Store at 4 °C.
2. Villous cytotrophoblast isolation
    Note: Use sterile surgical equipment, glassware, pipettes, flasks, etc.
    1. On the day of villous cytotrophoblast isolation, in a 37 °C water bath, warm the digestions solutions (from step 1.1.5) and 70 mL of FBS. Note: Use 50 mL of FBS to interrupt the digestions and the remaining 20 mL for the freezing step (1.2.22).
    2. After delivery, bring the placenta to the laboratory in ice-cold transport medium (from step 1.1.1) as quickly as possible (less than 1 hour).
    3. Discard transport medium and placental blood in liquid dustbin. Weigh the placenta and immerse in cold saline solution.
    4. Measure and analyze the following features: umbilical cord length; umbilical cord localization; placental length, width, shape (oval, discoid); membrane color; cotyledon structure pathologies. Note: Data are presented in the results section.
    5. Cut the umbilical cord alongside its placental insertion (*i.e.* at its base with an additional 1 cm radius circle around the cord). Immerse in 300 mL of histological tissue fixative solution (formalin 10%) for later histological analysis.
    6. Cut the entire placenta into cubes of 5 x 5 x 5 cm. Wash thoroughly (4 times x ~ 1 min) in saline solution (0.9%) to remove blood cells until saline solution is clear. Discard rinsing liquid.
    7. In a watch glass, remove placental membranes and mince tissues to remove blood vessels and calcifications. Hold blood vessels firmly with forceps and remove tissues using the back of Metzenbaum scissors.
        1. Place minced placenta in a Büchner funnel. Rinse with approximately 100 mL of saline buffer. Continue mincing until 30-35 g of minced tissue is obtained (use plastic weighing boat and scale). If needed, mince the remainder of the placenta to obtain up to three additional 30-35 g preparations. During this time, put minced tissue in a weighing boat on ice.
            Note: This step will take around 45 min to 1 h.
    8. Add the 30-35 g minced placental tissue to a trypsinizing flask. Transfer 150 mL of the prepared digestion solution 1 (Table 1) to the trypsinizing flask and mix well.
    9. Place the trypsinizing flask in a shaking water bath for 30 min at a speed of no more than 50 cycles/min and manually mix the trypsinizing flask every 5 min for homogenous digestion.
    10. At the end of the first digestion, remove the trypsinizing flask from the water bath and tilt it (45°) for 1 min to sediment the placental tissue. With a 10 mL sterile pipette, remove and discard approximately 80 mL of supernatant. Avoid aspirating the tissue.
    11. Transfer 100 mL of digestion solution 2 (Table 1) to the trypsinizing flask; mix well and repeat step 1.2.9.

12. At the end of the second digestion, remove the trypsinizing flask from the water bath and tilt it 45 ° for 1 min. With a 10 mL sterile pipette, remove 80 mL supernatant and gently transfer to a centrifuge tube with a cell strainer (100 µm mesh). Transfer the filtered supernatant to a beaker containing 2 mL of FBS every time the centrifuge tube is full.
13. Perform the third digestion as described for the second digestion (1.2.11 and 1.2.12) using 75 mL digestion solution 3. In parallel, perform steps 1.2.15 to 1.2.16.1 for digestion 2.
14. Perform the fourth digestion exactly as the third digestion using the digestion solution 4 (75 mL), but collect a maximum amount of supernatant. Perform steps 1.2.15 to 1.2.16.1 in parallel for digestion 3, and finally for digestion 4.
15. Aliquot the supernatant (from digestions 2, 3 and 4) into 13.5 mL parts, each part into one 15 mL centrifuge tube. With a 22.8 cm long glass Pasteur pipette, very gently and slowly place 1.5 mL of FBS at the bottom of each tube in order to create a separate layer. Do not mix FBS and supernatant. Centrifuge the tubes without brake for 20 min at 1250 x g at room temperature.
Note: After centrifugation, 4 layers are visible in the tube, as shown in Figure 1. The separation of trypsin and trophoblast cells avoids excessive cellular digestion.
16. With a vacuum pump, aspirate and discard the supernatant (digestion solution) and FBS layers, including the whitish film between the two (composed of trypsin and DNase). Resuspend the pellet (the trophoblasts and red blood cell layers) with 1 mL of warm cell culture medium (step 1.1.2).
    1. Collect resuspended cells from all tubes and combine them in 1 tube. Let the tube stand at room temperature until the end of all digestions. Note: After all digestions, the usual yield is 3 tubes of resuspended cells (one per digestion).
17. Make up the volume to 15 mL with warm cell culture medium. Centrifuge at 1250 x g for 10 min at room temperature. Remove the supernatant with a vacuum pump. Avoid aspirating the pellet.
18. Gently resuspend the pellet obtained from the 3 tubes with 1 mL of warm cell culture medium. Pool their content in 1 tube. To obtain 8 mL, complete the volume with warm cell culture medium.
19. Very gently layer the cell suspension on a separation gradient with a Pasteur pipette. Centrifuge without brake for 30 min at 507 x g at room temperature.
20. After centrifugation, identify the different layers of cells in the gradient with back-lighting. Locate the layers containing trophoblast and contaminating cells between 40-50% of density centrifugation medium. With a vacuum pump, remove upper layers (> 50%).
21. Collect cells located in the layers of interest with a Pasteur pipette and transfer them to a 50 mL centrifuge tube. Make up the volume to 50 mL with cell culture medium. Centrifuge for 10 min at 1250 x g at room temperature.
22. Under sterile conditions, discard the supernatant, resuspend the pellet with 20 mL of FBS and count the number of cells using a hemocytometer. On ice, add 2.22 mL of sterile dimethyl sulfoxide (DMSO) and mix gently by flipping. Aliquot 1.5 mL of cell suspension into cryogenic vials, freeze overnight at -80 °C and transfer to a liquid nitrogen tank.

3. Trophoblast purification
    1. Install the rinsing and running buffers and a new filter column on the magnetic purification instrument according to manufacturer's instructions. Perform the "clean program" to clean negative 1, positive 1 and positive 2 ports, and then introduce the 15 mL tubes under each port according to manufacturer's instructions.
    2. Thaw the cells that were frozen in step 1.2.22 quickly in a 37 °C water bath. Transfer cells to a 50 mL tube and resuspend cells gently with 20 mL of cold running buffer solution. Centrifuge the tube for 5 min at 450 x g and 4 °C.
    3. Discard the supernatant. Repeat the wash step with cold running buffer. Count cells using a hemocytometer to determine viability. Repeat the centrifugation (for 5 min, 450 x g at 4 °C). Carefully remove the supernatant. Add 1 mL of cold running buffer containing 1% *vol/vol* of mouse anti-HLA-ABC antibodies. Incubate at 4 °C for 30 min, mixing gently every 5 min.
    4. Add 6 mL of cold running buffer. Centrifuge for 5 min at 450 x g and 4 °C. Discard the supernatant and repeat this step. Resuspend cells in 1 mL of cold running buffer containing 10% *vol/vol* of anti-mouse secondary antibody-coupled magnetic beads. Incubate at 4 °C for 30 min, mixing gently every 5 min.
    5. Add 6 mL of cold running buffer. Centrifuge for 5 min at 450 x g and 4 °C. Discard the supernatant and resuspend in 5 mL cold running buffer.
    6. Separate the trophoblast cells using the magnetic purification instrument. Collect cells at the negative port and add 20 mL of cold running buffer.
    Note: Trophoblast cells do not contain the complex HLA-ABC, and are thus separated from other cell types and directed towards the negative 1 port.
    7. Centrifuge for 5 min at 450 x g and 4 °C. Discard the supernatant and gently resuspend the cells in 20 mL warm primary culture medium. Count the cells using a hemocytometer to determine viability.
    8. Plate the cells at the following densities: 0.15 x 10^6 cells / well in 96-well plates, 1.6 x 10^6 cells / well in 24-well plates and 4.5 x 10^6 cells / well in 6-well plates. Incubate plates at 37 °C and 5% CO_2.
    9. Confirm the purity of the trophoblast cells by flow cytometry[16,17] and/or by immunocytochemistry[18].
    Note: The purity of the immunopurified cells was determined using FITC-conjugated monoclonal antibodies against cytokeratin-7 and vimentin[17-19]. This protocol is well detailed in Lanoix *et al.*, 2008[7].
    10. After at least 4 hr, rinse the cells twice with warm culture medium to remove unattached cells and then transfer the plates to the normoxia chamber, which is composed of 8% O_2 (see Figure 2 and section 2).

2. *In vitro* induction of normoxia and hypoxia/reoxygenation

1. Incubator chamber operation (see Figure 2A for set-up).
    1. In a laminar flow hood, place a Petri dish containing sterile water at the bottom of the incubator chamber to avoid dryness; then place the previously prepared cell culture plates or flasks (step 1.3.10) on the superior shelves of the chamber.
    2. Outside the hood, attach the chamber (inlet port) to the gas hose (Figure 2A: 5a, b and c) to reach the tube of gas (8% O_2 or 0.5% O_2) (Figure 2A: 7). Open both inlet and outlet ports of the chamber. At this moment, the gas regulator (Figure 2A: 4) should remain closed.

3. Carefully open the gas regulator valve (Figure 2A: 4). Flush for 4 min with an air flow of 25 L/min to completely replace the air inside the chamber.
4. After flushing the chamber, close the gas regulator then the inlet and outlet ports of the chamber.
5. Unplug the flow meter outlet hose (Figure 2A: 5c) from the inlet port of the chamber and place the chamber in a cell culture incubator at 37 °C.
6. Replace the air currently present in the plates, flasks and dissolved in the culture medium by filling the chamber with gas 1 hr after step 2.1.3.
7. Repeat steps 2.1.1 to 2.1.6 for the other gas compositions (*e.g.* 2% O_2 for first trimester trophoblast culture[9]).

2. Confirm the oxygen percentage (Figure 2B-C)
   1. To confirm the concentration of oxygen in the cell culture medium (without cells) inside the chamber, use an oxygen electrode connected to an oxygen adapter. Connect the oxygen electrode to a voltmeter.
   2. Create a calibration curve in the same solution (*i.e.* cell culture medium) by exposing the solution to gases with known oxygen contents (*e.g.* 0% and 21% oxygen). After the readings are stabilized for each concentration, introduce the electrode into the medium in the chamber.
      Note: Take all measurement at the same depth in order to avoid any bias in oxygen concentration[10].

3. Induction of normoxia and hypoxia/reoxygenation in trophoblasts.
   1. After adding primary trophoblast cells to the appropriate cell culture flasks, plates or Petri dishes, perform treatments as necessary.
   2. In parallel, inside the chamber, expose cells to the desired gas mixture to reproduce a specific condition every 24 hr (Figure 3).

Representative Results

Isolation and immunopurification of villous cytotrophoblast cells from a normal term placenta obtained by vaginal delivery yielded 1 x 10^8 viable cells. The placenta weighed 350 g, was 19 cm in diameter, 4 cm tall with discoid shape and transparent membranes. No cotyledon malformation was detected. The umbilical cord had paracentral localization and a length of 56 cm. The purity was evaluated by flow cytometry using vimentin and cytokeratin-7 markers. More than 98% of the cells were negative for vimentin and positive for cytokeratin-7, confirming the purity of villous trophoblasts cells obtained from the immunopurification. Villous cytotrophoblast cells were added to 96-well culture plates under normoxic conditions in the presence or absence of 1 mM melatonin. The biochemical differentiation of villous cytotrophoblasts was monitored by determining levels of β-human chorionic gonadotropin (β-hCG) secretion as described previously[1,7,20,21]. The morphological differentiation and apoptosis were assessed by immunofluorescence using anti-desmoplakin and anti-caspase-cleaved cytokeratin 18 intermediate filaments[7,22]. Cell culture media from day 1 (mainly villous cytotrophoblasts) to day 4 (mainly syncytiotrophoblasts) were collected, centrifuged and β-hCG levels were measured in the supernatants. Production of β-hCG, which is exclusive to the syncytiotrophoblast, increased with culture time (Figure 4). Not only hypoxia/reoxygenation, but hyperoxia (> 20% O_2) also activated apoptosis[23]. Thus, adoption of an 8% O_2 concentration was representative of the quantity of oxygen to which a villous trophoblast cell would be exposed during the third trimester of pregnancy[10]. The peak of β-hCG levels observed at 72 hr confirmed the capacity of villous cytotrophoblasts to differentiate under these conditions. Melatonin did not alter β-hCG secretion under these study conditions. The decrease of β-hCG levels at 96 hr was likely caused by apoptosis of trophoblast cells, which increases after prolonged periods in primary culture[5,7,22,24,25] (Figure 4). DMSO (0.1% *vol/vol*) was selected because it did not affect β-hCG levels[26,27]. The protective role of melatonin was strongly related to its antioxidant properties. Hypoxia/reoxygenation after 72h of culture induced oxidative stress in villous trophoblast cells. The protective effect of melatonin was assessed with Reactive Oxygen Species (ROS) Detection Reagent (Figure 5A). After 96 hr of culture, trophoblast cells were incubated for 45 min with 10 µM of 5-(and-6)-carboxy-2',7'-dichlorodihydrofluorescein diacetate (carboxy-H2DCFDA) to detect the total amount of ROS produced[8]. Villous trophoblast cells that underwent hypoxia/reoxygenation had significantly increased ROS levels (54%) compared to those under normoxia. This increase was reversed by treatment with 1 mM melatonin. Moreover, under normoxia melatonin did not modulate ROS levels (homeostasis), which was similar to non-treated villous trophoblast cells (Figure 5A). Figure 4 and 5A show that under normoxia melatonin did not alter levels of oxidative stress or β-hCG secretion in the trophoblast cells, which corroborates previous studies showing no modulation of cell homeostasis under normal conditions[28,29].

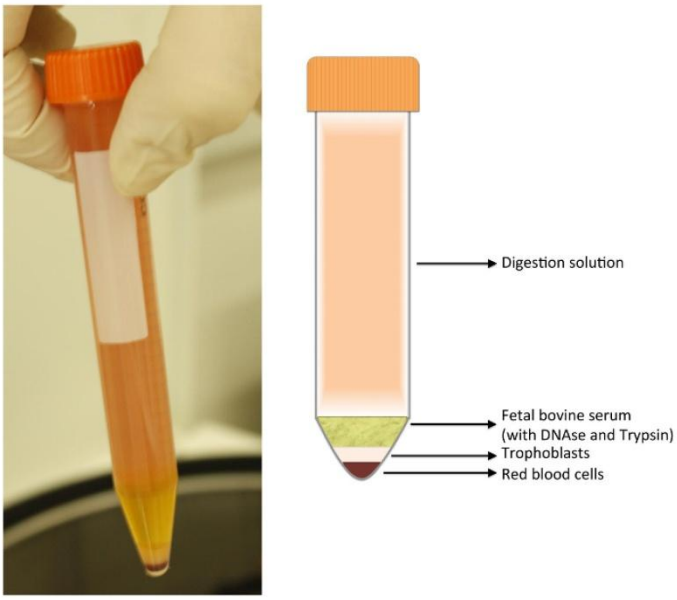

Figure 1: Digestion tube. After centrifugation, 4 layers are formed. The upper layer is composed of digestion solution; just below, the fetal bovine serum (FBS) layer contains a white film composed of trypsin and DNase, both layers should be discarded with a vacuum pump. The lower layers are composed as follow: a white layer containing fibroblasts, leukocytes, macrophages, and trophoblasts; and a bottom layer composed of red blood cells. Please click here to view a larger version of this figure.

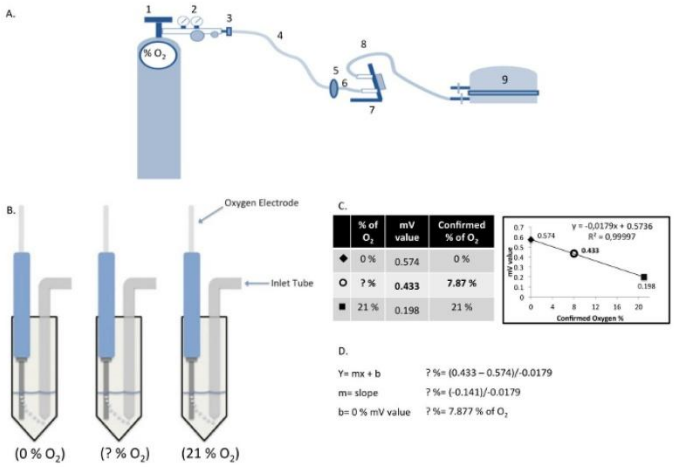

Figure 2: Components of the hypoxia chamber and measurement/calculation of dissolved oxygen concentration. (A) Hypoxia chamber and gas cylinder assembly: (1) Gas cylinder; (2) Gas regulator; (3) Gas hose clamp; (4) Cylinder gas hose; (5) Inlet filter; (6) Inlet hose; (7) Flow meter; (8) Outlet hose; (9) Modular incubator chamber. (B) Calculation of actual oxygen concentration in cell culture medium using a standard curve produced with known oxygen concentrations. (C and D) The relative values obtained in the solutions "0% O_2" and "21% O_2", are plotted graphically as a linear function to determine the oxygen concentration in the cell culture medium "?% O_2". Please click here to view a larger version of this figure.

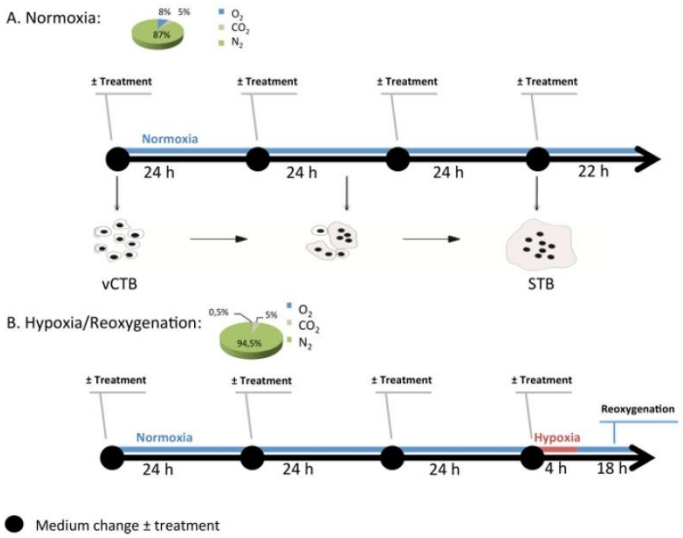

Figure 3: Generic experimental design of cell culture in the modular incubation chamber. Normoxia (8% O_2; 5% CO_2; 87% N_2) and hypoxia/reoxygenation (H/R) (0.5% O_2; 5% CO_2; 94.5% N_2) are conditions used to study pathological conditions in villous cytotrophoblast (vCTB) and syncytiotrophoblast (STB) cells. Every 24 hr, medium with or without melatonin (1mM) is changed and the gas mixture is renewed. Under H/R, STB cells undergo hypoxia (0.5% O_2) for 4 hr and then return to normoxia (8% O_2). Please click here to view a larger version of this figure.

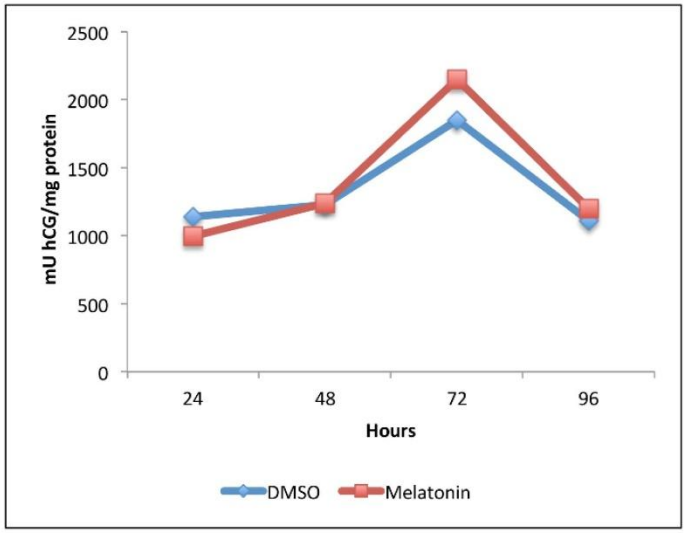

Figure 4: Effect of melatonin on beta-human chorionic gonadotropin (β-hCG) secretion during villous trophoblast differentiation. Villous cytotrophoblast cells were isolated and purified from human healthy term placentas. Cells were treated for 96 hr with 1 mM melatonin or dimethyl sulfoxyde (DMSO 0.1%: vehicle control) under normoxic conditions (8% O_2; 5% CO_2; 87% N_2). β-hCG levels in culture medium were measured by enzyme-linked immunosorbent assay (ELISA) after 24, 48, 72 and 96 hr of primary culture. Levels were normalized to the protein content of the whole-cell lysate from each corresponding well. Please click here to view a larger version of this figure.

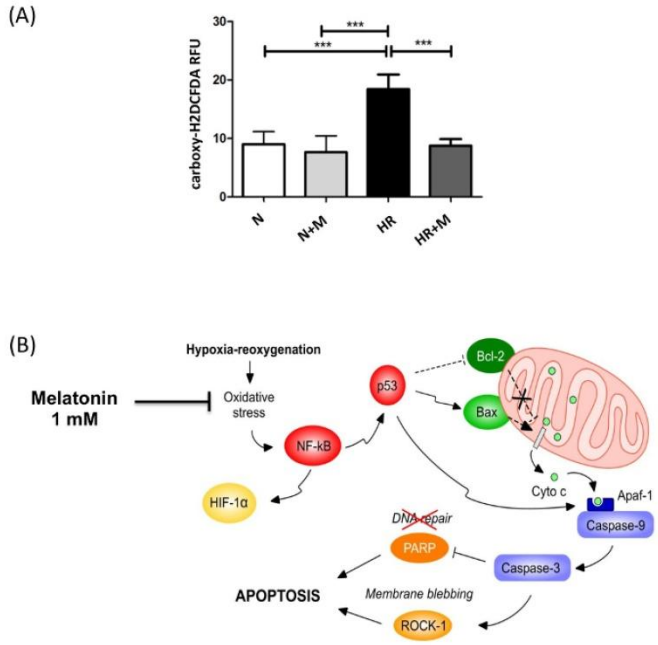

Figure 5: Anti-oxidant effect of melatonin in syncytiotrophoblast exposed to hypoxia/reoxygenation. (A) The effect of melatonin (M; 1 mM) on intracellular reactive oxygen species (ROS) levels in syncytiotrophoblast cells under normoxia (N) or hypoxia/reoxygenation (HR), induced after 72 hr of culture, was assessed by 5-(and-6)-carboxy-2′,7′-dichlorodihydrofluorescein diacetate (carboxy-H2DCFDA) fluorescence. Results are expressed as the mean ± SD of 3 different placentas; *** $P < 0.001$ (Lanoix, *et al.*[8]). (B) The cellular pathways involved in the putative protection of melatonin against hypoxia/reoxygenation-induced apoptosis. Primary villous cytotrophoblast cells were cultured for 72 hr under normoxia (8% O_2) to allow differentiation into syncytiotrophoblast. Cells were exposed to 1 mM of melatonin or vehicle control and then subjected to hypoxia (0.5% O_2) for 4 hr followed by an 18 hr reoxygenation period (8% O_2). Hypoxia/reoxygenation-induced oxidative stress activates redox sensitive transcription factors such as nuclear factor kappa B (NF-κB) and hypoxia inducible factor 1 (HIF-1). NF-κB induces p53, which triggers the Bax/Bcl-2 pathway of mitochondrial apoptosis involving the cleavage and activation of caspases 9 and 3. Caspase 3 activates Rho-associated, coiled coil-containing protein kinase 1 (ROCK-1), the cleavage of poly(ADP-ribose) polymerase (PARP) and the impairment of DNA repair. Melatonin prevents the induction of mitochondrial apoptosis by acting as a powerful antioxidant to reduce the oxidative stress caused by hypoxia/reoxygenation. This figure has been modified from Lanoix *et al.*, 2013[8]. Please click here to view a larger version of this figure.

	Digestion 1	Digestion 2	Digestion 3	Digestion 4
Modified HBSS (ml)	150	100	75	75
DNAse (µl)	300	200	150	150
(0.1 mg/µl)				
$MgSO_4$ (µl)	150	100	75	75
(800 mM)				
$CaCl_2$ (µl)	150	100	75	75
(100 mM)				
Trypsin (U)	1,824,000	1,200,000	960,000	960,000
P/S (ml)	1	0	0	0

Table 1: Quantities of ingredients for the digestion solution. Penicillin and streptomycin (P/S); magnesium sulfate ($MgSO_4$); calcium chloride ($CaCl_2$); deoxyribonuclease IV (DNase IV).

Gradient Concentration (%)	70	65	60	55	50	45	40	35	30	25	20	15	10	5
Density centrifugation media solution (ml)	2.33	2.17	2	1.83	1.67	1.5	1.33	1.17	1	0.83	0.67	0.5	0.33	0.17
Modified HBSS (ml)	0.67	0.83	1	1.17	1.33	1.5	1.67	1.83	2	2.17	2.33	2.5	2.67	2.83

Table 2: Volumes of density centrifugation media solution and modified HBSS required for preparation of the gradient solution.

Discussion

In mammals, fetal development is directly dependent on adequate placental function. The developmental origins of health disorders are based on the hypothesis that the cause of diseases manifested later in life can be traced back to early development and that the placenta has a mechanistic role in fetal programming[30-32]. The placenta is the key mediator of fetal growth and development: it regulates nutrient transfer, protects against harmful exposures, and has major endocrine functions. The development by Kliman *et al.* of a reproducible technique to isolate viable primary cytotrophoblasts is a milestone in the study of normal and abnormal placental functions[6]. Many researchers have adapted this technique to reproduce specific conditions *in vitro* to understand placental physiology[18,20,33,34]. As described by Petroff *et al.*, many steps are important to guarantee a purified and robust yield of isolated cytotrophoblast cells[18]. For example, the digestion steps have undergone several modifications since the development of the technique in 1988, such as an increased number and length of the digestions, as well as to the composition and quantity of digestion enzymes, resulting in greater numbers of viable isolated cytotrophoblasts[18]. This current protocol has three main advantages: cryopreservation, which allows for the possibility to continue the protocol later immunomagnetic purification, which increases cytotrophoblast purity and the use of pre-coated microplates improving cell attachment[7,34-36]. The existing literature contains several examples of diverse modifications of the isolation technique for cytotrophoblast cells, but the characteristics of the density centrifugation media has remained virtually unmodified[37]. The presented technique of isolation/immunopurification has several critical steps. Hence, it is important to evaluate the purity of the villous cytotrophoblast cells at the end of each immunopurification. This can be done by flow cytometry using appropriate antibodies as markers: cytokeratin-7 (trophoblastic marker), cluster of differentiation 45 (CD45) and vimentin (non-trophoblast cells markers)[18,38,39]. Other critical aspects are the quality of the obtained placentas, the FBS and density centrifugation media gradient, as well as centrifugation speed, which can all influence the yield and quality of the cells[20,40].

Although widely used, the present technique has unavoidable limitations. Firstly, the amount of viable cytotrophoblasts cells obtained after immunopurification is relatively low and is the main limiting factor in the number of possible conditions/treatments that can be tested. Secondly, the life-span of primary trophoblast cells is short and *in vitro* differentiation into syncytiotrophoblast is closely followed by a reduction of cell viability and an increase of apoptosis. The short length of trophoblast cell viability which does not proliferate *in vitro*, limits the evaluation of longer term treatments, because apoptosis is irreversibly triggered after about 4 days of culture[7,41]. Thirdly, interplacental variability is large, so a relatively large number of placentas is required to obtain statistically interpretable results. On the other hand, primary trophoblast culture has unique advantages, such as the capacity of the cells to differentiate into syncytium, which allows for the study of conditions and treatments in different cell phenotypes according to the various stages of differentiation. The oxygenation method presented in this protocol is highly adaptable and its configuration can be tailored for other situations, such as the culture of primary trophoblast cells from first trimester pregnancy, which should be exposed to a lower oxygen tension for normoxia[9,42,43].

There are other approaches to study human placental function *in vitro*. Snap-freezing placental tissues allows for multi-omics analyses, but requires placental multisite sampling, to avoid for example metabolic variations due to the oxygenation gradient, which decreases from the central vili to the periphery[44]. However, live trophoblast cell biology and behavior cannot be studied using this approach[45]. Villous explants have the advantage of maintaining the whole villous structure with the constituent cell types and their communication, but responses to treatments are not specific to trophoblast cells[23]. Commercially available trophoblast-like choriocarcinoma cell lines, such as BeWo, Jeg-3 and JAR can be used to study placental functions, such as fusion, differentiation, and transplacental transport. However, recent studies show that gene expression in primary cytotrophoblasts and BeWo tumor cells are poorly correlated[46-48]. Thus, primary villous trophoblast cell culture, despite its limitations, possess the unique advantage of mimicking the *in vivo* environment of the normal or abnormal placenta.

Studies using villous trophoblast cell in primary culture and villous explants show that hypoxia and hypoxia/reoxygenation systematically decrease trophoblast cell viability, concomitant with increased levels of oxidative stress, inflammation, autophagy and apoptosis[43,49-52]. This hypoxia/reoxygenation cell culture model, specifically, has allowed us to demonstrate the antioxidant and anti-apoptotic effects of melatonin in villous trophoblast cells. In primary villous trophoblast cells exposed to hypoxia/reoxygenation, melatonin prevents the following: induction of oxidative stress, decreased antioxidant enzyme activities, increased activity of redox-sensitive signalling pathways, and induction of mitochondrial apoptosis (Figure 5B)[8]. The hypoxia/reoxygenation model is a unique tool to ascertain the preventive role of melatonin in oxidative stress-induced damage and its possible protective role in pregnancy complications such as preeclampsia, where placental melatonin synthesis is reduced[53]. Melatonin is a powerful antioxidant with a wide range of targets[54]. Also, the safety of melatonin as a treatment has been largely established. The beneficial results with melatonin have been reproduced by several researchers and melatonin is currently in clinical trials as a potential preventive or therapeutic treatment in pregnancies complicated by preeclampsia or intrauterine growth restriction[55,56].

In conclusion, the isolation, purification and primary culture of high quality cytotrophoblast cells, together with the technique of hypoxia/reoxygenation enable a wide range of promising experimental approaches to better understand pregnancy complications related to oxidative stress and improve placental health.

Disclosures

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References

1. Vaillancourt, C., Lanoix, D., Le Bellego, F., Daoud, G., & Lafond, J. Involvement of MAPK signalling in human villous trophoblast differentiation. *Mini Rev Med Chem*. **9** (8), 962-973 (2009).
2. Gauster, M., Moser, G., Orendi, K., & Huppertz, B. Factors involved in regulating trophoblast fusion: potential role in the development of preeclampsia. *Placenta*. **30 Suppl A** S49-54 (2009).
3. Huppertz, B., Kadyrov, M., & Kingdom, J. C. Apoptosis and its role in the trophoblast. *Am J Obstet Gynecol*. **195** (1), 29-39 (2006).
4. Lanoix, D., Lacasse, A. A., Reiter, R. J., & Vaillancourt, C. Melatonin: the smart killer: the human trophoblast as a model. *Mol Cell Endocrinol*. **348** (1), 1-11 (2012).
5. Huppertz, B., Frank, H. G., Reister, F., Kingdom, J., Korr, H., & Kaufmann, P. Apoptosis cascade progresses during turnover of human trophoblast: analysis of villous cytotrophoblast and syncytial fragments in vitro. *Lab Invest*. **79** (12), 1687-1702 (1999).
6. Kliman, H. J., Nestler, J. E., Sermasi, E., Sanger, J. M., & Strauss, J. F., 3rd. Purification, characterization, and in vitro differentiation of cytotrophoblasts from human term placentae. *Endocrinology*. **118** (4), 1567-1582 (1986).
7. Lanoix, D., Beghdadi, H., Lafond, J., & Vaillancourt, C. Human placental trophoblasts synthesize melatonin and express its receptors. *J Pineal Res*. **45** (1), 50-60 (2008).
8. Lanoix, D., Lacasse, A. A., Reiter, R. J., & Vaillancourt, C. Melatonin: The watchdog of villous trophoblast homeostasis against hypoxia/reoxygenation-induced oxidative stress and apoptosis. *Mol Cell Endocrinol*. **381** (1-2), 35-45 (2013).
9. Tuuli, M. G., Longtine, M. S., & Nelson, D. M. Review: Oxygen and trophoblast biology--a source of controversy. *Placenta*. **32 Suppl 2** S109-118 (2011).
10. Chen, B., Longtine, M. S., & Nelson, D. M. Pericellular oxygen concentration of cultured primary human trophoblasts. *Placenta*. **34** (2), 106-109 (2013).
11. Roberts, J. M., & Hubel, C. A. Is oxidative stress the link in the two-stage model of pre-eclampsia? *Lancet*. **354** (9181), 788-789 (1999).
12. Burton, G. J., & Jauniaux, E. Oxidative stress. *Best Pract Res Clin Obstet Gynaecol*. **25** (3), 287-299 (2011).
13. Ji, L., Brkic, J., Liu, M., Fu, G., Peng, C., & Wang, Y. L. Placental trophoblast cell differentiation: Physiological regulation and pathological relevance to preeclampsia. *Mol Aspects Med*. **34** (5), 981-1023 (2013).
14. Redman, C. W., & Sargent, I. L. Placental stress and pre-eclampsia: a revised view. *Placenta*. **30 Suppl A** S38-42 (2009).
15. Sagrillo-Fagundes, L., Soliman, A., & Vaillancourt, C. Maternal and placental melatonin: actions and implication for successful pregnancies. *Minerva Ginecol*. **66** (3), 251-266 (2014).
16. Blaschitz, A., Weiss, U., Dohr, G., & Desoye, G. Antibody reaction patterns in first trimester placenta: implications for trophoblast isolation and purity screening. *Placenta*. **21** (7), 733-741 (2000).
17. Potgens, A. J., Gaus, G., Frank, H. G., & Kaufmann, P. Characterization of trophoblast cell isolations by a modified flow cytometry assay. *Placenta*. **22** (2-3), 251-255 (2001).
18. Petroff, M. G., Phillips, T. A., Ka, H., Pace, J. L., & Hunt, J. S. Isolation and culture of term human trophoblast cells. *Methods Mol Med*. **121** 203-217 (2006).
19. Maldonado-Estrada, J., Menu, E., Roques, P., Barre-Sinoussi, F., & Chaouat, G. Evaluation of Cytokeratin 7 as an accurate intracellular marker with which to assess the purity of human placental villous trophoblast cells by flow cytometry. *J Immunol Methods*. **286** (1-2), 21-34 (2004).
20. Le Bellego, F., Vaillancourt, C., & Lafond, J. Isolation and culture of term human cytotrophoblast cells and in vitro methods for studying human cytotrophoblast cells' calcium uptake. *Methods Mol Biol*. **550** 73-87 (2009).
21. Mounier, C., Barbeau, B., Vaillancourt, C., & Lafond, J. Endocrinology and cell signaling in human villous trophoblast. *Methods Mol Biol*. **550** 89-102 (2009).
22. Chen, B. *et al.* Pomegranate juice and punicalagin attenuate oxidative stress and apoptosis in human placenta and in human placental trophoblasts. *Am J Physiol Endocrinol Metab*. **302** (9), E1142-1152 (2012).
23. Reti, N. G. *et al.* Effect of high oxygen on placental function in short-term explant cultures. *Cell Tissue Res*. **328** (3), 607-616 (2007).
24. Pidoux, G. *et al.* Biochemical characterization and modulation of LH/CG-receptor during human trophoblast differentiation. *J Cell Physiol*. **212** (1), 26-35 (2007).
25. Pidoux, G. *et al.* ZO-1 is involved in trophoblastic cell differentiation in human placenta. *Am J Physiol Cell Physiol*. **298** (6), C1517-1526 (2010).
26. Williams, J. L., Fyfe, G. K., Sibley, C. P., Baker, P. N., & Greenwood, S. L. K+ channel inhibition modulates the biochemical and morphological differentiation of human placental cytotrophoblast cells in vitro. *Am J Physiol Regul Integr Comp Physiol*. **295** (4), R1204-1213 (2008).

27. Schild, R. L., Schaiff, W. T., Carlson, M. G., Cronbach, E. J., Nelson, D. M., & Sadovsky, Y. The activity of PPAR gamma in primary human trophoblasts is enhanced by oxidized lipids. *J Clin Endocrinol Metab*. **87** (3), 1105-1110 (2002).
28. Menendez-Pelaez, A., & Reiter, R. J. Distribution of melatonin in mammalian tissues: the relative importance of nuclear versus cytosolic localization. *J Pineal Res*. **15** (2), 59-69 (1993).
29. Perrone, S., Stazzoni, G., Tataranno, M. L., & Buonocore, G. New pharmacologic and therapeutic approaches for hypoxic-ischemic encephalopathy in the newborn. *J Matern Fetal Neonatal Med*. **25 Suppl 1** 83-88 (2012).
30. Nelissen, E. C., van Montfoort, A. P., Dumoulin, J. C., & Evers, J. L. Epigenetics and the placenta. *Hum Reprod Update*. **17** (3), 397-417 (2011).
31. Barker, D. J. Intrauterine programming of adult disease. *Mol Med Today*. **1** (9), 418-423 (1995).
32. Barker, J. R., Thomas, C. F., & Behan, M. Serotonergic projections from the caudal raphe nuclei to the hypoglossal nucleus in male and female rats. *Respir Physiol Neurobiol*. **165** (2-3), 175-184 (2009).
33. Yui, J. *et al.* Functional, long-term cultures of human term trophoblasts purified by column-elimination of CD9 expressing cells. *Placenta*. **15** (3), 231-246 (1994).
34. Kilani, R. T., Chang, L. J., Garcia-Lloret, M. I., Hemmings, D., Winkler-Lowen, B., & Guilbert, L. J. Placental trophoblasts resist infection by multiple human immunodeficiency virus (HIV) type 1 variants even with cytomegalovirus coinfection but support HIV replication after provirus transfection. *J Virol*. **71** (9), 6359-6372 (1997).
35. Knofler, M., Stenzel, M., & Husslein, P. Shedding of tumour necrosis factor receptors from purified villous term trophoblasts and cytotrophoblastic BeWo cells. *Hum Reprod*. **13** (8), 2308-2316 (1998).
36. Douglas, G. C., & King, B. F. Isolation of pure villous cytotrophoblast from term human placenta using immunomagnetic microspheres. *J Immunol Methods*. **119** (2), 259-268 (1989).
37. Li, L., & Schust, D. J. Isolation, purification and in vitro differentiation of cytotrophoblast cells from human term placenta. *Reprod Biol Endocrinol*. **13** 71 (2015).
38. Stenqvist, A. C. *et al.* An efficient optimized method for isolation of villous trophoblast cells from human early pregnancy placenta suitable for functional and molecular studies. *Am J Reprod Immunol*. **60** (1), 33-42 (2008).
39. Potgens, A. J., Kataoka, H., Ferstl, S., Frank, H. G., & Kaufmann, P. A positive immunoselection method to isolate villous cytotrophoblast cells from first trimester and term placenta to high purity. *Placenta*. **24** (4), 412-423 [pii] (2003).
40. Lanoix, D., & Vaillancourt, C. Cell culture media formulation and supplementation affect villous trophoblast HCG release. *Placenta*. **31** (6), 558-559 (2010).
41. Vaillancourt, C., & Lafond, J. Human embryogenesis: overview. *Methods Mol Biol*. **550** 3-7 (2009).
42. Armant, D. R. *et al.* Human trophoblast survival at low oxygen concentrations requires metalloproteinase-mediated shedding of heparin-binding EGF-like growth factor. *Development*. **133** (4), 751-759 (2006).
43. McCaig, D., & Lyall, F. Hypoxia upregulates GCM1 in human placenta explants. *Hypertens Pregnancy*. **28** (4), 457-472 (2009).
44. Burton, G. J. *et al.* Optimising sample collection for placental research. *Placenta*. **35** (1), 9-22 (2014).
45. Lanoix, D. *et al.* Quantitative PCR pitfalls: the case of the human placenta. *Mol Biotechnol*. **52** (3), 234-243 (2012).
46. Bilban, M. *et al.* Trophoblast invasion: assessment of cellular models using gene expression signatures. *Placenta*. **31** (11), 989-996 (2010).
47. Novakovic, B. *et al.* Wide-ranging DNA methylation differences of primary trophoblast cell populations and derived cell lines: implications and opportunities for understanding trophoblast function. *Mol Hum Reprod*. **17** (6), 344-353 (2011).
48. Burleigh, D. W. *et al.* Microarray analysis of BeWo and JEG3 trophoblast cell lines: identification of differentially expressed transcripts. *Placenta*. **28** (5-6), 383-389 (2007).
49. Hung, T. H., Skepper, J. N., Charnock-Jones, D. S., & Burton, G. J. Hypoxia-reoxygenation: a potent inducer of apoptotic changes in the human placenta and possible etiological factor in preeclampsia. *Circ Res*. **90** (12), 1274-1281 (2002).
50. Heazell, A. E., Moll, S. J., Jones, C. J., Baker, P. N., & Crocker, I. P. Formation of syncytial knots is increased by hyperoxia, hypoxia and reactive oxygen species. *Placenta*. **28 Suppl A** S33-40 (2007).
51. Heazell, A. E., Lacey, H. A., Jones, C. J., Huppertz, B., Baker, P. N., & Crocker, I. P. Effects of oxygen on cell turnover and expression of regulators of apoptosis in human placental trophoblast. *Placenta*. **29** (2), 175-186 (2008).
52. Chen, B., Longtine, M. S., & Nelson, D. M. Hypoxia induces autophagy in primary human trophoblasts. *Endocrinology*. **153** (10), 4946-4954 (2012).
53. Lanoix, D., Guerin, P., & Vaillancourt, C. Placental melatonin production and melatonin receptor expression are altered in preeclampsia: new insights into the role of this hormone in pregnancy. *J Pineal Res*. **53** (4), 417-425 (2012).
54. Galano, A., Tan, D. X., & Reiter, R. J. Melatonin as a natural ally against oxidative stress: a physicochemical examination. *J Pineal Res*. **51** (1), 1-16 (2011).
55. Alers, N. O., Jenkin, G., Miller, S. L., & Wallace, E. M. Antenatal melatonin as an antioxidant in human pregnancies complicated by fetal growth restriction--a phase I pilot clinical trial: study protocol. *BMJ Open*. **3** (12), e004141 (2013).
56. Hobson, S. R., Lim, R., Gardiner, E. E., Alers, N. O., & Wallace, E. M. Phase I pilot clinical trial of antenatal maternally administered melatonin to decrease the level of oxidative stress in human pregnancies affected by pre-eclampsia (PAMPR): study protocol. *BMJ Open*. **3** (9), e003788 (2013).

