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ATP-binding cassette transporters in reproduction: a new frontier

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BACKGROUND: The transmembrane ATP-binding cassette (ABC) transporters actively efflux an array of clinically relevant compounds across biological barriers, and modulate biodistribution of many physiological and pharmacological factors. To date, over 48 ABC transporters have been identified and shown to be directly and indirectly involved in peri-implantation events and fetal/placental development. They efflux cholesterol, steroid hormones, vitamins, cytokines, chemokines, prostaglandins, diverse xenobiotics and environmental toxins, playing a critical role in regulating drug disposition, immunological responses and lipid trafficking, as well as preventing fetal accumulation of drugs and environmental toxins.

METHODS: This review examines ABC transporters as important mediators of placental barrier functions and key reproductive processes. Expression, localization and function of all identified ABC transporters were systematically reviewed using PubMed and Google Scholar websites to identify relevant studies examining ABC transporters in reproductive tissues in physiological and pathophysiological states. Only reports written in English were incorporated with no restriction on year of publication. While a major focus has been placed on the human, extensive evidence from animal studies is utilized to describe current understanding of the regulation and function of ABC transporters relevant to human reproduction.

RESULTS: ABC transporters are modulators of steroidogenesis, fertilization, implantation, nutrient transport and immunological responses, and function as 'gatekeepers' at various barrier sites (i.e. blood-testes barrier and placenta) against potentially harmful xenobiotic factors, including drugs and environmental toxins. These roles appear to be species dependent and change as a function of gestation and development. The best-described ABC transporters in reproductive tissues (primarily in the placenta) are the multidrug transporters p-glycoprotein and breast cancer-related protein, the multidrug resistance proteins 1 through 5 and the cholesterol transporters ABCA1 and ABCG1.

CONCLUSIONS: The ABC transporters have various roles across multiple reproductive tissues. Knowledge of efflux direction, tissue distribution, substrate specificity and regulation of the ABC transporters in the placenta and other reproductive tissues is rapidly expanding. This will allow better understanding of the disposition of specific substrates within reproductive tissues, and facilitate development of novel treatments for reproductive disorders as well as improved approaches to protecting the developing fetus.

Key words: ABC transporters / p-glycoprotein / breast cancer-related protein / multidrug resistance-associated proteins / placenta / fetal membranes / decidua / myometrium / testes / ovary

Introduction

The ATP-binding cassette transporters (ABC transporters) are highly conserved ubiquitous transmembrane proteins that are abundant in prokaryote and eukaryote species (Dean and Annilo, 2005). ABC transporters utilize ATP hydrolysis to translocate a wide variety of substrates (Higgins, 1992). Several ABC transporters are also present in the membranes of diverse organelles such as endoplasmic reticulum, mitochondria, golgi, peroxisomes and lysosomes (Ruiz *et al.*, 2013). The primary function of these transporters is to modulate transfer of substrates from the cytosol toward the extracellular space (George and Jones, 2012; Tarling *et al.*, 2013). This efflux function limits exogenous substrate accumulation in the cytosol, or alternatively allows endogenous substrates and waste matter metabolites that have been formed in the cytosol, to be relocated to the extracellular space (Bellamy, 1996).

To date, over 48 ABC transporter genes have been identified and proved to be transcriptionally active and encode functional proteins. Based on their structural homologies, ABC transporters have been subdivided into seven sub-families labeled ABCA through ABCG (Wenzel *et al.*, 2007; Honorat *et al.*, 2011). The ABC transporters have a very broad substrate specificity, many of which are physiologically relevant both directly and indirectly to key reproductive processes. For example, ABC transporters efflux numerous steroid hormones, including androgens (testosterone, dihydrotestosterone, dehydroepiandrosterone sulfate), estrogens (17β-estradiol, estrone, estriol), glucocorticoids (cortisol, dexamethasone), mineralocorticoids (aldosterone) and progestogens (pregnenolone, 17 hydroxyprogesterone) (Table I). Further, a number of the ABC transporters also transport cholesterol, the common precursor of steroid hormones and fat-soluble vitamins (A,D,E,K) (Ikonen, 2008). Compounds involved in inflammatory and immunological responses, such as cytokines, chemokines and prostaglandins (PG), are also substrates for specific ABC transporters (Table I; van de Ven *et al.*, 2009; Iqbal *et al.*, 2012). These factors are crucial mediators of fertilization, embryo segmentation, implantation, placentation, fetal development and the birth process (Challis *et al.*, 2000; Devoto *et al.*, 2002; Keelan *et al.*, 2003; Sawicki *et al.*, 2006; Bazer *et al.*, 2009; Van Sinderen *et al.*, 2013).

While ABC transporters play a major role in biodistribution of many physiological factors involved in different reproductive processes, they also efflux clinically relevant drugs (e.g. anticancer, anti-human immunodeficiency virus drugs, synthetic steroids, antibiotics) and environmental toxins (e.g. bisphenol A—BPA, ivermectin) (Marquez and Van Bambeke, 2011; Iqbal *et al.*, 2012; Mazur *et al.*, 2012; Table I). ABC transporters play a critical role in barrier-tissues such as the placenta (Table II), blood-brain barrier (BBB) and blood-testis barrier (BTB). At the placenta and the fetal BBB, they limit the entry of xenobiotics and other factors in the maternal circulation into the fetal compartment and fetal brain (Hutson *et al.*, 2010; Iqbal *et al.*, 2012; Bloise *et al.*, 2013; Baello *et al.*, 2014). A number of the ABC transporters, including P-glycoprotein (P-gp; encoded by *ABCB1*) and breast cancer-related protein (BCRP; encoded by *ABCG2*) were first identified in cancer cells where they confer drug resistance to a plethora of anti-neoplastic drugs. As such, they were named 'multidrug resistance (MDR) proteins' (Xia and Smith, 2012).

To date, much attention has been focused on the function of ABC transporters in barrier-sites and neoplastic tissues. There has been far less emphasis on the regulation and function of these transporters in reproductive tissues. This review comprehensively examines ABC transporters as important mediators of key reproductive processes, including uterine function, pregnancy and embryo/fetal development (Fig. 1).

Methods

The expression, localization and function of all 48 ABC transporters have been systematically reviewed using PubMed and Google Scholar websites to identify relevant studies in the human and in animal models (i.e. mouse, rat, guinea pig, porcine, ovine, bovine) investigating ABC transporters in diverse intrauterine tissues. A combination of the following search terms was used as a strategy to identify relevant literature: zygote, embryos, endometrium, decidua, placenta, syncytiotrophoblast, cytotrophoblast, uterus, myometrium, fetal membranes, amnion, chorion, maternal recognition of pregnancy, maternal–fetal interface, oxygen tension, estradiol, progesterone, cortisol, toxins, infection/inflammation pre-eclampsia (PE) and preterm delivery in humans and in animal models (whenever relevant). Only reports written in English were incorporated with no restriction on year of publication. We describe and discuss the existing knowledge on ABC transporters as key mediators of important reproductive events and highlight the areas that require further research.

ABC transporters in early embryo development and preimplantation events

Embryo segmentation

For most multicellular organisms, sexual reproduction is fundamental to producing offspring. It begins with the fusion of two parental gametes

Table I **The best-described ABC transporters and their most clinically relevant physiological and pharmacological substrates in reproduction.**

ABC transporter	Physiological substrates/function	Pharmacological substrates	References
ABCA1 (TGD)	Cholesterol; surfactant phospholipid; MIF	Unknown	Agassandian *et al.* (2004), Klaassen and Aleksunes (2010), Ietta *et al.* (2010)
ABCB1 (P-gp, MDR1)	17-hydroxyprogesterone, aldosterone; anti-inflammatory cytokines (IL-4); chemokines (CCL2); endogenous (cortisol) and synthetic glucocorticoids; estradiol-17β-glucuronide; estriol; estrone; flavonoids; PAF, pregnenolone, pro-inflammatory cytokines (IL-1β, IL-2, IL-6, IFNγ, GM-CSF, TNF-α); sphingomyelin and short-chain phospholipid analogs	Amoxicillin, amprenavir, atorvastatin; bromocriptine; clarithromycin; carbamazepine; cimetidine; ciprofloxacin DEX; diclofenac; digoxin; doxorubicin; indinavir, itraconazole; ivermectin; L-dopa; levofloxacin; loratadine, losartan; morphine; nifedipine; nelfinavir; phenobarbital; prednisolone; prazosin; ranitidine; rifampicin; ritonavir; saquinavir; tetracycline	Folkers (2009), Marquez and Van Bambeke (2011), Kooij *et al.* (2011), Pawlik *et al.* (2005), Bleier *et al.* (2013), Wang *et al.* (2005), Mizutani *et al.* (2008), Sharom (2011), (2014), Bellarosa *et al.* (2009), Barnes *et al.* (1996), Aye *et al.* (2009)
ABCB4 (MDR-3)	Phosphatidylcholine	Digoxin; paclitaxel; verapamil; vinblastine	Folkers (2009), Smith *et al.* (2000)
ABCC1 (MRP1)	Bilirubin glucuronides; estradiol-17β-glucuronide; estrone 3-sulfate; dehydroepiandrosterone sulfate; glucuronide and sulfate conjugates of bile salts; folate; leukotriene C4, testosterone	Chloroquine; doxorubicin; fluoroquinolones; macrolides; methotrexate; nelfinavir; vinblastine	Augustine *et al.* (2005), Brown *et al.* (2006), Maher *et al.* (2005), Marquez and Van Bambeke (2011), Robillard *et al.* (2012), Sivils *et al.* (2010), Sodani *et al.* (2012), Klein *et al.* (2014), Bellarosa *et al.* (2009), Cho *et al.* (2014)
ABCC2 (MRP2)	17 β-glucuronosyl estradiol; dehydroepiandrosterone sulfate, estrone 3-sulfate; leukotriene C4; bilirubin−glucuronides; CCK-8; glucuronide and sulfate conjugates of bile salts	Methotrexate; pravastatin, doxorubicin, adefovir	Keppler (2014), Nies and Keppler (2007), Sodani *et al.* (2012)
ABCC3 (MRP3)	17 β-glucuronosyl estradiol; leukotriene C4; bilirubin−glucuronides; dehydroepiandrosterone 3-sulfate; glucuronide conjugates of bile salts	Methotrexate, acetaminophen glucuronide, fexofenadine	Keppler (2014), Zhou *et al.* (2008), Bellarosa *et al.* (2009)
ABCC4 (MRP4)	Estradiol-17β-glucuronide; dehydroepiandrosterone sulfate; folate; nucleotide; prostaglandins; thromboxane; urate, glucuronide and sulfate conjugates of bile salts	Beta-lactam; cetirizine; fluoroquinolones; mefloquine; methotrexate; pravastatin, tetracyclines, AZT	Augustine *et al.* (2005), Folkers (2009), Maher *et al.* (2005), Marquez and Van Bambeke (2011), Morgan *et al.* (2012), Robillard *et al.* (2012), Sodani *et al.* (2012), Borst *et al.* (2007), Cho *et al.* (2014)
ABCC5 (MRP5)	cAMP, cGMP, folate	Adefovir, methotrexate	Augustine *et al.* (2005), Folkers (2009), Long *et al.* (2011), Marquez and Van Bambeke (2011), Wielinga *et al.* (2005), Sodani *et al.* (2012)
ABCG1 (WHITE1)	Sterol, cholesterol	Unknown	Drouineaud *et al.* (2007), Puttabyatappa *et al.* (2010), Tarling and Edwards (2011), Wilcox *et al.* (2007), Aye *et al.* (2010)
ABCG2 (BCRP)	Estradiol-17β-glucuronide; dehydroepiandrosterone; estrone-3-sulfate; folate; porphyrins; sphingolipids; prostaglandins	Adefovir; beta-lactams; cimetidine; diclofenac; doxorubicin; fluoroquinolones; glyburide, nifedipine; nitrofurans; oxfendazole; prazosin; rosuvastatin; AZT	Dankers *et al.* (2013), Marquez and Van Bambeke (2011), Qian *et al.* (2013), Lemos *et al.* (2008), Evseenko *et al.*, (2007a, b), Cho *et al.* (2014)

AZT, zidovudine; cAMP, cyclic adenosine monophosphate; CCL2, chemokine (C-C motif) ligand 2; CCK-8, cholecystokinin peptide; cGMP, cyclic guanosine monophosphate; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, Interferon; IL, interleukin; MIF, macrophage migration inhibitory factor (MIF); PAF, platelet-activating factor, TNF, tumor necrosis factor.

Table II Placental ABC transporter localization and function.

Transporter	Tissue localization	Endocrine and cytokine regulation/alterations in obstetric disorders	References
ABCA1	Syncytium's BM in first trimester placentae and syncytium's AM in third trimester placentae; EV and V cytotrophoblasts; FBV's endothelia; Hofbauer cells	*E2* ↓ mRNA and protein in first trimester placentae; ↓ mRNA and protein in *antiphospholipid syndrome*; ↓ mRNA and protein in *PE* and *PE* with *IUGR*; ↑ mRNA and protein in early-onset *PE*; ↓ protein in *GDM*; *hypoxia* ↑ mRNA in first trimester placentae	Albrecht *et al.* (2007), Bhattacharjee *et al.* (2010), Baumann *et al.* (2013), Albrecht *et al.* (2010), Ietta *et al.* (2010), Plosch *et al.* (2010), Dube *et al.* (2013)
ABCB1 (P-gp/MDR1)	Syncytium's AM; syncytium-facing membrane of the cytotrophoblast	*LPS*, *IL-1*β and TNF-α ↓ mRNA and protein; *PGE2* ↓ mRNA with no changes in function; ↓ mRNA and protein in *SGA preterm infants*; mRNA and protein ↓ toward term; *DEX* and *BET* ↑ mRNA; *E2* and *P4* ↑ protein and function; BPA ↑ function; *hypoxia* ↑ mRNA and protein; *Zearalenone* (mycotoxin) ↑ protein; ↑ mRNA and protein in infective preterm labor; ↑ mRNA and staining intensity in *HIV-infected women*	Lye *et al.* (2013), Sun *et al.* (2006), Vahakangas and Myllynen (2009), Mason *et al.* (2014), Javam *et al.* (2014), Hodyl *et al.* (2013), Manceau *et al.* (2012), Camus *et al.* (2006), Coles *et al.* (2009), Evseenko *et al.* (2007a, b), Prouillac *et al.* (2009), Lye *et al.* (2015), Mason *et al.* (2011), Jin and Audus (2005)
ABCB4 (MDR3)	Syncytium's BM	*IL-6* ↑ *mRNA and protein*; *E2* ↑ *mRNA, protein and function*	Evseenko *et al.* (2007a, b)
ABCB5	First trimester VT; cytotrophoblast	Unknown	Volpicelli *et al.* (2014)
ABCC1 (MRP1)	Syncytium's BM; FBV's endothelia	*IL-6*, *TNF-α and P4* ↑ mRNA; *Zearalenone* (mycotoxin) ↑ mRNA and protein	Nagashige *et al.* (2003), St-Pierre *et al.* (2000), Evseenko *et al.* (2007a, b), Prouillac *et al.* (2009)
ABCC2 (MRP2)	Syncytium's AM	mRNA and protein ↑ toward term; *Zearalenone* (mycotoxin) ↑ mRNA and protein; ↑ mRNA and protein in *intrahepatic cholestasis of pregnancy*	St-Pierre *et al.* (2000), Azzaroli *et al.* (2007), Prouillac *et al.* (2009), Meyer zu Schwabedissen *et al.* (2005a, b)
ABCC3 (MRP3)	Syncytium's AM; FBV's endothelia	↓ mRNA in *intrahepatic cholestasis of pregnancy*	St-Pierre *et al.* (2000), Azzaroli *et al.* (2007)
ABCC5 (MRP5)	Syncytium's BM; FBV's endothelia	↓ with gestational age	Meyer zu Schwabedissen *et al.* (2005a, b)
ABCG1	Syncytium's BM; FBV's endothelia	↓ protein in obese *GDM*	Stefulj *et al.* (2009), Baumann *et al.* (2013), Dube *et al.* (2013)
ABCG2 (BCRP)	Syncytium's AM; FBV's endothelia	*LPS*, *IL-1*β and *TNF-α* ↓ mRNA and protein; ↓ mRNA in *idiopathic FGR*; *E2* ↓/↑mRNA and protein in BeWo cells; *P4* ↑ mRNA and protein in BeWo cells; *E2* ↑ protein; *PGE2*, *EGF*, *IGF-II* ↑ mRNA and protein; *Zearalenone* (mycotoxin) ↑ mRNA; *hypoxia*↑staining intensity; ↑ mRNA and protein in infective preterm labor	Lye *et al.* (2013), Yeboah *et al.* (2006), Wang *et al.* (2008), Mason *et al.* (2014), Evseenko *et al.* (2007a, b), Wang *et al.* (2006), Evseenko *et al.* (2007a, b), Prouillac *et al.* (2009), Lye *et al.* (2015), Mason *et al.* (2011), Yasuda *et al.* (2006)

AM, apical membrane; BM, basolateral membrane; BET, betamethasone; BPA, bisphenol A; DEX, dexamethasone; E2, estradiol; EGF, Epidermal growth factor; EV, extravillous; FBV, fetal blood vessels; FGR, fetal growth restriction; GDM, gestational diabetes mellitus; IGF-II, Insulin-like growth factor 2; interleukin-1beta, IL-1β; PE, pre-eclampsia; IUGR, intrauterine growth restriction; PG, prostaglandin; PM, plasma membrane; tumor necrosis factor-alpha, TNF-α; VT, villous trophoblast.

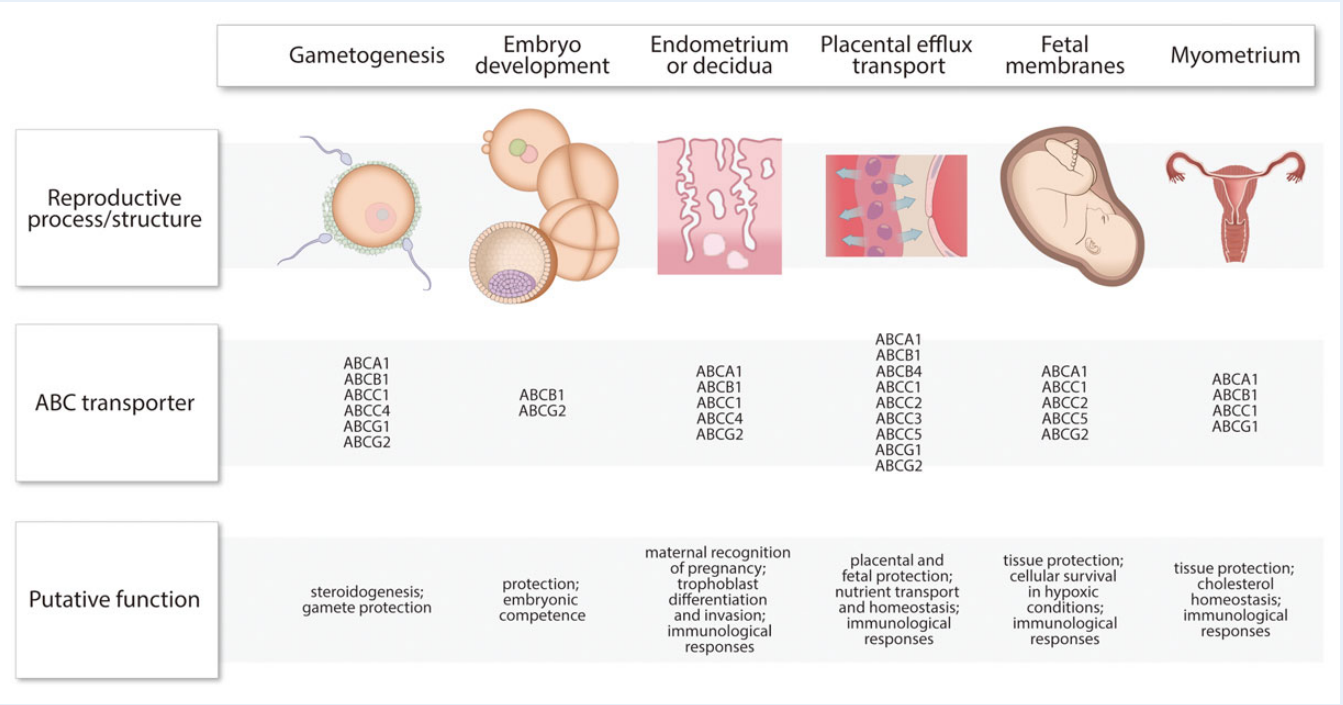

Figure 1 Selected ABC transporters are important mediators of key reproductive processes. ABC transporters actively efflux numerous clinically relevant compounds from cells, playing critical physiological and protective roles in regulating drug disposition, immunological responses and lipid trafficking, as well as preventing accumulation of environmental toxins. As such, ABC transporters are important factors in the regulation of gametogenesis, embryogenesis, embryo/fetal development and parturition.

resulting in the formation of a new genetically unique zygote. This zygote then undergoes multiple cell divisions to become the morula and then develops through the blastocyst stages. Subsequently, as the blastocyst hatches from the zona pellucida, the trophectoderm layer adheres to a receptive endometrium and implants into the uterine wall allowing the embryo to undergo gastrulation and somite segmentation. Emerging evidence demonstrates important roles for ABC transporters in early embryo development and differentiation.

The most well-described ABC transporters in peri-implantation embryos and endometrium are P-gp and BCRP (Fig. 1); other ABC transporters remain largely unexplored. In mice and rats, P-gp is encoded by two *Abcb1* gene isoforms, *Abcb1a* and *Abcb1b*, whereas in the human P-gp is encoded by a single *ABCB1* gene. In the mouse, significant *Abcb1a/b* mRNA expression was described in the 2-cell zygote and then again in embryos at 7–9 days postcoitum (dpc), however, expression was very low during the blastocyst stage. Even though 2-cell stage zygotes expressed *Abcb1a/b* genes, no transporter activity was detected in the blastomeres, indicating that P-gp activity is not required at the 2-cell stage. However, P-gp efflux activity was identified in the inner cell mass layer of blastocysts and in all embryonic cells at 7 and 9 dpc (Sawicki *et al.*, 2006). In contrast, in another study P-gp function was identified in mouse embryos from zygote to the 8-cell stage (Elbling *et al.*, 1993). These data suggest P-gp efflux activity of selected substrates maybe involved in the sequence of events leading to morphogenic progression. It is important to note that critical differences between *Abcb1a* and *Abcb1b* have been described, restricting the extrapolation from rodent findings to the human (Pappas *et al.*, 2014). BCRP is encoded by the *abcg2* gene and is increasingly expressed during early embryo development (Sawicki *et al.*, 2006), however, no studies have investigated BCRP function in the very early embryo. It is noteworthy that to our knowledge there is no evidence indicating early embryonic anomalies in the *Abcb1a/b* or *Abcg2* knockout mouse.

In the bovine embryo, P-gp protein was detected in the peri-implantation stage. *ABCB1* expression was higher in germinal vesicle- and second metaphase-stage oocytes compared with 8-cell and 16-cell embryos, and blastocysts. Moreover, bovine embryos treated with forskolin (an activator of adenylyl cyclase) plus rifampicin (an antibiotic) exhibited a 1.8-fold increase in *ABCB1* mRNA levels. After cryopreservation, blastocysts treated with forskolin and rifampicin, which expressed high levels of *ABCB1*, demonstrated significantly increased viability (16.8%), blastocyst hatching (15.4%) and cell proliferation rates compared with control blastocysts (Mori *et al.*, 2013), suggesting that embryonic developmental competence improves with increased expression of *ABCB1* during segmentation.

Improved embryonic developmental competence conferred by up-regulation of *ABCB1* may be, in part, elicited by increased efflux of environmental toxins. Metabolomics of plasticware and protein preparations used for embryo culture of different species, determined by gas chromatography mass spectrometry analysis, revealed the presence of plasticides and oil contaminants in embryo culture systems, including ditertbutylphenol and diethylene glycol (Krisher *et al.*, 2015). Since ABC transporters protect cells from a number of environmental toxins (Marquez and Van Bambeke, 2011; Iqbal *et al.*, 2012; Mazur *et al.*, 2012), it is possible that ABC transporters may confer embryo

protection from contaminants present in *in vitro* culture systems and this should be further explored.

Maternal recognition of pregnancy

Crosstalk between the preimplantation embryo and the maternal environment is essential for the establishment of a healthy pregnancy. A network of signaling through paracrine and autocrine factors released by the receptive endometrium and the developing conceptus is paramount in mediating blastocyst nidation and trophoblast invasion into the endometrium in earlier stages of pregnancy (Vigano *et al.*, 2003). In this context, selected ABC transporters are likely critical mediators of maternal-embryonic crosstalk.

P-gp expression has been detected in the non-pregnant human endometrium and in early gestation (Axiotis *et al.*, 1991). In the non-pregnant state, immunohistochemistry revealed a menstrual cycle-dependent pattern of expression. No P-gp was detectable in the early proliferative phase while P-gp was present in ~15% of the glandular epithelia of the endometrium in the mid-proliferative phase. Endometrial P-gp was increased in the late proliferative phase with high levels in the apical paranuclear regions. Luminal P-gp peaked in the early secretory phase, and was present in 80% of the glandular epithelial cells. Levels then decreased in the late secretory phase, with P-gp immunostaining detected in <35% of the endometrial glandular cells (Axiotis *et al.*, 1991). This pattern indicates that endometrial P-gp expression is highly responsive to hormonal changes in the menstrual cycle and that it may play a role in regulating the biodistribution of key substrates involved in the implantation process and maternal recognition of pregnancy. In this regard, the pro-inflammatory cytokine interleukin (IL)-6, a P-gp substrate, has been linked to uterine receptivity and blastocyst-uterine crosstalk (Bleier *et al.*, 2013). IL-6 is expressed by blastocysts and is thought to be involved in trophoblast differentiation and invasion into the endometrial wall (Bloise *et al.*, 2014). As such, high levels of P-gp would allow efflux of key compounds to the uterine lumen at specific points during menstrual cycle.

A related ABC transporter, ABCC4 or MRP-4, transports PGE1, PGE2 and PGF2α (Reid *et al.*, 2003; Lin *et al.*, 2008). Both PGE2 and PGF2α are non-invasive biomarkers of endometrial receptivity (Vilella *et al.*, 2013). MRP-4 in the endometrium is localized to the glandular epithelial cells (Gori *et al.*, 2013) and is regulated by oxytocin, a major modulator of myometrial contractility (Lacroix-Pepin *et al.*, 2011). In the porcine endometrium, *ABCC4* mRNA is most abundantly expressed on Day 12 of pregnancy and during late pregnancy, and MRP-4 is predominantly localized to the uterine luminal epithelial and glandular epithelial cells (Seo *et al.*, 2014). These emerging data collectively indicate important potential roles of ABC transporters in supporting the establishment and maintenance of pregnancy by promoting efflux of cytokines and PGs at the maternal-embryo/fetal interface.

ABC transporters in the maternal–fetal interface

A dialogue between the mother and the fetus starts during the preimplantation period and continues until birth. The most important macroanatomical structures that confer maternal–fetal communication are the placenta, decidua and fetal membranes (Cartwright *et al.*, 2010). Adaptations in placental phenotype and placental transport across pregnancy are of critical importance in allowing the conceptus to meet its growth demands (Desforges and Sibley, 2010; Sibley *et al.*, 2010; Bloise *et al.*, 2014). The syncytiotrophoblast layer of the human placenta is a multinucleated epithelial syncytium that functions as the primary barrier between mother and fetus. It is responsible for transporting nutrients (e.g. amino acids, glucose and lipids) and for preventing fetal accumulation of maternal steroids, toxins and xenobiotics, which could be detrimental to development (Iqbal *et al.*, 2012; Lager and Powell, 2012). Important nutrient carrier systems in the placenta are the system A and system L amino acid transporters (Bloise *et al.*, 2012; Lager and Powell, 2012; Dilworth and Sibley, 2013), GLUT1, GLUT3 and GLUT4 glucose transporters (Ericsson *et al.*, 2005; Bloise *et al.*, 2012) and fatty acid transport proteins (Lager and Powell, 2012). Transcription of 42 of the ABC transporter genes has been identified in the human placenta, though very few have been identified at the level of protein (Nishimura and Naito, 2005; Staud *et al.*, 2012; Table II); the latter likely results from the paucity of reliable-specific antibodies. Given their important role in transporting physiologically important factors as well as maternally derived toxins and other xenobiotics (Table I), it is critical to determine which ABC transporters are present (at the level of mRNA, protein and function) in intrauterine tissues, together with their cellular localization and their temporal pattern of expression. It is also important to determine how the regulation of the ABC transporters is modified in pregnancy pathologies. In this section, we review the current knowledge available for ABC transporters in the placenta (Fig. 2 and Table II), fetal membranes, decidua (Table III) and myometrium (Fig. 1).

Placenta

In order to reach the fetal compartment, substances present in the maternal blood must traverse the microvillous plasma membrane and basal plasma membrane of the syncytiotrophoblast, and pass through the extracellular matrix and cytotrophoblast cells prior to gaining access to the fetal capillary endothelium (Aye and Keelan, 2013; Dilworth and Sibley, 2013). Solutes can cross the placental barrier via different processes, including simple diffusion, active or facilitative transport, uptake induced by receptor activation and pinocytosis (Aye and Keelan, 2013). The rate of solute diffusion is highly dependent upon its molecular size, lipophilicity, surface thickness of the exchange barrier, concentration gradients in maternal and fetal compartments and placental/umbilical blood flow rates (Dilworth and Sibley, 2013).

Fetal accumulation of certain substances must be avoided throughout pregnancy; or during specific time windows of developmental sensitivity. Extrusion transporters represent an important physiological strategy preventing excessive fetal exposure to selected maternally derived substances (endogenous and exogenous). Steroid transfer from mother to fetus is tightly regulated across pregnancy since temporal production of steroid hormones is critical for normal fetal growth and development (Kaludjerovic and Ward, 2012). In the developing fetus, endogenous glucocorticoids (cortisol and corticosterone) function as a developmental trigger and are critical for maturation of the fetal brain, lung, kidney, liver and thyroid at specific developmental stages (Moisiadis and Matthews, 2014a, b). Premature exposure to high glucocorticoid levels has been associated with effects on growth as well as long-term endocrine and behavioral functions in offspring (Moisiadis and Matthews, 2014a, b). Endogenous glucocorticoid transfer from the maternal to fetal side is modulated by placental 11β-hydroxysteroid dehydrogenase 1 and 2 (11β-HSD1 and 11β-HSD2, respectively;

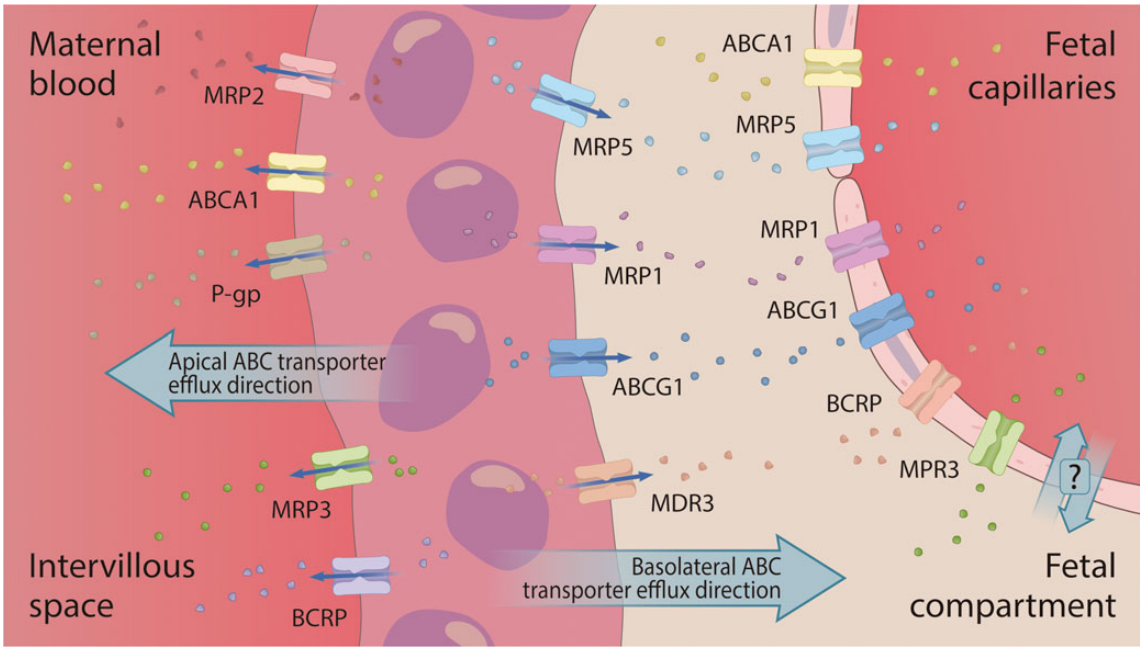

Figure 2 ABC transporters in the maternal–fetal interface. The best-described placental ABC transporters are the multidrug transporters P-gp, BCRP, the multidrug resistance protein 3 (MDR3), the MRPs 1,2,3 and 5 and the cholesterol transporters ABCA1 and ABCG1. ABC transporters localized to the syncytiotrophoblast can efflux substrates toward the fetal or maternal sides. Those on the basolateral membrane will assist in the transfer of factors from the mother to the fetus, while those on the apical surface prevent transfer of factors across the placenta toward the fetus. A number of transporters have been localized to the endothelial cells of the fetal capillaries, however, little is known as to their specific cellular localization, as well as the direction of efflux.

Table III ABC transporter localization and function in fetal membranes and decidua.

Transporter	Tissue localization	Endocrine and cytokine regulation/ alterations in obstetric disorders	References
ABCA1	Amnion cells; epithelium of secreting endometrial glands, decidua	Unknown	Nikitina *et al.* (2011), Albrecht *et al.* (2010)
ABCB1 (P-gp/MDR1)	Amnion and chorion cells (very low mRNA); deciduas' cytoplasm and membrane	Unknown	Aye *et al.* (2007), Axiotis *et al.* (1991)
ABCB4 (MDR-3)	Amnion (very low mRNA)	Unknown	Aye *et al.* (2007)
ABCC1 (MRP1)	Apical and basolateral surface of the amnion epithelium; chorionic membranes; decidua	*Arsenium* ↑ mRNA in both cell types	Yoshino *et al.* (2011), Aye *et al.* (2007)
ABCC2 (MRP2)	Apical surface of the amnion epithelium; amnion and chorion cells	*Arsenium* ↑ mRNA in amnion cells and chorion cells (time-dependent); *Arsenium* ↓ protein in C-cells	Yoshino *et al.* (2011), Aye *et al.* (2007)
ABCC5 (MRP5)	Apical and basolateral surfaces of the amnion epithelium	Unknown	Aye *et al.* (2007)
ABCC10 (MRP7)	Amnion (mRNA)	Unknown	Aye *et al.* (2007)
ABCG2 (BCRP)	Apical surface, cytoplasm and membrane between cells of the amnion epithelium; PM and nucleus of chorionic trophoblast; PM of decidua stroma	Unknown	Aye *et al.* (2007), Yeboah *et al.* (2008)

Chapman *et al.*, 2013). Placental 11β-HSD2 limits endogenous glucocorticoid accumulation in the fetus by converting maternal cortisol to inactive cortisone, however, synthetic glucocorticoids (sGC) are poor substrates for these enzymes (Kapoor *et al.*, 2008). Importantly, cortisol and sGC are P-gp substrates (Yates *et al.*, 2003) and compelling evidence suggests P-gp plays an important role in protecting the

conceptus from overexposure to maternally derived glucocorticoids and particularly sGC (Kapoor *et al.*, 2008; Moisiadis and Matthews, 2014a, b).

Other steroid hormones, such as estradiol, estriol, estrone and dehydroepiandrosterone sulfate (DHEA-S) are also substrates for a number of the ABC transporters (Table I). During pregnancy, the placenta is the major site for estrogen synthesis which is dependent upon DHEA and its sulfoconjugate DHEA-S derived from the maternal and fetal adrenal glands (Kaludjerovic and Ward, 2012). Estradiol has many actions in the maternal compartment, including myometrial and mammary gland development (Jaffer *et al.*, 2009; Macias and Hinck, 2012) but also plays important roles in the developing fetus (Wood, 2014). Therefore, it is tempting to speculate that ABC transporters may also play as yet unexplored roles in estrogen biodistribution at the maternal–fetal interface. In the following section, we consider the role of specific ABC transporters in the placenta in uncomplicated and pathophysiological pregnancies.

Placental ABCB1/P-gp

The ABCB subset is the most intensively studied subset of the ABC transporters because of its role in MDR and has important conserved functions that are similar in all mammals (Dean and Annilo, 2005). The most well characterized ABCB transporter in the placenta is P-gp (ABCB1). In the human placenta, P-gp is localized to the apical surface of the microvillus membrane of the syncytium and at the syncytium-facing membrane of the cytotrophoblast, adjacent to the basolateral membrane of the syncytiotrophoblast (Sun *et al.*, 2006; Lye *et al.*, 2013). Placental P-gp expression has been reported in different species, including the human (Sun *et al.*, 2006; Lye *et al.*, 2013), mouse (Kalabis *et al.*, 2005; Bloise *et al.*, 2013), rat (Mark *et al.*, 2009), guinea pig (Kalabis *et al.*, 2009) and bovine (Waterkotte *et al.*, 2011).

P-gp tissue localization in the syncytiotrophoblast is consistent with its role in fetal protection, but its presence in the cytotrophoblast suggests a role in delivering substrates to the syncytium (Lye *et al.*, 2013; Table II). We and others have demonstrated that placental *ABCB1* mRNA and P-gp protein are present at high levels during first trimester, with reduced levels at term (Mylona *et al.*, 1999; Gil *et al.*, 2005; Mathias *et al.*, 2005; Sun *et al.*, 2006; Lye *et al.*, 2013). This gestation-dependent pattern of expression suggests placental P-gp plays an important role in protecting the fetus from exposure to potentially harmful factors, especially in the first trimester, when the developing conceptus is most vulnerable to teratogenicity. Further, it also demonstrates that placental P-gp mediated fetal protection decreases with advancing gestation. In this context, [3H]digoxin fetal accumulation (a benchmark substrate for assessing P-gp activity) increases with advancing pregnancy in the mouse (Petropoulos *et al.*, 2007). Conversely, P-gp expression in the guinea pig fetal BBB and mouse fetal brain increases toward term (Petropoulos *et al.*, 2010a, b; Iqbal *et al.*, 2011; Baello *et al.*, 2014) indicating fetal brain protection increases as placental fetal protection decreases. Expression and function of P-gp in the fetal BBB is an important component of fetal protection. P-gp in the guinea pig fetal BBB is induced by glucocorticoids (Iqbal *et al.*, 2011) and growth factors such as transforming growth factor-β (Baello *et al.*, 2014), and inhibited by the pro-inflammatory cytokines IL-1β, IL-6 and tumor necrosis factor-α (TNF-α) (Iqbal *et al.*, 2012), in a gestational age-dependent manner.

Dramatic phenotypes have been associated with disruption of placental P-gp. Mice carrying a spontaneous mutation in the *Abcb1a* gene, demonstrate reduced P-gp levels in the placenta, brain and intestine (Lankas *et al.*, 1998). Following heterozygous crosses, pregnant mice carried fetuses of all 3 *Abcb1a* genotypes, +/+, +/− and −/−. Pregnant mice were exposed to L-652,280, an avermectin derivative, and known teratogen that induces a cleft palate phenotype, from embryonic day (E)6 to E15. Fetal examination on E18 revealed that 100% of *Abcb1a* (−/−) deficient fetuses had developed cleft palate. *Abcb1a* heterozygote (+/−) littermates were less sensitive, and there was no structural impact of the avermectin in the wild-type fetuses (Lankas *et al.*, 1998). This elegant study showed direct evidence that P-gp is a key gatekeeper of the maternal–fetal interface, preventing the fetus from accumulating maternal blood-borne teratogens.

Owing to its role in fetal protection, there has been considerable interest in the regulation of P-gp expression and function in the placenta. Dexamethasone (DEX) treatments of human trophoblast cell lines (JEG3 and BeWo) and human primary trophoblast cell cultures (derived from term placentas) increased *ABCB1* mRNA expression (Pavek *et al.*, 2007; Manceau *et al.*, 2012). Importantly, this effect was abolished by glucocorticoid receptor (GR) antagonists (Pavek *et al.*, 2007; Manceau *et al.*, 2012). In the murine placenta, DEX treatments from either E9.5–E15.5 or E12.5–E18.5 significantly increased *Abcb1a* and P-gp (E12.5–E18.5) expression but did not alter P-gp function (Petropoulos *et al.*, 2010a, b). In contrast, in rats DEX administration in drinking water from E13 to E22 decreased labyrinthine expression of *Abcb1a* mRNA (∼45%) but did not alter junctional zone expression of *Abcb1a* (Mark *et al.*, 2009). The fact that sGCs can modify levels of ABC transporter gene expression and protein has implications for women treated with these steroids in the clinical management of preterm birth. The relevance of these findings to changes in endogenous glucocorticoids (cortisol/corticosterone) in the maternal and fetal circulation are less clear, as while sGCs bind the GR and the pregnane-X-receptor (PXR), endogenous glucocorticoids activate the GR and the mineralocorticoid receptor, but not the PXR. Further studies are clearly required to determine the relative impact of sGCs and endogenous glucocorticoids on ABC transporters in the human placenta.

Other steroid hormones have also been linked to placental P-gp. In the mouse, maternal plasma progesterone levels correlated to placental *Abcb1b* mRNA expression levels (Kalabis *et al.*, 2005). Nonetheless, pregnant mice treated chronically with progesterone (from E14.5 to E18.5) did not exhibit alterations in fetal accumulation of P-gp substrate ([3H] digoxin) or altered placental *Abcb1a/b* mRNA expression (Petropoulos *et al.*, 2007). Placental *Abcb1a/b* mRNA levels were unaltered in ovariectomized pregnant rats submitted to full estrogen and partial progesterone replacement from E16 to E22 (Mark *et al.*, 2009). These studies suggest progesterone does not modulate P-gp expression and activity, at least at the end of pregnancy. However, in human placental choriocarcinoma JAR cells, P-gp protein expression was stimulated by progesterone, which corresponded with a decrease in cellular uptake of the P-gp substrates saquinavir and paclitaxel (Coles *et al.*, 2009). Estrogen also increased P-gp protein expression and activity in JAR and human primary trophoblast cells (Evseenko *et al.*, 2007a, b; Coles *et al.*, 2009). Together, these findings suggest that actions of steroid hormones on placental P-gp expression and function are species-specific and time-dependent, though further studies are necessary.

Infection and inflammation have also been shown to regulate placental P-gp. Recently, we determined the role of lipopolysaccharide (LPS—a component of the outer membrane of Gram-negative bacteria) in

regulating placental P-gp (Bloise et al., 2013). In the mouse, LPS, at doses that induced a robust inflammatory response but not immediate preterm delivery, inhibited P-gp activity and increased fetal drug exposure (Bloise et al., 2013). Moreover, LPS treatment of first trimester human trophoblast explants down-regulate *ABCB1* mRNA expression and P-gp protein levels (Lye et al., 2015). However, polyinosinic:polycytidylic acid (Poly IC) treatments (which mimics viral infection) did not result in changes of *ABCB1* mRNA and P-gp expression in first trimester but decreased *ABCB1* expression in third trimester placentas (Lye et al., 2015). Consistent with these findings, human primary placental trophoblast cells challenged with the pro-inflammatory cytokines (TNF-α or IL-1β) exhibited decreased levels of *ABCB1* mRNA and P-gp (Evseenko et al., 2007a, b). However, placentas from patients undergoing preterm labor with inflammation [diagnosed stage III histological chorioamnionitis (HCA)] exhibited increased *ABCB1* mRNA and P-gp expression compared with non-infective preterm labor placentas (Mason et al., 2011). It is somewhat puzzling that *in vitro* experiments probing the effects of pro-inflammatory agents (i.e. pro-inflammatory cytokines, LPS and Poly IC), described decreased *ABCB1* and P-gp expression while placentas from preterm labor with inflammation exhibited an opposite outcome. It is important to highlight there are two types of HCA; infective and non-infective (Conti et al., 2015). The percentage of non-infective HCA ranges from <30% to more than 50% and importantly, the etiology of non-infectious HCA is largely unknown and is probably multifactorial. Infective HCA in turn is generally polymicrobial with over 65% of amniotic fluid cultures positive for two or more organisms (Conti et al., 2015). This demonstrates that regulation of placental *ABCB1* and P-gp expression by infection and inflammation is complex and deserves further investigation. We can also conclude that selective infective agents and intrauterine inflammatory events, such as those associated with HCA, have the potential to change drug disposition in intrauterine tissues and more importantly, in the fetus, and they do this in a manner that is dependent on the stage of gestation.

Experiments conducted in human peripheral blood mononuclear cells and primary cultures of human astrocytes demonstrated that P-gp transports pro-inflammatory cytokines, namely IL-2, interferon-ϒ and TNF-α and chemokines toward the extracellular space (Pawlik et al., 2005; Kooij et al., 2011). Importantly, P-gp mediated release of the chemokine (C-C motif) ligand 2 (CCL2) is capable of regulating trafficking of CD8(+) T cells into parenchyma tissues (Kooij et al., 2014). We can therefore conclude that P-gp is likely to exert key functions in the secretion of pro-inflammatory/chemotactic factors into the extracellular space and regulate immunological responses. Indeed, trophoblast tissue can secrete a plethora of cytokines/chemokines, which are directly or indirectly involved with immune chemotaxis and the inflammatory response (Torricelli et al., 2009; Bloise et al., 2010, 2013; Novembri et al., 2011; Dowling et al., 2012). Hence, it can be postulated that P-gp may be part of the trophoblast-specific immunological responses to infection, which are gestational age- and infective agent-dependent (Lye et al., 2015).

P-gp expression is associated with fetal and placental size. P-gp expression is lower in human placentas from small for gestational age preterm infants (Hodyl et al., 2013) and in smaller placentas in mice (Bloise et al., 2013). This interesting link between fetal and placental size/weights and P-gp is important but should be expanded to include assessment of the other ABC transporters. It would also be important to determine whether altered ABC transporter levels results from altered fetal/placental growth or that altered ABC expression is responsible for reduced growth. Such findings would be clinically relevant as reduced fetal/placental weights are common in infectious and non-infectious preterm labor as well as in intrauterine growth restriction (IUGR) and PE.

Compromised placentation can lead to abnormal placental perfusion and chronic placental ischemia associated with PE, IUGR, or even preterm delivery (Chaddha et al., 2004; Burton et al., 2009; Pringle et al., 2010). Given that oxygen plays a major role in placental development and fetal growth (Red-Horse et al., 2004), experiments have investigated the role of oxygen on placental P-gp expression. When first trimester placental villous explants were incubated in hypoxic conditions (3% O_2), there was an increase in P-gp immunostaining in proliferating cytotrophoblasts with no changes in *ABCB1* mRNA (Lye et al., 2013). Similarly, term placental explants cultured in 3% oxygen (compared with 20% oxygen tension) exhibited significant increases in *ABCB1* mRNA and P-gp protein expression (Javam et al., 2014), demonstrating an important effect of oxygen in modulating placental P-gp expression. These data suggest chronic placental ischemia and hypoxia have the potential to alter fetal exposure to drugs, xenobiotics and endogenous substrates, especially in obstetric complications, such as IUGR and PE, where inadequate placental blood flow may produce insufficient oxygen delivery to the maternal/fetal interface (Lye et al., 2013; Javam et al., 2014).

Clearly, placental P-gp is regulated by a number of different factors acting together or independently at distinct gestational time points. P-gp dysregulation may occur in pregnancy pathologies associated with hormonal imbalance, local hypoxia and or infection/inflammation with the potential to impact negatively on the developmental health of the conceptus.

Placental ABCG2/ BCRP

The ABCG family genes are involved in transporting cholesterol and other sterols, with the exception of *ABCG2*, which is highly related to MDR (Dean and Annilo, 2005). BCRP, like P-gp, is abundantly expressed in the placenta. Whilst BCRP and P-gp have many common substrates, both transporters have a spectrum of specific chemically unrelated substrates (Schinkel and Jonker, 2003; Iqbal et al., 2012; Table I). BCRP is localized to the syncytiotrophoblast and cytotrophoblast cells and to endothelial cells of the fetal blood vessels. Protein expression increases toward term with no change in mRNA expression (Yeboah et al., 2006; Lye et al., 2013; Table II). This would suggest increased fetal protection against BCRP substrates as gestation proceeds.

A number of compounds involved in erythropoiesis and Fe^{2+} (ferrous) ion metabolism are physiological substrates of BCRP (Table I). In particular, BCRP exports porphyrins (such as heme) and porphyrin metabolites such as protoporphyrin IX (Desuzinges-Mandon et al., 2010). An excess of porphyrins can damage DNA structure and cellular membranes therefore BCRP mediated-porphyrin efflux plays an important protective role in preventing porphyrin intracellular toxicity (Krishnamurthy et al., 2004). In this context, BCRP promotes survival of blood stem cells exposed to hypoxia, via regulation of intracellular porphyrin levels (Krishnamurthy et al., 2004). Studies assessing the protective role of BCRP in preventing intracellular porphyrin accumulation in reproductive tissues are very limited. However, silencing of BCRP expression in BeWo cells did not alter intracellular protoporphyrin IX levels after insult with pro-apoptotic cytokines (Evseenko et al., 2007a, b), suggesting BCRP does not promote trophoblast survival under these conditions. There is recent evidence for a role of placental heme iron

utilization in supporting fetal iron demands (Cao *et al.*, 2014). Further studies are required to elucidate the role of BCRP in preventing porphyrin toxicity in trophoblast cells and to determine if dysregulation of BCRP alters porphyrin accumulation in the trophoblast.

In vitro studies have demonstrated that estrogen and progesterone modulate placental BCRP expression. Human placental BeWo cells treated with progesterone displayed increased *ABCG2* mRNA and BCRP protein, whereas estrogen decreased expression. Importantly, the estrogen receptor antagonist ICI-182,780 abrogated the decrease in BCRP expression after estrogen treatments (Wang *et al.*, 2006). In the same cell line, it has been reported that progesterone effects on BCRP expression are mediated by progesterone receptor B (Wang *et al.*, 2008). Another study reported that term human primary trophoblast cells treated with estrogen and progesterone did not show differences in *ABCG2* mRNA expression (Evseenko *et al.*, 2007a, b). In a mouse model, progesterone administered daily from ED14.5 until ED18.5 did not modify placental *Abcg2* mRNA and BCRP protein levels (Kalabis *et al.*, 2007). These variable results do not provide a definitive answer as to the role of estrogen and progesterone in regulation of placental BCRP. The human studies where modulatory effects were identified were undertaken in cancer-derived cell lines, but there was no effect in primary human trophoblasts or in the mouse placenta, *in vivo*. Further studies are clearly required particularly at earlier stages of human gestation.

With respect to infection and inflammation, human first trimester placental explants challenged with LPS exhibited reduced *ABCG2* mRNA and BCRP protein expression 24 h after challenge. Interestingly, poly IC [which models viral infection via toll-like receptor (TLR)-3 activation] did not alter expression of either transporter in first or third trimester placentas indicating the effects of TLR activation in regulation of BCRP are insult-specific and gestational age-dependent (Lye *et al.*, 2015). This study also suggests that in cases of maternal infection, the first trimester conceptus is at greater risk of exposure to BCRP substrates than the third trimester fetus. However, in another report, TNF-α and IL-1β challenge of primary cultures of term human placentas decreased BCRP expression and activity, whereas IL-6 challenge had no effect (Evseenko *et al.*, 2007a, b). Also, PGE2, which is produced by the placenta and is an important mediator of the innate immune response, increases placental BCRP expression and function in JAR cells and primary human trophoblast cells via EP1 and EP3 receptor activation (Mason *et al.*, 2014). Placentas from preterm labor with inflammation also exhibited increased *ABCG2* and BCRP expression compared with preterm labor without inflammation (Mason *et al.*, 2011). It is therefore evident that BCRP response patterns in the human placenta may depend upon specific inflammatory agents.

Studies have also been undertaken in rodents. In a mouse model of maternal infection (simulated by LPS), there was no effect on placental *Abcg2* mRNA expression in mid-pregnancy (Bloise *et al.*, 2013). However, TLR-3 activation following poly IC treatment was effective in decreasing rat placental *Abcg2* mRNA expression in a dose-dependent manner during mid-late pregnancy (Petrovic and Piquette-Miller, 2010). Since pro-inflammatory agents may exert their effects through distinct pathways (Hauguel-de Mouzon and Guerre-Millo, 2006; Koga and Mor, 2008) and some cytokines may produce paradoxical responses depending on which side of the maternal–fetal interface the inflammatory insult occurs (Mitchell *et al.*, 2004; Bloise *et al.*, 2010), it is plausible that the BCRP response to inflammatory agents is complex and highly dependent on stage of pregnancy, type of insult and the species investigated.

Placental BCRP expression is also affected by oxygen tension. Hypoxia resulted in a reduction in BCRP immunostaining in first trimester placental explants (Lye *et al.*, 2013). In contrast, hypoxia in third trimester term placental explants had no effect on *ABCG2* mRNA and BCRP protein expression (Javam *et al.*, 2014; Table II). This gestation-dependent response to hypoxia suggests that BCRP substrates are more likely to accumulate in first trimester conceptus under this condition than in later stages of gestation. These studies also indicate that oxygen is an important regulator of placental BCRP expression across pregnancy.

Placental multidrug resistance-associated proteins

The multidrug resistance-associated proteins (MRPs) share considerable sequence and functional homology and comprise the C subset family of the ABC transporters superfamily (Sodani *et al.*, 2012; Kunjachan *et al.*, 2013). To date, 13 transporters have been classified as members of the MRP family, nine of which are involved in MDR (MRP1-9) (Zhou *et al.*, 2008; Sodani *et al.*, 2012). mRNA for all 13 MRP transporters have been detected in human placenta (Yabuuchi *et al.*, 2002; Nishimura and Naito, 2005). However, only four MRP transporters have been fully characterized in the placenta thus far (Table II). Determining the precise localization and function of the different members of the MRP transporter family in the placenta is of importance to our understanding of drug and nutrient disposition in intrauterine tissues and the fetus across pregnancy.

MRP2 and MRP3 are localized to the apical membrane of the syncytiotrophoblast suggesting a role in efflux into the maternal compartment. In contrast, MRP1 and MRP5 are present at the basolateral membrane of syncytiotrophoblast suggesting efflux into the fetal compartment (Table II). This subset of the ABC superfamily can modulate transport of a wide range of physiological and pharmacological substrates of obstetric relevance (Table I). For example, folate (vitamin B9) is a substrate of MRPs-1-4 (encoded by *ABCC1-4*) (Lemos *et al.*, 2008; Sodani *et al.*, 2012) and other ABC transporters as well (Table I). Folate is an important nutrient involved in one-carbon metabolism and is taken as a supplement during the periconceptional period and early pregnancy to reduce the risk of neural tube defects and congenital anomalies (Ramakrishnan *et al.*, 2014). It is also a critical factor in the process of DNA methylation.

Further examples of physiological MRP substrates are glucuronide conjugates of bile salts (Bellarosa *et al.*, 2009) (Table I). The placenta is the major route of elimination for biliary compounds produced by the fetal liver (Macias *et al.*, 2009). MRPs are important transporters of biliary compounds at the maternal–fetal interface. MRP1–3 are present in the placenta and have the ability to transport biliary compounds (Macias *et al.*, 2009). MRP1 is localized to the basolateral membrane, whereas MRP2 and 3 are localized to the apical membrane of the syncytiotrophoblast (Table II); therefore efflux of biliary compounds may occur in both directions. Since accumulation of biliary compounds in the fetus can be potentially harmful (Macias *et al.*, 2009), it is important to better understand biliary transport in the placenta and how expression and localization of placental MRP transporters, throughout pregnancy, mediate fetal bile salt excretion toward the maternal side. Examples of pharmacological substrates are the antibiotics fluoroquinolone and macrolide and the antiviral drugs zidovudine (AZT) and adefovir

(Table I). Changes in function of the MRP transporters, especially in the first trimester, have the potential to impact early embryo development. The specific functional roles of the MRPs in the placenta require further careful characterization.

Little is known about the regulation of MRP-related transporters in the placenta. IL-6, TNF-α and progesterone have been shown to up-regulate levels of *ABCC1* mRNA in primary cultures of human trophoblasts (Evseenko *et al.*, 2007a, b), However, there was no associated increase in MRP1 protein, demonstrating disconnect between mRNA and protein expression (Evseenko *et al.*, 2007a, b). In contrast, LPS treatments of pregnant rats on E17.5 significantly decreased placental *Abcc1*, *Abcc2* and *Abcc3* mRNA expression (Petrovic *et al.*, 2008). Zearalenone, a fungal mycotoxin with estrogen-like properties that is found in cereals and other plant products, increased placental *ABCC1* mRNA and MRP1 as well as *ABCC2* mRNA and MRP2 expression in human choriocarcinoma cell lines (Table II). As such, food contaminants may alter placental drug transporter expression (Prouillac *et al.*, 2009) and in doing so modify fetal drug and toxin exposure. Further work is required to determine the impact of common pregnancy states, including infection, hypoxia and maternal stress (glucocorticoid exposure), as well as maternal diet on the MRP-related transporters in the human placenta. In addition, the impact of pregnancy pathologies on these transporters should also be investigated.

Placental ABC transporters-mediated lipid exchange

Placental lipid exchange is critical for normal placental function and fetal development, however, the mechanisms of placental cholesterol trafficking are not well understood. Members of the A and G ABC transporter family have been identified as important transporters of cholesterol, phospholipids and other lipophilic molecules present in the plasma membrane and intracellular compartments (Oram and Vaughan, 2006). They are also known as reverse cholesterol transporters because their biological function is to efflux cholesterol and phospholipids from within peripheral cells to lipid-free apolipoprotein AI (apoAI), constituting a central step in high-density lipoprotein (HDL) biosynthesis (Selva *et al.*, 2004). The first functional evidence relating these transporters to cholesterol homeostasis in the placenta was reported in *Abca1* null mice. Functional loss of *Abca1* resulted in severe placental malformation, embryo growth retardation, fetal loss and neonatal death; all attributed to altered local steroidogenesis (Christiansen-Weber *et al.*, 2000). In the first trimester human placenta, ABCA1 was localized to the basolateral membrane and to a lesser extent, the apical membrane of villous cytotrophoblast cells (Bhattacharjee *et al.*, 2010). Conversely, in the third trimester human placenta, ABCA1 protein was localized to the apical syncytium of placental villi and the apical membrane of endothelial cells of fetal blood vessels within the villi (Table II). This suggests cytotrophoblast cells support cholesterol disposition to the embryo/fetus in early pregnancy via ABCA1, whereas toward term, ABCA1 supports cholesterol transport from the placenta to the mother (Albrecht *et al.*, 2007; Stefulj *et al.*, 2009; Aye *et al.*, 2010; Aye and Keelan, 2013). ABCA1 is functionally active in primary human trophoblast cells where it effluxes cholesterol and cytotoxic oxysterols from within the syncytiotrophoblast toward the maternal side (Aye *et al.*, 2010). ABCG1 protein is localized to the basolateral membrane of the syncytiotrophoblast in the term human placenta and displays efflux activity in human primary trophoblast cells (Aye *et al.*, 2010), suggesting it mediates cholesterol and phospholipid transport to the fetus.

In vitro experiments have begun to reveal important functions of these transporters in placental biology. *ABCA1* mRNA and protein levels increase during trophoblast syncytialization *in vitro*, indicating that increased cholesterol efflux is important during cytotrophoblast differentiation into syncytiotrophoblast cells (Aye *et al.*, 2010; Keelan *et al.*, 2011). Importantly, placental cholesterol transport via ABCA and ABCG transporters require the extracellular cholesterol acceptors apoAI, apoE and HDL (Aye *et al.*, 2010; Dube *et al.*, 2013). To this end, both microvillous and basal membranes of the syncytiotrophoblast display binding sites for HDL particles (Aye and Keelan, 2013). Evidence, to date, supports a role for ABCA1 and ABCG1 as cholesterol carriers at the maternal fetal interface, probably acting in concert to efflux cholesterol in different directions; to the maternal side via ABCA1 and to the fetal side via ABCG1, in a gestational and age-dependent manner. It is important to emphasize that ABCA1 and ABCG1 are not the only cholesterol transporters in the placenta. Placental cholesterol transfer and fetal cholesterol requirements vary across pregnancy and occur by the interaction of different transport systems [reviewed in Baardman *et al.* (2013)]. However, compelling evidence suggests that cholesterol trafficking mediated by selected ABC transporters in the apical and basolateral membrane of the trophoblast is important to placental cholesterol homeostasis and transfer to both sides of the maternal fetal interface.

Dysregulation of placental ABCA1 and ABCG1 cholesterol transporters may have important implications. Small interfering RNA-mediated silencing of *ABCA1* or *ABCG1* mRNA expression in human term primary trophoblasts cells decreased cell viability by sensitizing trophoblast cells to toxic cholesterol metabolites originating from oxidative processes, namely 25-hydroxycholesterol and 7-ketocholesterol (Aye *et al.*, 2010). Alterations in ABCA1 and ABCG1 may be detrimental to syncytialization of cytotrophoblast cells, by impacting basic syncytiotrophoblast functions such as hormonal output and placental nutrient transport. In fact, alterations in ABCA1 and ABCG1 mRNA and protein have been demonstrated in different obstetric pathologies (Table II). Further investigation is necessary to elucidate the role of placental ABC cholesterol transporters in different obstetric pathologies.

ABC transporters in the fetal membranes and decidua

Fetal membranes provide a physical barrier between the intrauterine environment and the developing fetus and are involved in the labor process. At term, two layers constitute the human fetal membranes: an outermost layer of chorion adjacent to an innermost layer of amnion tissue. The fetal membranes exhibit barrier-like functions and provide a paracrine-signaling matrix at the maternal–fetal interface, between the maternal and fetal compartments (Myatt and Sun, 2010). Decidua is a specialized reproductive tissue containing endometrial stromal cells attached to the uterine wall. They secrete factors related to endovascular trophoblast invasion and immune chemotaxis responsible for the genesis and maintenance of a functional fetal–maternal interface (Kajihara *et al.*, 2013). Relatively little is known as to the localization and function of the ABC transporters in human fetal membranes and decidua (Table III).

ABCB1 mRNA was detected by microarray-based expression profile of ABC xenobiotic transporter genes at very low levels in amnion at

term, however, qPCR and western blot analysis failed to identify *ABCB1* mRNA or P-gp in amnion tissue (Aye *et al.*, 2007). Very low levels of P-gp protein were identified in cultured chorion cells isolated from human fetal membranes (Yoshino *et al.*, 2011). This suggests P-gp has very little biological function in term fetal membranes. In contrast, BCRP and MRPs have been identified in the fetal membranes. BCRP was localized to the apical surface, cytoplasm and membrane between amnion cells, whereas in chorion trophoblasts and attached decidua stromal cells, BCRP immunostaining was detected in the plasma membrane and in the nucleus of the chorion trophoblast cells (Yeboah *et al.*, 2008). In another report, BCRP was detected in the cytoplasm of the amnion epithelium (Aye *et al.*, 2007). No specific apical or basolateral staining was identified, thus the direction of BCRP efflux activity in amnion cells (i.e. in or out of the fetal compartment) remains to be determined. *ABCG2* mRNA and BCRP expression levels in amnion cells do not change with labor (Yeboah *et al.*, 2008) (Table III), suggesting that BCRP does not play a role in the initiation of labor, though further functional studies are necessary. Fetal membranes are not vascularized (Myatt and Sun, 2010) and are thus subjected to a relatively low oxygen environment. BCRP has a well-described role in promoting cellular survival in hypoxic conditions (Krishnamurthy *et al.*, 2004; Lye *et al.*, 2013; Javam *et al.*, 2014); therefore, it is possible that BCRP plays a role in the survival and function of amnion, chorion and decidual cells in a low oxygen environment.

A comprehensive report has identified expression of MRP1, MRP2 and MRP5 mRNA and protein in amnion. MRP1 and MRP5 protein were localized to the apical and basolateral surfaces of amnion cells, while MRP2 was present at very low levels on the apical surface of amnion cells. MRP1 localization was also present in the chorionic and attached decidual cells of fetal membranes (Aye *et al.*, 2007). Localization of MRP1 and MRP5 to both apical and basolateral membranes suggests a putative role in cellular (amnion) protection rather than a barrier-like function (Table III). The localization of MRP2 to the apical membrane of amnion cells would be consistent with substrate transfer into the amniotic fluid. However, the very low expression of MRP2 raises questions as to its biological significance in amnion cells. It is important to note that amnion and decidual cells synthesize PGs, including PGE2 and PGE2α (Gibb and Sun, 1996; Alam *et al.*, 2007; Myatt and Sun, 2010). PGs are important factors triggering and maintaining labor (Gibb, 1998; Challis *et al.*, 2000) and, more specifically, MRP2 and MRP4 are efflux carriers of PGE2 and PGE2α (Rius *et al.*, 2005; de Waart *et al.*, 2006; Lin *et al.*, 2008). Whether MRP-mediated efflux of PGs in fetal membranes is related to the induction and maintenance of labor is currently unknown and requires further investigation.

The cholesterol/phospholipids transporter ABCA1 has been localized to the cell membrane and cytoplasmic compartments of human amnion epithelial cells (Nikitina *et al.*, 2011). ABCA1 was also present in decidual cells and epithelial cells of endometrial glands in early gestation (Albrecht *et al.*, 2010; Table III), suggesting ABCA1 is an important transporter mediating lipid trafficking in these specialized intrauterine cell types.

In addition to playing a direct role in fetal protection, ABC transporters in human fetal membranes are likely important in amnion/chorion development, proliferation, homeostasis, nutrition and local barrier-like functions. Future studies should investigate if expression and localization of ABC transporters are dependent on gestational age and how different obstetric disorders, such as infectious and non-infectious preterm delivery and PE, might alter ABC-mediated transport in the fetal membranes.

Uterine ABC transporters

The uterus comprises two functionally distinct zones: the endometrium (discussed earlier) and the myometrium. The uterus undergoes dramatic changes in both size and number of myometrial smooth muscle cells and other uterine cell types in order to allow development and growth of the conceptus throughout pregnancy (Ciarmela *et al.*, 2011). The myometrium remains a relatively unexplored organ in the context of ABC transporter expression and function. However, given the key role these transporters play in the efflux of substrates that are important in maintaining uterine quiescence and inducing uterine contractility (i.e. steroids, cytokines and chemokines) further studies are critical.

Abcb1 mRNA expression was >100-fold reduced in the myometrium after the initiation of labor compared with late pregnancy in the rat (Helguera *et al.*, 2009). This suggests P-gp may be involved in myometrial quiescence and that its down-regulation results in increased myometrial contractility (Helguera *et al.*, 2009). In late pregnancy, myometrial MRP1 transports the bioactive lipid sphingosine-1-phosphate (S1P) (Tanfin *et al.*, 2011). S1P induces the expression of cyclooxygenase-2 (COX-2) in the rat myometrium in late gestation (Serrano-Sanchez *et al.*, 2008). COX-2 drives PG synthesis, which in turn induce myometrial contractions and labor (Serrano-Sanchez *et al.*, 2008). Therefore, myometrial MRP1 likely plays an important role in extruding factors related to the induction of contractility and hence initiation of labor. Apart from being the precursor of steroid hormones and fat-soluble vitamins, cholesterol is essential to modulate human oxytocin receptor membrane activity, stability and affinity to oxytocin (Mouzat *et al.*, 2007). Oxytocin mediates loss of uterine quiescence and triggers uterine contractility. It is therefore paramount to the onset of labor. In this regard, altered myometrial cholesterol content has been associated with abnormal oxytocin-induced uterine smooth muscle contraction in the rat (Mouzat *et al.*, 2007). Therefore, understanding the regulation of cholesterol trafficking in the myometrium is likely key to understanding the mechanisms leading to uterine contractility and labor.

Uterine expression of the cholesterol/phospholipid transporter *ABCA1* has been reported in non-gravid human and bovine uterus (Langmann *et al.*, 2003; Farke *et al.*, 2006). However, the specific localization was not described. Nuclear receptor liver X receptor-ß (LXRß) acts to prevent accumulation of cholesteryl esters in mouse myometrium via regulation of ABCA1 and ABCG1. Indeed, LXRß knockout mice exhibit increased uterine cholesteryl ester accumulation and defective contractile activity induced by oxytocin or PGF2α in the myometrium associated with decreased uterine expression of *ABCG1* mRNA (Mouzat *et al.*, 2007). This suggests ABCG1 may be an important regulator of myometrium contractility and thus onset of labor. Together, the above studies suggest that a number of the ABC transporters are important in the regulation of labor-related processes. Future experiments are required to determine the role of the ABC transporter family in the initiation of labor and which obstetric disorders or conditions alter myometrium ABC transporter expression and activity across pregnancy.

Gonadal ABC transporters

ABC transporters have been identified in the male and female gonads of a number of species, though relatively little is known as to their specific functions at these sites. In the following section, the expression and

localization of ABC transporters in the testis and ovary and their possible roles in gonadal physiology and gametogenesis are reviewed.

Testes

One of the most important structures in the testis is the BTB. The physical properties of the BTB prevent the entry of large or hydrophilic molecules from the bloodstream into seminiferous tubules (Bart *et al.*, 2002). ABC efflux transporters prevent accumulation of potentially harmful smaller or lipophilic compounds within the testes and are critical in protecting germ cells from circulating harmful factors (Bart *et al.*, 2002). One important example is depletion of P-gp expression in *Abcb1a* (−/−) deficient mice. When exposed to the P-gp substrate [3H]ivermectin, *Abcb1a* (−/−) deficient mice displayed increased accumulation of the drug in the testis and epididymis (Schinkel *et al.*, 1995), highlighting its important role as gatekeeper in the BTB.

Across multiple species, the most abundant ABC transporters in the testes include P-gp, BCRP, MRP1 and MRP4 (Cordon-Cardo *et al.*, 1989; Bart *et al.*, 2002; Bart *et al.*, 2004), though others are also present. P-gp and BCRP are localized to the plasma membrane of myoid cells and on the luminal surface of the capillary endothelial cells. P-gp is also localized to Leydig cells. MRP1 and MRP4 have been identified on the basolateral membrane of Sertoli cells (Bart *et al.*, 2004), and MRP1 is also localized in Leydig cells in human and non-human primates (Klein *et al.*, 2014).

Dysregulation of ABC transporters in barrier sites has the potential to disrupt hormonal output. Endocrine-disrupting chemicals (EDCs) have the ability to alter efflux function of testicular ABC transporters. The EDCs tetrabromobisphenol A, perfluorooctanoic acid and perfluorooctanesulfonic acid inhibit testicular function of P-gp, MRP1 and MRP4 but not BCRP. BCRP is inhibited by BPA (Dankers *et al.*, 2013). These data show that environmental toxins disrupt the function of selected ABC transporters of the BTB.

ABC transporters also play a role in testicular steroidogenesis. Recently, it has been postulated that testosterone is an MRP1 substrate. MRP1 immunostaining is present in Leydig cells located in the interstitial region of rat, macaques and human testes (Klein *et al.*, 2014). MRP1 null mice show reduced serum and testicular testosterone levels despite unaltered expression or activity of testicular steroid synthesizing enzymes or steroid inactivating hepatic enzymes (Sivils *et al.*, 2010). Thus, reduced systemic testosterone levels in MRP1 null mice results from impaired testosterone release into the bloodstream (Sivils *et al.*, 2010). Further, murine Leydig (MA-10) cells treated with an MRP1 inhibitor (MK-571) failed to produce testosterone (Dankers *et al.*, 2013). Together these studies suggest that MRP1 plays an important role in testosterone efflux from Leydig cells.

ABC lipid transporters also appear to be important determinants of male fertility. ABCA1 is highly expressed in Sertoli cells and to a lesser extent in Leydig cells of mice (Selva *et al.*, 2004). Sertoli cells have the ability to engulf and degrade tubular cytoplasmic residues shed from elongated spermatids, as well as debris from apoptotic spermatogenic cells. Such lipid residues can accumulate in excess within Sertoli cells and must be removed in order to avoid lipotoxic-mediated cellular damage (Selva *et al.*, 2004). In this context, ABCA1 mediates lipid efflux from Sertoli cells, protecting the cells from lipotoxicity. Dysfunction of Sertoli cell ABCA1 decreases intratesticular testosterone levels and sperm counts leading to impaired fertility of Abca1 null mice (Selva *et al.*, 2004). Collectively, these data demonstrate that ABC transporters play a critical role in male gametogenesis, steroidogenesis and reproductive function. Disruption of selected testicular transporters has the potential to impair male gonadal function and be detrimental to male reproductive performance.

Ovary

The ovary is the major site of hormonal output responsible for orchestrating menstrual cycle and secondary gender characteristics, as well as supporting oogenesis. In this context, accumulating evidence indicates that certain ABC transporters are important in ovarian physiology. P-gp is present at both the luminal and abluminal membranes of ovarian endothelium (Stewart *et al.*, 1996). Similarly, BCRP immunostaining was localized to endothelial cells lining the lumen of the murine ovarian vasculature (Dankers *et al.*, 2012). Since the ovarian capillaries do not form a classic blood-tissue barrier, P-gp and BCRP endothelial localization in the ovaries indicates that these transporters play local cellular roles in the endothelium, rather than a barrier role-protecting the ovary from accumulating detrimental toxins and metabolites (Stewart *et al.*, 1996).

P-gp was also detected in the rat granulosa cells derived from preovulatory follicles and in the corpora lutea following equine chorionic gonadotrophin or/hCG treatments (Lee *et al.*, 1998). LH treatment of granulosa cells, isolated from porcine ovarian follicles, rapidly induced ABCB1 expression and P-gp activity. Interestingly, no effect was observed following FSH treatment; showing specific responsiveness to selected pituitary gonadotrophins (Fukuda *et al.*, 2006). Progesterone significantly increased ABCB1 mRNA expression and P-gp activity, whereas estrogen had no stimulatory effect in LH-treated porcine granulosa cells (Fukuda *et al.*, 2006). Evidence that progesterone regulates ABCB1 expression in porcine granulosa cells was corroborated in another study. The PR antagonist RU486 prevented the effect of progesterone on ABCB1 expression. Interestingly, this was a transient effect and the authors suggested that granulosa cells may develop resistance to RU486 exposure by increasing ABCB1 expression (Fukuda *et al.*, 2007). The fact that P-gp is highly responsive to LH and progesterone suggests a role for this transporter in granulosa cell luteinization. Dysregulation of P-gp in the granulosa cells has the potential to impair folliculogenesis and ovarian steroidogenesis, although future investigations are necessary to investigate these possibilities.

Expression of other ABC transporters has been identified at the protein and mRNA levels in the ovary or related cell lines. However, the biological activity of these transporters and their regulation remains largely unexplored. Understanding the role of ABC transporters in the ovary will shed light on the regulation of key reproductive events such as oogenesis and ovarian steroidogenesis.

Conclusion

It is clear that ABC transporters play critical physiological and protective roles during gametogenesis, embryogenesis and embryo/fetal development. They are involved in modulation of steroidogenesis, uterine cyclicity, implantation, nutrient transport, immunological responses and act as 'gatekeepers' against potentially harmful factors in the systemic circulation. The most well-described ABC transporters in reproductive tissues are the multidrug transporters, P-gp, BCRP and MRP1-5, and

the cholesterol transporters ABCA1 and ABCG1. Understanding ABC efflux activity, expression patterns, substrate specificity and regulation of these and the remaining 40 ABC transporters will provide substantial insight into normal reproductive function and associated pathologies, including infertility and pregnancy complications. It may also hold the key to development of innovative new interventions to treat these pathologies.

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E.B. and S.G.M. conceived and designed the review. E.B performed the literature searches. E.B., T.M.O.-C., F.M.R., S.J.L., W.G. and S.G.M. each contributed to writing the manuscript.

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Conflict of interest

The authors have nothing to declare.

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