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REVIEW

Androgen and estrogen receptors in placental physiology and dysfunction

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BACKGROUND: The placenta is recognized as an endocrine organ, largely due to its secretions of steroid hormones, including progesterone, androgens, and estrogens. Steroid hormones play an essential role in the progression of pregnancy, fetal development, and growth. Furthermore, steroids are necessary for establishment and maintenance of a normal pregnancy, preparing the endometrium for implantation, stimulating endometrial secretions, and regulating uterine blood flow, however the exact mechanism of sex steroid signaling through their receptors in placental function is unknown.
OBJECTIVE: In this review, we will provide an overview of the current knowledge on sex steroid receptors in normal placental development, as well as evidence of abnormal signaling associated with placental dysfunction.
METHODS: A systematic literature search was performed using the NCBI PubMed search engine, including the following key works: estrogen receptor, androgen receptor, placenta, placental development, cytotrophoblast, and differentiation.
RESULTS: Of the over 700 articles that were returned, 125 studies focused on estrogen and androgen receptors in human placenta development and function during normal and abnormal pregnancy, as well as in rodents and ruminants placentae.
CONCLUSION: Receptors for both estrogens and androgens have been localized within the mammalian placenta, but surprisingly little is known about their signaling in trophoblast cell differentiation and function. An emerging picture is developing in which estrogen receptors possibly play role in cytotrophoblast proliferation and extravillous trophoblast invasion, whereas androgen receptors are involved in syncytiotrophoblast differentiation and function.

Keywords Placenta, ESR, AR, preeclampsia, IUGR, PCOS

Placental growth and fetal development

The placenta is a transitory endocrine organ that secretes hormones such as androgens, estrogens, progestins, chorionic gonadotropins and placental lactogen, and is essential to the health and development of a fetus. The placenta is also responsible for the exchange of nutrients, gas, and waste between the fetus and mother, and improper placental development can lead to disorders such as preeclampsia (PE) and intrauterine growth restriction (IUGR) (Morgan, 2016). Over the past 20-five years, the maternal morbidity and mortality rate in the United States has continued to rise due to PE and IUGR (CDC, 2016). Often, these placental disorders are linked to metabolic syndromes, such as gestational diabetes mellitus (GDM), obesity, and polycystic ovarian syndrome (PCOS) (Bartnik et al., 2016; Koster et al., 2015), and abnormal differentiation of trophoblast cells is thought to be the underlying cause of these disorders (Sibai et al., 1997; Goldenberg et al., 2007). However, not only is the placenta a source of hormones, it also contains receptors and as such, is a target of hormone action.

The formation and development of the placenta is a complex process that involves cell migration, cell proliferation and angiogenesis. Placental steroids have been recognized as regulators of trophoblast differentiation and development by stimulation of vascular endothelial growth factor (VEGF) and angiogenesis during pregnancy (Albrecht et al., 2010). In humans, a fertilized oocyte develops into a multi-cell blastocyst approximately 96 h after fertilization. On day three post-fertilization, the blastocyst enters the uterine cavity and the trophectoderm, comprised of trophoblast cells, begin to invade the maternal decidua around seven days after fertilization (Knobil and Neill 1998; Kaufmann et al., 2003). The trophoblast cells give rise to cytotrophoblast cells, which function as proliferative bipotential progenitor cells of the

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placenta (Enders 1968; Kaufmann et al., 2003). Cytotrophoblasts can further differentiate into two sublineages, extravillous trophoblast (EVT) and syncytiotrophoblast (ST). Extravillous trophoblast cells are involved in maternal spiral artery remodeling, to accommodate increased uterine blood flow to the placenta and growing fetus (reviewed in Chang et al., 2018) A continuous, multinuclear layer of syncytiotrophoblast is formed where continued cell fusion leads to development of a syncytium that will be in direct contact with maternal blood (Fig. 1) (Brosens et al., 1967; Pijnenborg et al., 1981; Benirschkle and Kaufmann, 2000; Hirano et al., 2002; Guibourdenche et al., 2009).

The fetal portion of the placenta, known as the chorion, contains floating and anchoring villi that cover the surface of the placenta and are the functional units that transfer nutrients and waste between mother and fetus (Bonagura et al., 2008; Aberdeen et al., 2010). Early in placental development, cytotrophoblasts proliferate in a hypoxic environment and begin to invade the uterus. As oxygen levels increase, cells are driven toward differentiation and rapid invasion and migration into the spiral arteries (Red-Horse et al., 2004). This hypoxic environment stimulates estrogen and progesterone secretion from trophoblast cells in order to support uterine spiral artery remodeling and steroid production throughout pregnancy. For chorionic development to occur in humans, cytotrophoblasts cells must differentiate and invade into the maternal spiral arteries to divert and enhance blood flow to the placenta (Red-Horse et al., 2004).

By week 12 of pregnancy, the arterioles have expanded to adapt to the increase in blood flow. Failed invasion and expansion of the arterioles would lead to an inability of the placenta to support a growing fetus. During the process of spiral artery invasion, cytotrophoblasts express many angiogenesis-related factors including VEGF, insulin-like growth factor (IGF) and placental growth factor (PGF) (Zhou et al., 2002). IGFs and VEGF are expressed throughout the placenta of various species, including the chorion, cytotrophoblasts and maternal decidualized cells, and function as key regulators of fetal growth (Zhou et al., 2002; Lash et al., 2003). In pregnancies complicated by IGUR and PE, there is impaired blood flow, potentially due to inappropriate maternal artery invasion by the extravillous trophoblasts. Using a well-established sheep model for IUGR, it has been reported that IUGR is associated with abnormal vasculature, decreased vessel organization (Regnault et al., 2002), and increased VEGFA levels early in gestation, suggesting a compensatory mechanism to maintain normal blood flow.

Early chorionic villus development involves invasion and migration of trophoblasts into discrete columns composed of fetal vessels and mesenchyme (Kay et al., 2011). Villi are further characterized as primary, secondary or tertiary. Primary villi contain a central core of cytotrophoblasts, secondary contain cytotrophoblasts and a mesenchymal core while tertiary villi have a surface of syncytiotrophoblasts and a complete layer of cytotrophoblasts (Kay et al., 2011). At day 21 post conception, placental vasculogenesis begins in the core of the secondary villi (Kay et al., 2011). Pluripotent mesenchymal cells are recruited, and can differentiate into hematopoietic/blood cell precursors or angioblastic cells that will form the first vessels (Kay et al., 2011). At day 32,

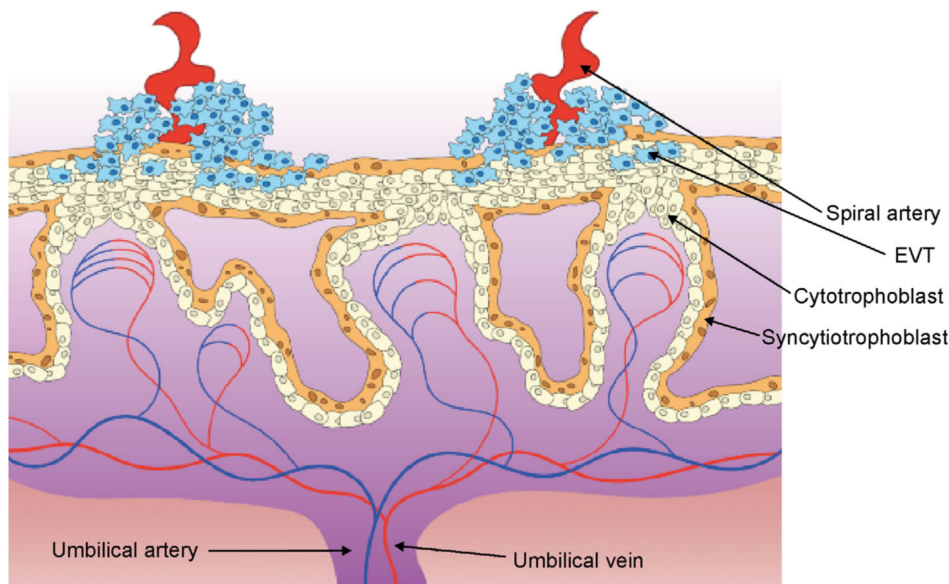

Figure 1 Trophoblast cell differentiation in the human placenta. Proliferating progenitor cytotrophoblast cells differentiate and give rise to extravillous trophoblast (EVT) and multinucleated syncytiotrophoblast (ST). Extravillous trophoblast cells invade maternal interstitial uterine tissue as well as spiral arteries and are important for uterine artery remodeling necessary for increased blood flow to the placenta. Syncytiotrophoblast cells fuse and form a multinucleated syncytium and are critical for nutrient and gas exchange as well as hormone production and secretion.

vasculogenesis gives way to angiogenesis to create the complex network of vessels that comprise the placenta. This is achieved by two main types of angiogenesis, branching and non-branching.

Non-branching angiogenesis refers to the growth of a vessel by extension while branching occurs by either sprouting or intussusception and require additional recruitment of perivascular cells for stabilization and is mediated by many growth factors including; VEGF, PGF, and IGF (Kay et al., 2011). As pregnancy progresses, *VEGFA* and its receptors, *VEGFR1* and *2* levels decrease and PGF levels begin to rise around 12 weeks post-conception (Regnault et al., 2002; Reynolds et al., 2005; Hagen et al., 2005). As PGF expression peaks, non-branching angiogenesis takes over to form the terminal villi (Kaufmann et al., 1985). Although the role and importance of the placenta as an endocrine organ during pregnancy is well established and receptors for both estrogen and androgen sex steroids have been described within the placenta, surprisingly little is known about estrogen and androgen signaling in trophoblast cell differentiation and function. In this review, we will provide an overview of the role of estrogen-estrogen receptor (ESR) and testosterone-androgen receptor (AR) in placental physiology and dysfunction.

Estrogen signaling

Estrogens are steroid hormones that contain an 18-carbon structure and include; estrone (E_1), estradiol (E2), estriol (E_3), and 16-hydroxy estrone, with the most biologically active estrogen being E2. Estrogen signaling plays an important role in placental development, pregnancy and parturition. During pregnancy, the placenta is responsible for the secretion of large amounts of systemic estrogens necessary for placental cell proliferation, differentiation and angiogenesis (Hall, 2011). Similar to other steroid hormones, estrogen function requires signaling through a member of the nuclear receptor superfamily of transcriptional regulators; estrogen receptor (ESR). There are two ESR's- ESR1 and ESR2 derived from separate genes. *ESR1* is located in the long arm of chromosome 6 and *ESR2* is located on chromosome 14.

As with all members of the nuclear receptor superfamily, these receptors are structurally divided into 6 domains; an N-terminal transactivation function 1 (A and B) domain, a DNA binding (C) domain, a short hinge (D) domain containing nuclear localization signals, a ligand binding and transactivating function 2 (E) domain, and a C-terminal (F) domain (recently reviewed in Arnal et al., 2017). Estrogen binding to its receptors leads to a conformational change that causes it to dissociate from heat shock proteins and translocation of ligand bound-ER into the nucleus. This complex binds to estrogen response elements, GGTCAnnnTGACC (Mangelsdorf et al., 1995) near or in the promotor region of target genes to regulate transcription (Lodish et al., 2008). Ligand binding activates multiprotein coactivator complexes through LxxLL motifs to regulate transcriptional activity by forming a homodimer or heterodimer with ESR2. Ligand activated ESR can also interact with DNA binding proteins such as AP1 and SP1 to promoter regions in DNA binding domain-independent fashion.

In addition to its genomic actions via nuclear ESR's, estrogens also produce non-genomic effects, which is common among steroid hormones. These effects are rapid, and occur by binding to membrane bound ESR1/2 or a G-protein-coupled receptor such as GPER1 or GPR30 (Bjornstrom et al., 2005). Binding to these membrane receptors leads to increased intracellular calcium, cyclic adenosine monophosphate (cAMP) production or phosphorylation of protein kinase B (Akt) or mitogen-activated protein kinase (MAPK) (Björnström et al., 2005). Studies have also shown that nuclear receptors, including ESR and AR can translocate to the plasma membrane when a conserved 9 amino acid motif in the ligand binding domain becomes palmitoylated (Acconcia et al., 2005; Albrecht and Pepe, 2010). Once localized to the plasma membrane, these receptors act as a scaffolding complex for other signaling molecules that are activated by E2 (Acconcia et al., 2005).

Estrogen and ESR in placental function

The human placenta is capable of producing large amounts of estrogen necessary for placental and fetal growth as well as for the initiation of parturition (Gibb et al., 2006). By seventh week of pregnancy, there are adequate numbers of syncytiotrophoblasts to become the main producers of hormones in the placenta (Guibourdenche et al., 2009). Maternal cholesterol is incorporated into trophoblast cells via endocytosis, where it can be used as free cholesterol within cellular membranes, stored in lipid droplets, or utilized for steroidogenesis (Guibourdenche et al., 2009). The syncytiotrophoblasts are incapable of efficiently synthesizing cholesterol via *de novo* synthesis and therefore a maternal source is required during pregnancy (Guibourdenche et al., 2009). Cholesterol is converted to pregnenolone by P450scc via side-chain cleavage, and then progesterone (Guibourdenche et al., 2009). ESR1 and 2 have both been localized to specific cells of the placenta (Table 1); ESR1 is expressed in differentiating cytotrophoblasts while ESR2 is found in syncytiotrophoblasts and extravillous trophoblasts (Bukovsky et al., 2003; Schiessl et al., 2006; Kumar et al., 2009).

For estrogen synthesis to occur in the human placenta, there needs to be adequate levels of maternal and fetal dehydroepiandrosterone sulfate (DHEA-S) secreted by the adrenal glands. DHEA-S is converted to androstenedione by 3β-HSD and then to testosterone by 17β-HSD. Testosterone is further processed into estradiol by aromatase (Bousquet et al., 1984; Strauss et al., 1996; Wooding et al., 1996; Guibour-

Table 1 Localization of ESRs and AR in human trophoblast cells

Location	Receptor		
	AR	ESR1	ESR2
Differentiating cytotrophoblast	—	++	— +
CT	— +	++	— +
ST	++	—	+
EVT	NA	— +	++

++, Positive immunolocalization. +, Limited/low immunolocalization. — +, Low/variable immunolocalization. — , Not present. NA, Not determined.

denche et al., 2009; Hu et al., 2010). An hypoxic environment can regulate aromatase activity and as such, estradiol synthesis (Thompson and Siiteri, 1974; Goto and Fishman, 1977; Zachariah and Juchau, 1977). This contributes to the varying roles of estrogen between pregnancy trimesters where increased hypoxia during the first trimester leads to rapid proliferation and differentiation of the trophoblasts as well as remodeling of the maternal spiral arteries.

Estrogen also influences uterine receptivity and blastocyst implantation by interacting directly with the uterus to regulate growth factors, cytokine release and prostaglandins (reviewed in Bazer et al., 2009). For example, there is a finite amount of time the uterus is receptive to an implanting embryo, and estrogen and progesterone work in tandem to regulate factors necessary for this to occur (Bazer et al., 2008). Estrogens are also responsible in part, for the regulation of leptin expression in placental cells via genomic and non-genomic pathways (Gambino et al., 2010). Leptin is a nonglycosylated peptide of 146 amino acids that mediates thermogenesis, angiogenesis, arterial blood pressure, hematopoiesis osteogenesis, chondrogenesis as well as immune and neuroendocrine functions (reviewed in Schanton et al., 2018). Leptin is secreted by adipose tissue and the placenta and is a known inducer of trophoblast proliferation (Gambino et al., 2012). In first trimester trophoblast cells treated with E2, there is increased leptin expression and estrogen binding of ESR1 activates the LEP promoter in JEG-3 choriocarcinoma cells (Chardonnens et al., 1999; O'Neil et al., 2001). In addition to transcriptional activation of *LEP*, treating mutant MEK or MAPK cells with E2 did not illicit a response in leptin expression, suggesting these pathways are necessary for estrogen-induced leptin synthesis (Gambino et al., 2010; Maymo et al., 2011).

As the uterine spiral arteries are remodeled, an extensive vascular network is created to allow for appropriate fetal growth. Estrogens stimulate angiogenesis by increasing expression of VEGF as well as increasing vascular permeability and endothelial cell mitosis (Albrecht et al., 2010, Astwood, 1938; Friederici, 1967). Early in the second trimester, an increase in estrogen blocks additional differentiation of cytotrophoblasts into EVTs, by downregulating VEGF (Albrecht et al., 2006; Bonagura et al., 2008). This process may be due to the increased blood pressure and flow from the now remodeled maternal spiral arteries, and estrogens are no longer required to maintain the established uteroplacental blood flow (Aberdeen et al., 2010). ESR1 signaling also has a positive feedback role by regulating aromatase expression through its actions on CYP19A1 transcription and thus promoting placental estrogen synthesis (Kumar et al., 2009).

As indicated above, estrogens also signal through membrane receptors, including the G-protein coupled receptor GPR30. Using placental explants and immortalized human trophoblast cells HTR8/SVneo, Tong and colleagues reported that estrogen signaling through GPR30 activates the PI3K-Akt signaling pathway, upregulates MMP9 expression and increased trophoblast cell invasion (Tong et al., 2016). Furthermore, GPR30 levels are lower in placentas from preeclamptic women compared to placentas from uncomplicated pregnancies (Tong et al. 206; Feng et al., 2017).

Estrogen and ESR in placental dysfunction

Estrogens and ESR have also been implicated in various placental disorders such as PE, GDM and IUGR (Poidatz et al., 2015). In 2018, Wan et al. described a reduction in systemic estrogen and progesterone plasma levels in 86 women with diagnosed preeclampsia with fetal-growth restriction (FGR). Of these, 35 women were characterized as being severely preeclamptic and 31 with early onset PE (Wan et al. 2018). No significant differences were found in plasma E2 and progesterone in women with mild and severe PE patients or between early versus late onset PE. Interestingly, levels of E2 and progesterone from PE placental explants were lower compared to normal tissue. In first trimester placental extracts incubated with an oxidative species to mimic oxidative stress, they found lower E2 in culture media. These findings suggest that deficiency of the placenta leads to decreased sex steroid secretion (Wan et al., 2018). In women with GDM, placental estrogens and aromatase are decreased (McRobie et al., 1997; Uzelac et al., 2010). Decreased levels of E2 and E_3 have been observed in both mild and severe cases of PE and preeclampic placental tissue (Hertig et al., 2010; Açıkgöz et al., 2013; Jobe et al., 2013) while E_1 has generally been observed as decreased only in severe cases of PE (Hertig et al., 2010; Jobe et al., 2013). E_3 was also found to be significantly lower in PE placental tissue compared to normal pregnancies (Açıkgöz et al., 2013).

The role of ESR in placental dysfunction is less well known and the literature has described conflicting results. For example, Molvarec et al. found two *ESR* polymorphisms

associated with severe preeclampsia. Peripheral blood samples revealed homozygous T-A haplotype carriers of ESR1 polymorphisms that were associated with an increased risk for severe preeclampsia (Molvarec et al., 2006). Contradictory to this study, no association between ESR polymorphisms and severe PE was described in a study in 2009 (Zhang et al., 2009). Finally, Park and colleagues examined ESR1 and ESR2 expression in preeclamptic placenta, and found that ESR1 expression is reduced and ESR2 activity appeared to be inactivated in preeclamtic placenta (Park et al., 2018). Currently, it is unknown whether abnormal E2 levels or ESR signaling is a cause, or the result of abnormal placental differentiation or function.

Testosterone signaling

Androgens are a family of 19 carbon steroid hormones that includes adrenal androgens, dehydroepiandrosterone (DHEA) and DHEA-S, androstenedione, androstenediol, testosterone, and dihydrotestosterone (DHT). Similar to progesterone and E2, maternal testosterone plasma levels increase during pregnancy (Castracane et al., 1998; O'Leary et al., 1991) and the placenta itself is a source of testosterone (Rhind et al., 2011; Bellingham et al., 2012; Padmanabhan and Veiga-Lopez, 2014). Recent studies revealed that the endometrium is also a source of the more potent, DHT. In fact, androgens are proposed to play a role in endometrial cell proliferation and decidualization (endometrial stromal cell differentiation) by regulating expression of decidualization marker IGFBP1 and receptivity marker SPP1 (Gibson et al., 2016; Simitsidellis et al., 2018).

AR has been investigated as both a transcription factor and a signal transducer in a variety of tissues. Notably AR acts as a ligand activated transcription factor to regulate gene expression by binding to the androgen response element (ARE: AGAACAnnnTGTTCT) in promoter regions of AR target genes. AR classically binds androgens to produce genomic effects involving translocation of the androgen-AR complex to the nucleus, leading to regulation of gene transcription. AR can also interact with co-regulators or DNA-bound transcription factors to regulate gene transcription. The AR gene is localized to the X chromosome and encodes a protein made up of 919 amino acids and like ESR contains an N-terminal domain, AF1 transactivation factor domain, DNA binding and hinge domain, a ligand binding domain, AF2 transactivation domain and C-terminal domain (Park, 2005).

AR signaling was first described in *Xenopus* oocytes as a membrane signaling mechanism through activation by a bound ligand. This ligand-receptor complex repressed G-protein signaling leading to a decrease in cAMP and ERK signaling. Migeon et al. (1981) localized AR to the X chromosome by establishing a cell line that expressed the testicular feminization locus (*Tfm*) from mouse kidney cells that lacked androgen binding ability. Experiments using these cells showed that when the AR locus was present, binding affinity was similar to what is observed in human cells (Migeon, 1981). Like estrogens, androgens can also illicit rapid non-genomic effects including Ca^{2+} influx and cAMP pathway activation through binding of membrane receptors (Pi et al., 2010; Wang et al., 2014). For example, recent evidence suggests that the G-protein coupled receptor GPRC6A is a membrane receptor for androgens (Pi et al., 2010; Wang et al., 2014). GPRC6A is better known for its role in mediating the effects of Osteocalcin, an osteoblast-specific hormone that plays a role in bone calcification and calcium ion homeostasis (Pi et al., 2011). Recent studies have indicated a role for GPRC6A in stimulating testosterone production in Leydig cells (Chamouni et al., 2014). Knockout of GPRC6A in male mice leads to testicular feminization (Pi et al., 2008) while overexpression in HEK 293 cells negative for AR treated with testosterone induced extracellular-related kinase (ERK) activity (Kang et al. 2004).

Testosterone and AR in placental function

Very little is known about the androgens and androgen signaling in the placenta. Most pregnancy-related research regarding androgens centers on infertility or placental disorders such as PE. During early embryonic development, testicular androgen production is required for appropriate male sex differentiation (Hughes et al., 2012; Metzler et al., 2017). In 1979, Barile et al. reported the first evidence for a testosterone binding protein in human placental tissue. In 1980, Hirota et al. found AR in the cytosol of human placental villi and it has since been localized to differentiated syncytiotrophoblast, cytotrophoblasts and placental stroma (Table 1) (Iwamura et al., 1994; Hsu et al., 2009). Research from our laboratory demonstrates that AR binds to an ARE in the VEGF promoter in ovine placental tissue. This data suggests that placental androgens may play and important role in regulating trophoblast function (Cleys et al., 2015).

In pregnant women, androstenedioine levels increase starting on day 14 of pregnancy (O'Leary et al., 1991; Castracane et al., 1998) while testosterone was found to decrease blood flow to the placenta (Abbas and Gupta 2008). Human fetuses with complete androgen insensitivity syndrome due to mutations in AR survive fetal development (Hughes and Evans, 1987; Bangsboll et al., 1992; Brown, 1995; Quigley et al., 1995; reviewed by Ahmed et al. 2000), suggesting that lost or perturbed AR signaling may not be detrimental to placental development, despite findings of increased maternal serum androgens in compromised pregnancies (Atamer et al., 2004; Ghorashi and Sheikhvatan, 2008; Hsu et al., 2009; Lorzadeh and Kazemirad, 2012). This suggests that maternal androgen signaling may be sufficient to maintain androgen-mediated angiogenesis in the placenta during pregnancy.

Alternatively, compensatory mechanisms likely exist to support placental development and differentiation, and possibly include non-genomic signaling, heterodimerization with other nuclear receptors, and/or overlapping functions of both estrogen and androgen receptor signaling. For example, estrogen regulation of LEP expression in trophoblast cells involves both genomic and non-genomic processes and both ESR and AR are known regulators of cell proliferation and angiogenesis. Finally, like androgens, maternal serum levels of osteocalcin also increase during gestation (Seki et al., 1991; Maliqueo et al., 2016). Although AR has been localized to the decidua and placental trophoblast cells in humans (Table 1), nothing is known about GPRC6A localization in the placenta (Uzelac et al., 2010; Srichomkwun et al., 2015).

Testosterone and AR in placental dysfunction

There is limited information about a functional role of androgens and AR during pregnancy and atypical placental development. In women with PCOS, increased serum testosterone has a profound impact on insulin action, increased adiposity, increased risk of developing type II diabetes (reviewed Morford et al., 2018) and increased adverse pregnancy outcomes, including miscarriage (Bahri et al., 2018 and references therein). Increased maternal glucose has been shown to cause increased fetal birthweight and neonatal adiposity (reviewed by Meng et al., 2016; Bahri et al., 2018). In PE patients there is increased serum testosterone levels and decreased aromatase activity compared to normal pregnancies which is non-dependent on fetal gender (Thoumsin et al., 1982; Atamer et al., 2004; Ghorashi and Sheikhvatan, 2008; Hsu et al., 2009). AR levels have been shown to be increased in the syncytiotrophoblast and stroma of PE patients (Hsu et al., 2009; Sathishkumar et al., 2011) and like ER, polymorphisms in AR have been correlated with an increased risk for the development of PE, miscarriage, and spontaneous pre-term birth (Lim et al., 2011; Jahaninejad et al., 2013).

Specifically, polymorphisms greater than 16 GGC trinucleotide repeats in the transcriptional activation domain of the *AR* gene can lead to decreased AR function and expression (Lim et al., 2011). PE patients have also been found to have increased free testosterone and androstenedione at both 17 and 33 weeks of gestation (Carlsen et al., 2005) but by six weeks postpartum, testosterone levels in the serum return to normal (Serin et al., 2001). This association suggests an alternative source of androgens during pregnancy, most likely the placenta. In women with GDM, increased testosterone has been used as an early indicator of developing the disease and increased AR in the placental tissues of these patients has been reported (Uzelac et al., 2010; Gözükara et al., 2014). Decreased *VEGFR2* and *VEGFRA* are also decreased in GDM placentas compared to normal tissue (Uzelac et al., 2010) suggesting a derangement in placental angiogenesis, potentially mediated by androgens.

Excess testosterone during pregnancy such as seen in women with PCOS has been reported to negatively affect placental angiogenesis, but not lead to fetal virilisation of female fetuses (O'Leary et al., 1991; Castracane et al., 1998; Sir-Petermann et al., 2002; Fornes et al., 2016). For example, in rats treated with exogenous testosterone, placental VEGFR1 expression was decreased compared to controls (Fornes et al., 2016). Similarly, in a prenatal androgenization PCOS model in sheep increased AR expression in the placenta was observed, as well as increased levels epigenetic regulators including DNMT1 and H19 (Cleys et al., 2015). Interestingly, contrary to the observation in rats treated with testosterone and in GDM placentas that VEGFA receptor decreased, placental expression of VEGFA protein were increased both on maternal and fetal sides in this PCOS model. This suggests potential compensatory exist in trophoblast cells to regulate proper placental angiogenesis.

ESR and AR in placenta of other mammals

Sex steroids and their receptors have also been found in placenta of other species, such as rodents and ruminants, and similar to humans little is known about ESR and AR function in this organ. For example, studies in mice have reported that ESR1 is expressed in uterine stromal and epithelial cells and is important in mediating decidualization and implantation (e. g., Pawar et al., 2015). However, very little is known about ESR in placenta aside from its reported presence according to RT-PCR (Mouse Genome Informatics reference ID J:46439). In mouse embryos, it was found that ESR may regulate trophectoderm cell differentiation (Cheng et al., 2016). Similarly, AR was found to be located in nuclei of placental stroma at day 1-2 of gestation and increased days 3-4. After day 5 of pregnancy, AR protein levels decrease (Xu et al., 2015). In vivo AR is inhibited during decidualizion, possibly due to the increase of progesterone during this process, while estrogen was found to increase AR expression in uterine stroma (Xu et al., 2015). In obese mice exposed to DHT at gestational day 15.5, AR protein expression increased in the placenta, similar to what occurs in women suffering from polycystic ovarian syndrome (PCOS) (Fornes et al., 2017).

In sheep, both ESR1 and ESR2 have been localized to maternal and fetal placental tissues during early pregnancy (days 14-30) by immunofluorescence (Bairagi et al., 2018). *ESR1* levels are decreased after day 16 of pregnancy in caruncular (maternal) tissue, whereas *ESR2* levels are generally low (Reynolds et al., 2015). Interestingly, *ESR1* levels increase in between day 16 and 20, after which they drop in cotyledons (fetal). Previously, we reported AR presence in sheep placentomes at mid-gestation both in maternal and fetal compartments (Cleys et al., 2015). Moreover, chromatin immunoprecipitation experiments indi-

cated that VEGFA is a direct target of AR in trophoblast cells (Cleys et al., 2015), suggesting AR signaling may play a role in regulating placental angiogenesis through its action on expression of angiogenic factors such as VEGFA.

Finally, both ESR1 and ESR2 (mRNA and protein) have been reported in bovine placentomes. Interestingly, in cows ESR1 appears to be confined primarily to caruncles, whereas ESR2 is present in cotyledons during mid- and late gestation. ESR1 localized to caruncular epithelial cells and capillary pericytes (Hoffmann and Schuler, 2002). Due to its higher expression on the fetal side, and localization in mature trophoblast giant cells and vascular cells, ESR2 is thought to play a role in trophoblast giant cell differentiation and vascular function (Schuler et al., 2002, 2005). An intracrine function for local ESR signaling in placentomes is further supported by the observation that inactive estrone sulfate entering maternal circulation maybe converted locally into (active) free estrogens by steroid sulfatase present in caruncles, as well as upregulation of aromatase during trophoblast giant cell differentiation (Schuler et al., 2008).

Similarly, AR is present cow placentomes from day 50 until term. Moreover placental testosterone concentrations increase during gestation suggesting a role for androgen signaling through AR in placental physiology (Khatri et al., 2013). Nuclear staining for AR is observed in trophoblast giant cells, and immunoreactivity appeared to increase in immature and mature trophoblast giant cells, uninucleated trophoblast cells and stromal cells from mid-gestation until term. This overlap in expression between ESR2 and AR in trophoblast giant cells suggest possible complimentary functions in trophoblast cell function or differentiation.

Summary and conclusion

The placenta is well known for its ability to secrete hormones during pregnancy thereby promoting fetal growth and development as well as maintaining pregnancy. The placenta synthesizes and secretes estrogens and androgens throughout pregnancy, and both ESR and AR localize to different trophoblast cells types suggesting diverse roles in placental development and function. Placenta development and trophoblast differentiation involves many processes commonly seen in cancer, such as proliferation, migration, invasion, and angiogenesis, and ESR and AR signaling are known to be involved in these cellular processes.

Similar to their role in cancer and tumorigenesis, we postulate that estrogen and androgen signaling play a diverse role in trophoblast differentiation (Fig. 2). To date studies

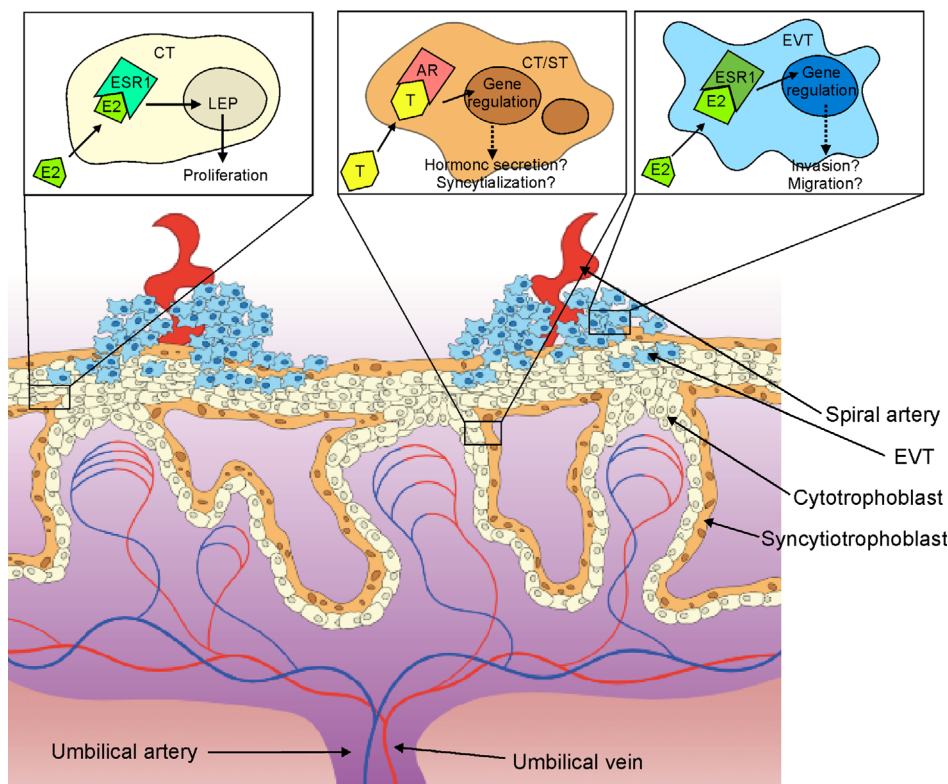

Figure 2 Proposed roles of estrogen (ESR) and androgen receptor (AR) in differentiating trophoblast cells. Solid arrows indicate reported functions, according to the literature. Dashed arrows indicate proposed functions. Based on available expression and localization data we postulate that ESR1 and AR have important functions in cytotrophoblast function (proliferation) and differentiation (syncytialization), respectively. Furthermore, ESR1 expression in extravillous trophoblast cells (EVT) suggests a possible role in EVT functions such as migration and invasion.

have demonstrated that E2 through non-genomic or genomic (ESR) signaling regulates LEP expression thereby in turn controlling trophoblast proliferation, and is a known regulator of placental angiogenesis. However its role in trophoblast differentiation into invasive EVT's or cell fusion and syncytialization is less clear. Very little is known about AR function in the placenta. Recent evidence indicates AR signaling plays a role in endometrial cell differentiation (decidualization), and our own data suggests a role in regulating VEGFA and placental angiogenesis. Whether additional or unique roles exist in trophoblast proliferation, differentiation and/or syncytialization is still unclear.

Finally, the observations that pregnancy disorders such as PE, GDM, and PCOS are associated with placental dysfunction and abnormal estrogen/ESR and androgen/AR levels suggests involvement of these steroids in normal placental physiology. Furthermore, although both ESR's and AR have been localized in placental tissue, the exact contribution of abnormal of ESR and AR signaling in placental dysfunction in these pregnancy disorders remains to be fully explored. Ultimately, this knowledge will provide the foundation for future studies designed to uncover underlying mechanisms in which for example metabolic diseases associated with abnormal steroid levels lead to placental dysfunction. Moreover, it also provides novel avenues that focus on estrogen and androgen signaling that can be targeted/investigated to alleviate pregnancy disorders associated with placental dysfunction and abnormal steroid hormones levels.

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