Chapter

Androgen Signaling in the Placenta

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Abstract

The placenta is a multifunctional, transitory organ that mediates transport of nutrients and waste, gas exchange, and endocrine signaling. In fact, placental secretion of hormones is critical for maintenance of pregnancy, as well as growth and development of healthy offspring. In this chapter, the role of androgens in placental development and function is highlighted. First, a brief summary will be provided on the different mammalian placental types followed by an overview of placental steroidogenesis. Next, the chapter will focus on genomic and non-genomic androgen signaling pathways. Finally, an overview will be provided on the current status of androgen signaling in the placenta during normal and abnormal pregnancies.

Keywords: pregnancy, placenta, testosterone, dihydrotestosterone, androgen receptor

1. Introduction

Establishing and maintaining pregnancy requires a finely regulated series of physiological events involving mother, fetus, and placenta. The essential role of steroid hormones in the production and maintenance of many of these changes is well characterized. For example, important effects of progesterone include preparation of the endometrium for implantation [1], modulation of the maternal immune response to tolerate the fetal allograft, maintenance of myometrial quiescence [2], and preparation of mammary glands for lactation. Estrogens appear to influence uterine blood flow and neovascularization, increase the expression of critical proteins that are involved in progesterone production and steroid metabolism and participate in preparation of mammary glands for lactation [3, 4]. Throughout pregnancy, levels of maternal circulating androgens, including testosterone (T), dihydrotestosterone (DHT), dehydroepiandrosterone (DHEA) and androstenedione (A4) increase, with concentrations three-fold higher by the third trimester when compared to non-pregnant levels in women [5]. Although T is a well-known precursor for estrogens (E2) synthesis, the placenta can both be a source and a target for androgens. The goal of this chapter is to summarize what is known about androgens and androgen receptor in pregnancy and compare it between species and between different types of placenta.

2. Placenta classification

The placenta is a multifunctional, transitory organ that mediates transport of nutrients and waste, gas exchange, and endocrine signaling. In fact, placental

secretion of hormones is critical for maintenance of pregnancy, as well as growth and development of a healthy offspring. Despite fulfilling similar functions, there is a wide range of diversity in placental anatomo-histology among species.

During early embryogenesis, the first cells to differentiate are trophoblast cells, which form the chorion or fetal portion of the placenta. Villous trophoblast cells have two distinct cell populations; undifferentiated cytotrophoblast cells and differentiated syncytiotrophoblast tissue. The syncytiotrophoblast tissue is a continuous, multinucleated, specialized layer of epithelial cells, which covers the villous surface and is in direct contact with maternal blood. This layer is formed by fusion of cytotrophoblast cells.

Placental gross morphological classification is based on the shape and the area of contact between fetal and maternal tissue [6]. There are four commonly describe placental shapes among mammals:

1. **Diffuse placenta**, present in the horse and pig, has chorionic villi in contact with the uterine endometrium throughout the entire surface of the allantochorion, forming either folds (pig) or microcotyledons (horse).

2. **Cotyledonary placenta**, present in ruminants, is made up of multiple discrete areas of attachment called cotyledons, which are formed by the interaction between the fetal allantochorion and the maternal endometrium. The fetal side of this type of placenta is called cotyledons, the maternal side is called caruncles, and the cotyledon-caruncle complexes are known as placentomes.

3. **Zonary placenta**, present in carnivores such as dogs and cats, has the shape of a complete (dog and cat) or incomplete band (ferrets and raccoons) of tissue surrounding the chorionic sack.

4. **Discoid placenta**, present in rodents and primates, is formed by a collection of villi on a single (mice and human) or double (rabbit) disc.

In addition to the gross morphological classification, placentas are also categorized by histology (**Figure 1**) which is based on the different number of cell layers separating fetal from maternal circulation [7]. Before the placenta is formed, there are a total of six layers of tissue separating maternal and fetal blood. Three of these layers are fetal extraembryonic membranes in the chorioallantoic placenta of all mammals, all of which are components of the mature placenta. These three layers include endothelium lining allantoic capillaries, connective tissue in the form of chorioallantoic mesoderm, and chorionic epithelium, derived from trophoblast cells. There are also three layers on the maternal side, but the number of these layers which are retained after placentation varies greatly among species. The three potential maternal layers in a placenta are endothelium lining endometrial blood vessels, connective tissue of the endometrium, and endometrial epithelial cells.

Based on this degree of separation, or number of layers separating the fetal and maternal tissues, there are four different types of placenta (see **Figure 1**):

1. **Epitheliochorial placenta**, present in pig and horse, consists of all six layers separating maternal from fetal blood throughout gestation. The trophoblast cells make contact with the uterine epithelium forming microcotyledons (horse) or chorionic folds (pig). Microcotyledons contain highly vascularized chorionic villi that extend into elaborate invaginations of the endometrium. Chorionic folds are formed by the lining of the chorionic villi into the wrinkled surface of the uterine epithelium.

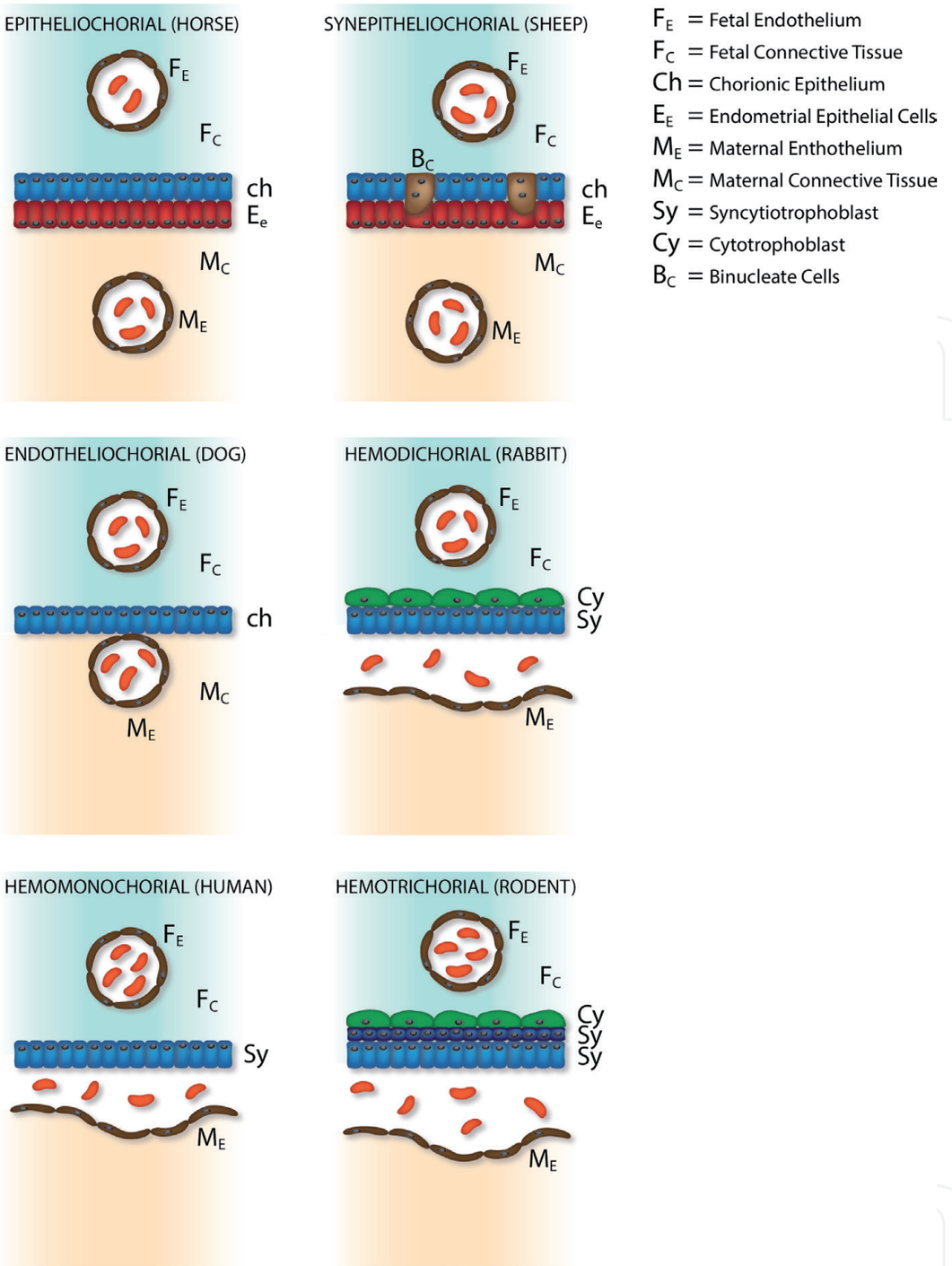

Figure 1.
Histological classification of the placenta.

2. **Synepitheliochorial placenta**, present in ruminants, contains the same layers as an epitheliochorial placenta. In this type of placenta, the uterine epithelium is modified by invasion and fusion of binucleate cells forming the syncytium, which contains embryonic and maternal nuclei. More recently, multinucleated trophoblast giant cells (TGC), formed by incomplete cytokinesis of mono-nucleated trophoblast cells, are believed to remove endometrial epithelial cells and fuse and contribute to the syncytial trophoblast layer [8].

3. **Endotheliochorial placenta**, present in carnivores (cats and dogs), is formed when the endometrial epithelium is disrupted during placentation, and fetal chorionic epithelial cells come in contact with maternal endothelial cells.

During implantation, cytotrophoblast cells surround the central third of the chorioallantois and proliferate to form a syncytium called the syncytiotrophoblast layer. The syncytiotrophoblast layer erodes through the endometrial epithelium and grows around maternal capillaries. Initially, the invading fetal cells are in the form of villi, but villi soon coalesce to form a labyrinthine-type placenta. For this reason, only four tissue layers separate the maternal from the fetal blood.

4. **Hemochorial placenta**, present in humans and rodents, is the most invasive form of placentation. Fetal chorionic epithelium is bathed in maternal blood because chorionic villi have invaded through endometrial epithelium and eroded through maternal endothelium. The number of trophoblast layers in contact with the maternal circulation shows variation between species. It is hemomonochorial in humans, with one layer of syncytiotrophoblast, and hemodichorial in primates, with one layer of syncytiotrophoblast upon one layer of cytotrophoblast cells. Finally, it is hemotrichorial in rodents with one layer of cytotrophoblast and two layers of syncytiotrophoblast separating maternal and fetal blood.

3. Placenta steroidogenesis

A key function of the placenta is the secretion of hormones. Like other steroid hormones, T is derived from cholesterol and the synthesis involves several enzymatic steps. The first and fundamental step in its biosynthesis involves the oxidative breakdown of the cholesterol side chain by the enzyme P450scc (side-chain cleavage), a mitochondrial cytochrome oxidase, resulting in the loss of six carbon atoms to give rise to pregnenolone. Only certain cell types in humans are capable of pregnenolone synthesis, including testicular Leydig cells, ovarian theca and corpus luteal cells, placental trophoblast cells, cells of the adrenal cortex, and specific cells in the brain, such as the pyramidal and granular neurons of the hippocampus and the Purkinje cells from the cerebellum [9]. The resulting pregnenolone is either converted to progesterone or 17 α-hydroxypregnenolone via 3β-hydroxysteroid dehydrogenase (HSD3B) or cytochrome P450 17A1 (CYP17A1), respectively. Progesterone is secreted into maternal circulation, and 17α-hydroxypregnenolone can be metabolized to DHEA via CYP17A1. DHEA is oxidized into A4 via HSD3B. A4 is then reduced to 5 α-androstenedione via 5 α-reductase (SRD5A). DHEA and A4 can be converted by 17 β-hydroxysteroid dehydrogenase into androstenediol and T, respectively. Subsequently, T is converted into DHT via 5 α-reductase (SRD5A). T and 5 α-androstenedione can further be metabolized to estrogens via aromatase (CYP19A1) [10]. A summarized overview of placental steroidogenesis is provided in **Figure 2**.

Androgens are synthesized in tissues where 17α-hydroxylase/17,20-lyase cytochrome P450 (P450c17) exists. This enzyme is located in different tissues such as fetal and maternal adrenal glands, fetal ovaries and testes, and the corpus luteum, depending on to the animal species. In non-pregnant woman, 50% of all DHEA is secreted by the adrenal glands, 20% from the ovarian theca and 30% is derived from metabolism of circulating DHEA sulfate [11]. Adrenal glands and ovaries produce equal amounts of A4, with the total daily production rate 1.4-6.2 mg/day [12]. 50% of T is synthesized in the ovaries and adrenals and the other half is produced from A4 in the peripheral tissues. Daily production rate of T in non-pregnant women is in the order of 0.1–0.4 mg/day. Finally, the conversion of T to

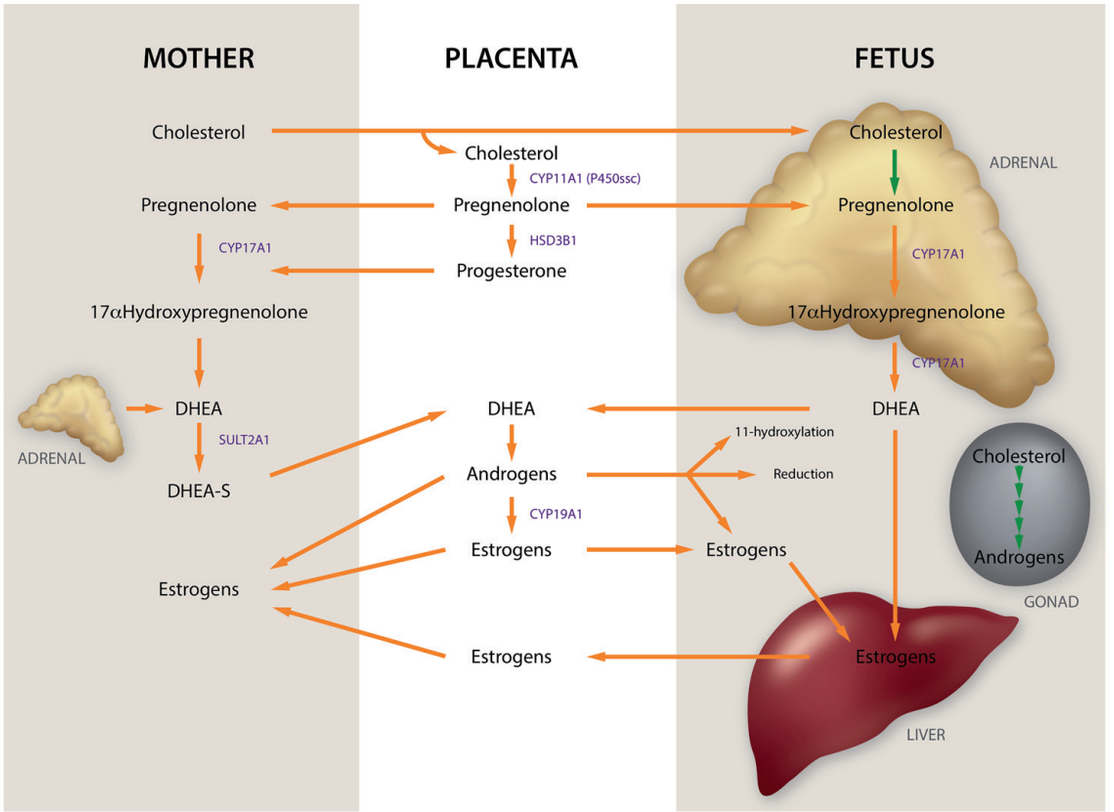

Figure 2.
Summarized overview of the functional interaction between the placental, maternal and fetal compartments for the biosynthesis of progesterone, estrogens and androgens by the human placenta. Progesterone is produced mainly from maternal cholesterol. Progesterone can be metabolized into DHEA by the maternal and fetal adrenal gland. DHEA can be converted into T. Subsequently, T can further be metabolized to estrogens via aromatase (CYP19A1). In horses, the placenta does not appear to express P450c17 and thus cannot convert de novo *c21 progestogens (pregnenolone and progesterone) to the c19 androgens (DEHA and A4). It is for this reason that the reaction occurs in the fetal adrenal.*

DHT occurs in peripheral tissues, such as ovaries and skin, with a daily production rating between 4.3 and 12.5 mg/day.

During pregnancy, an additional source of androgens comes from the fetus and placenta (**Figure 2**). Androgens are principally synthesized in the corpus luteum during early stages of gestation in rats and dogs and this function is taken over by the placenta in late stages of gestation in rats [13]. In sheep and goats, P450c17 is present in the placenta [14]. Some studies revealed that the absence of P450c17 in human and horse placentas results in negligible androgen synthesis [15]. However, protein and mRNA levels of CYP17A1 have been detected in primary human trophoblast cells and the human trophoblast cell line JEG-3 and trophoblast cells were able to generate testosterone *de novo* [16]. Placentas associated with a male fetus at term have increased expression of 5 α-reductase compared to a female fetus [17]. This enzyme is involved in reducing T to DHT, a potent androgen with a higher binding affinity to AR than T, suggesting the placenta may play a role in the hormonal differences between pregnancies between female and male fetuses.

In horses, the placenta does not appear to express P450c17 and thus cannot convert *de novo* c21 progestogens (pregnenolone and progesterone) to the c19 androgens (DEHA and A4). In this case, the fetal gonads are the main androgen source as estrogens precursor during mid to late gestation in the horse. Removal of fetal gonads results in an immediate fall in maternal plasma concentrations of conjugated and unconjugated estrogens whereas progestogens levels remain unchanged [18].

4. Androgen signaling and placenta function

To exert a cellular response, steroid hormones need to bind to either a membrane receptor or an intracellular, nuclear or cytoplasmic receptor. T and DHT can bind to either type of receptor, AR (encoded by *AR*, in the human Xq11-12), or a membrane-bound receptor, such as G protein-coupled receptor family C group 6 member A (GPRC6A) [19]. DHEA and A4 require conversion to T or DHT to exert their androgenic effects.

AR is expressed at all levels of the female hypothalamic-pituitary-gonadal axis, including the brain, ovarian stroma, ovarian follicles and corpus luteum. Furthermore, AR is present in first trimester and term placenta, and localizes to the cytosol of placental villi, and in cytotrophoblast, differentiated syncytiotrophoblast, and placental stroma [20]. In ruminant placentomes, nuclear signals are predominantly observed in invasive TGC and uninucleate trophoblast cells, stromal cells of the chorionic villi, caruncular epithelial, and stromal cells during late gestation [21, 22].

AR belongs to the steroid hormone intracellular receptor family. Exon 1 of the *AR* gene encodes the N-terminal domain, which contains an activation function 1 (AF1) region that interacts with coregulatory proteins to enhance transcriptional regulation of AR target genes. Exons 2 and 3 encode two distinct zinc-fingers (DNA-binding domain) required for interaction with a palindromic androgen response elements (ARE) of the core sequence, 5′-TGTTCT-3′, separated by 3 nucleotides located within the promoter regions of AR target genes. The remaining exons encode a hinge region which contains the nuclear localization signal, and the ligand binding domain [23].

When localized to the cytoplasm, AR is bound by a number of chaperone proteins including heat shock protein 90 (Hsp90) as well as immunophilins. When ligands, T or DHT, bind to AR, there is a conformational change which exposes the nuclear localization signal, allowing the interaction with importin-α, which facilitates nuclear translocation. Once inside the nucleus, two subunits of the AR dimerize and bind the ARE on promoter regions of AR-target genes, resulting in transcriptional regulation, leading to either activation or suppression of expression. Co-regulatory proteins, such as histone lysine demethylases (KDMs), modulate transcriptional activity of AR-target genes. In sheep for example, KDMs have been found to act as co-regulators in trophoblast cells [22, 24]. This interaction with regulator factors is critical for signaling processes in the placenta.

Androgens are known to stimulate proliferation of human umbilical vein endothelial cells, indicating a key role for androgens during pregnancy. During establishment of pregnancy, androgens play a role in embryo implantation. Early in pregnancy, before implantation, T is converted to DHT which regulates transcription of factors necessary for initiation of decidualization and early endometrial receptivity. Near the time of implantation, T itself promotes endometrial remodeling, and soon after implantation it serves as an important precursor for E2 which regulates vascular remodeling [25]. Studies in mice reveal that insufficient androgens may delay embryo implantation, whereas excess androgens lead to aberrant gene expression at implantation sites.

Studies on ovine placentas revealed vascular endothelial growth factor A (VEGFA) expression to be androgen responsive, and androgens are thought to regulate the expression of VEGFA and play a key role in placental angiogenesis [21, 23]. More specifically, AR and the KDM1A coregulator are recruited to an ARE in the ovine VEGFA promoter. On gestational day 90, placenta VEGFA mRNA and VEGFA and AR protein levels increased in testosterone-treated ewes compared to control placentas [22].

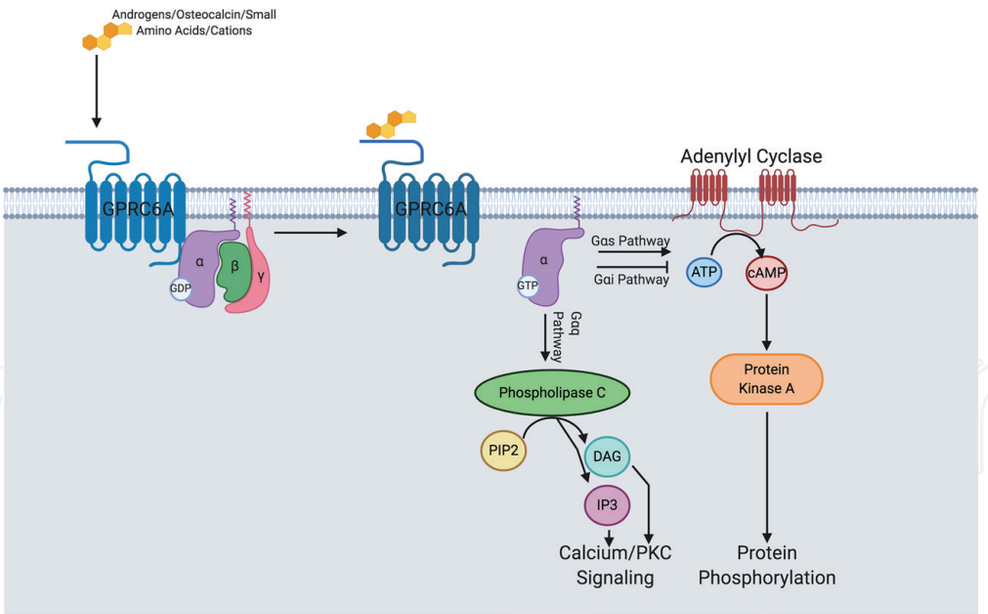

Figure 3.
Androgen signaling through GPRC6A in target tissue. Image created with BioRender.com.

In addition to the classical genomic intracellular AR mediated signaling pathways, androgens also act through membrane receptors. GPRC6A is a G protein-coupled receptor (GPCR) that functions as a membrane receptor for small amino acids, cations, osteocalcin, and androgens [26]. GPRC6A is known to have a long extracellular domain to allow for the binding of these different ligands [26]. GPRC6A mediates the effects of osteocalcin, a protein hormone released by osteoblasts, and results in the activation of the cAMP pathway and subsequently, testosterone synthesis by the Leydig cells of the testis. GPRC6A ligand binding can result in the activation of the Gαs, Gαi, and Gαq pathways (**Figure 3**). The presence of GPRC6A has been identified in placental trophoblast cell membranes, indicating the possibility of androgens eliciting a non-genomic effect on cells of the placenta.

Another membrane receptor that androgens elicit a non-genomic effect through is Zrt- and Irt-like protein 9 (ZIP9) [27]. ZIP9 is a zinc transporter that also acts as a receptor for androgens via G-protein coupling. Studies have revealed the presence of ZIP9 in ovarian tissue of Atlantic croakers, and act as a receptor for androgens inducing apoptosis in follicular cells, as well as promoting zinc uptake. Studies also show a similar action in breast and prostate tissue [28]. Ultimately, these two studies reveal that androgens binding to ZIP9 results in the activation of pro-apoptotic genes and the regulation of zinc homeostasis within target tissues [28].

Similarly, Transient receptor potential cation channel subfamily M (melastatin) member 8 (TRPM8) and Oxoeicosanoid receptor 1 (OXER1) bind a variety of ligands including androgens [29]. However, their expression during pregnancy or in placental cells is currently unknown.

5. Androgens and pregnancy

Androgens play a fundamental role in female physiology, particularly during pregnancy. In women, androgens are synthesized by cells within the ovaries, the adrenal glands, fat, and also in placenta, acting in an endocrine or paracrine fashion [30]. DHEA, mainly from the adrenal glands, acts as a crucial precursor for E2 and T in the ovary and other target tissues such as fat [31]. Depending on the intracellular availability of steroidogenic enzymes in target tissues, DHEA is converted to A4

which is a precursor for T, both of which can be aromatized to estrogens [32]. Some studies have reported an elevated level of T during pregnancy. An increase in T levels occurs from the first trimester of pregnancy, becoming more pronounced towards the third trimester, being three-folds higher than observed in non-pregnant women (**Table 1**) [36]. In contrast, maternal circulating DHEA levels decrease in pregnant women due to it being converted to T and E2 [37].

Steroid production varies widely among species, with these differences becoming more pronounced during pregnancy. Each species have their own distinct pattern of steroid serum levels, steroidogenic enzymes, receptors, and transporters to support their individual physiological requirements. For example, in dairy cows, maternal serum T levels increase ~100-fold during the last trimester of gestation (**Table 1**), as well as a ~50-fold increase in milk testosterone levels [38].

In the horse, T elevation during pregnancy presents a biphasic curve (**Table 1**). The first elevation is caused by luteal androgen production, which is stimulated by equine chorionic gonadotropin (eCG). The late rise and fall are temporally related to the development and regression, respectively, of the fetal gonads. The equine placenta has little capacity to synthesize androgens, as it lacks CYP17A1. Hence, androgens in the form of DHEA are substrates for E2 synthesis, and must be supplied mostly by fetal gonads, forming a true feto-placental unit [39].

As androgen levels increase during pregnancy, the mother and developing fetuses usually are protected from excess bioactive androgens by increased secretion of sex hormone-binding and placental aromatase, which converts T into E2. Hyperandrogenism can result from a number of conditions, the most common being luteomas and theca-lutein cysts within the ovary. Luteomas are benign tumors that occur during pregnancy with excess androgen production in 25-35% of the cases [33, 40, 41]. These often go unnoticed and in most cases are non-virilizing.

Humans	**Testosterone (ng/ml)**	
	Pimiparous	**Multiparous**
1st trimester	1.34	0.66
2nd trimester	1.98	0.73
3rd trimester	2.56	0.71
7-20 wks	0.69	
21-40 wks	1.095	
Horse		
Days from ovulation		
0-35	0.04	
35-120	0.14	
180–240	2.5	
240-300	3.5	
>300	1.5	
Cow		
Days from ovulation		
0-90	0.020-0.050	
90-270	0.22	

Table 1.
Testosterone serum levels during pregnancy in human [33], horses [34], and cows [35].

Additional causes of excess androgen production during pregnancy are conditions such as polycystic ovarian syndrome (PCOS), one of the most common endocrine disorders in women of reproductive age, and congenital adrenal hyperplasia (CAH). Both conditions result in pregnancy complications, including pregnancy induced hypertension and pre-eclampsia, a human pregnancy syndrome characterized by the onset of hypertension and proteinuria after 20 weeks of gestation, and can lead to maternal or fetal mortality [42]. In humans, clinical observations have established that women with PCOS exhibit similar features as seen in classical 21-hydroxylase deficiency in CAH, such as anovulation, ovarian hyperandrogenism, LH hypersecretion, polycystic appearing ovaries, and insulin resistance, despite normalization of adrenal androgen excess after birth [43]. Furthermore, animal studies have demonstrated that intrauterine exposure to excessive amounts of androgens can lead to development of PCOS after birth (reviewed in [44, 45]). In fact, prenatal androgenization in pregnant ewes has revealed reproductive and metabolic phenotypes in female offspring that closely resemble PCOS in women. These observations suggest that androgen excess during early life, whether derived from fetal or maternal sources, may provide one possible mechanism to explain the occurrence of PCOS in adulthood.

Less common causes leading to androgen excess during pregnancy include placental aromatase deficiency. Aromatase is encoded by the CYP19A1 gene, and is responsible for converting T to E2. At least 10 different promoters have been identified in its regulatory region, enabling regulation in a tissue-specific manner [46]. Mutations in CYP19A1 prevent aromatization of testosterone, leading to hyperandrogenism and phenotypes similar to androgen excess, including maternal and fetal virilization and development of ambiguous genitalia at birth [47]. Of particular interest is the observation that placental aromatase deficiency is associated with pre-eclampsia [48, 49]. Women with pre-eclampsia have significantly lower levels of placental aromatase, and significantly lower levels of both 17β-estradiol:testosterone and estrone:androstenedione ratios, as well as higher levels of T. In fact, this placental defect in steroidogenesis appears before clinical symptoms of pre-eclampsia and thus may serve as a diagnostic marker.

6. Conclusions

The focus of this chapter was on androgens and their potential role in pregnancy and placental development and function. Normal pregnancy in women is associated with increased maternal serum levels of androgens, which are derived from the adrenal glands, adipose tissue, ovaries, and placenta. Species differences in androgen production exist reflecting species-specific needs for pregnancy maintenance and/or placental function. Furthermore, the placenta contains classical androgen receptors as well as non-classical membrane receptors, indicating the placenta is a target of androgen signaling. Preliminary and ongoing studies suggest a role for androgen signaling in trophoblast cell differentiation and placental angiogenesis.

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