Regulation of androgen action during establishment of pregnancy

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Abstract

During the establishment of pregnancy, the ovarian-derived hormones progesterone and oestradiol regulate remodelling of the endometrium to promote an environment that is able to support and maintain a successful pregnancy. Decidualisation is characterised by differentiation of endometrial stromal cells that secrete growth factors and cytokines that regulate vascular remodelling and immune cell influx. This differentiation process is critical for reproduction, and inadequate decidualisation is implicated in the aetiology of pregnancy disorders such as foetal growth restriction and preeclampsia. In contrast to progesterone and oestradiol, the role of androgens in regulating endometrial function is poorly understood. Androgen receptors are expressed in the endometrium, and androgens are reported to regulate both the transcriptome and the secretome of endometrial stromal cells. In androgen-target tissues, circulating precursors are activated to mediate local effects, and recent studies report that steroid concentrations detected in endometrial tissue are distinct to those detected in the peripheral circulation. New evidence suggests that decidualisation results in dynamic changes in the expression of androgen biosynthetic enzymes, highlighting a role for pre-receptor regulation of androgen action during the establishment of pregnancy. These results suggest that such enzymes could be future therapeutic targets for the treatment of infertility associated with endometrial dysfunction. In conclusion, these data support the hypothesis that androgens play a beneficial role in regulating the establishment and maintenance of pregnancy. Future studies should be focussed on investigating the safety and efficacy of androgen supplementation with the potential for utilisation of novel therapeutics, such as selective androgen receptor modulators, to improve reproductive outcomes in women.

Key Words

- ▶ androgens
- ▶ decidualisation
- ▶ pregnancy
- ▶ fertility
- ▶ intracrine

Journal of Molecular Endocrinology
(2016) **57**, R35–R47

Introduction

The human endometrium is a complex multicellular tissue that undergoes cyclical remodelling in response to fluctuating levels of sex steroid hormones produced by the ovaries (Critchley & Saunders 2009). The endometrium has epithelial cells on its luminal surface with a multicellular stroma containing fibroblasts, blood vessels (lined with endothelial cells and surrounded by vascular smooth muscle cells), a dynamic immune cell population (including uterine natural killer (uNK) cells and macrophages) as well as glands bounded by a secretory epithelium (King 2000). Women of

reproductive age have high concentrations of circulating hormones derived predominantly from the ovaries and adrenal glands (Abraham 1974). Although concentrations of adrenal hormones are relatively constant, the growth of ovarian follicles and formation of the corpus luteum after ovulation result in fluctuations in the concentrations of ovarian-derived sex steroid hormones across the menstrual cycle. The post-ovulatory rise in progesterone promotes remodelling of the endometrial tissue characterised by decidualisation of endometrial stromal cells (ESCs),

vascular remodelling and progression to a limited state of receptivity that permits implantation. The regulation of these processes is highly coordinated, both temporally and spatially, within the endometrium. In addition to the established roles of ovarian-derived progesterone and oestradiol, recent evidence supports a role for locally produced intrauterine sex steroids in the regulation of decidualisation, endometrial remodelling and endometrial receptivity. A critical component of this is the regulation of the bioavailability and action of local androgens.

The role of androgen signalling in the regulation of endometrial function

Androgens in female physiology

In women, circulating androgens are synthesised predominantly from the ovaries and the adrenal glands, as well as from conversion in peripheral tissues. Circulating concentrations of androgens remain relatively constant throughout the menstrual cycle, exceeding those of oestrogens (Burger 2002, Rothman *et al.* 2011). The adrenal gland is responsible for secretion of androgen precursors such as dehydroepiandrosterone (DHEA) and its sulphate (DHEAS) as well as the weak androgen androstenedione (A4) and the active androgen testosterone. The ovary produces androgens throughout the menstrual cycle, with peak circulating concentrations of A4 and testosterone detected midcycle coincident with follicular development. Circulating concentrations of the potent endogenous androgen dihydrotestosterone (DHT) are low, but this is largely due to local metabolism within target tissues at the site of action. Sensitive liquid chromatography tandem mass-spectrometry assays have recently been used to detect intratissue androgen concentrations in human endometrium. Initial studies suggest that steroid concentrations in the endometrium are distinct from those in the circulation and may be dysregulated in endometrial pathologies such as endometriosis (Huhtinen *et al.* 2012, 2014). These new data highlight the capacity for local steroid biosynthesis, metabolism and action within the endometrium, which may impact on endometrial function in health and disease.

AR-dependent signalling and AR expression in the endometrium

Androgens mediate their actions by binding to the cognate androgen receptor (AR). AR, a member of the nuclear receptor superfamily, is encoded by a single gene

on the X chromosome (Kuiper *et al.* 1989). The spatial organisation of the gene corresponds to the following protein domains: an N-terminal domain (exon 1) with roles in transactivation and interaction with co-factors, a DNA-binding domain responsible for sequence specificity of DNA binding (exons 2–3), a hinge region for nuclear localisation (exon 4) and a C-terminal ligand-binding domain (exons 5–8) that binds steroidal and synthetic ligands (for a review on the roles of AR domains, see Claessens *et al.* (2008)). Upon ligand binding, AR undergoes conformational changes that result in receptor dimerisation and translocation to the nucleus followed by binding to response elements on enhancers and promoters of target genes and recruitment of co-factors leading to changes in gene expression.

Detection of AR is reported in several female tissues, including the ovaries, adipose, skin, brain, muscle and uterus (Kimura *et al.* 1993). In the cycling human endometrium, AR is expressed predominantly in stromal fibroblasts of the basal and functional layers during the oestrogen-dominated proliferative phase, with receptor expression reduced during the progesterone-dominated secretory phase of the menstrual cycle (Mertens *et al.* 2001, Marshall *et al.* 2011). Although AR is expressed at low levels in endometrial epithelial cells during the secretory phase, the endometrium of women administered with the anti-progestin mifepristone exhibits an elevated expression of AR in epithelial and stromal cells (Slayden *et al.* 2001, Narvekar *et al.* 2004). Notably, analysis of full-thickness uterine biopsies demonstrates that AR immunoexpression in endometrial epithelial cells is increased following progesterone withdrawal at the time of menses, consistent with role for progestins in regulating the expression of AR (Marshall *et al.* 2011). Following the establishment of pregnancy, immunohistochemistry studies have reported that AR is detected in decidual stromal cells and in endothelial cells lining endometrial arteries in first-trimester decidua (Horie *et al.* 1992, Milne *et al.* 2005, Critchley & Saunders 2009).

These findings demonstrate the capacity of the endometrium to function as an androgen-target organ. Furthermore, recent evidence suggests a role for local synthesis of androgens and signalling via intracrine mechanisms within the endometrium (Gibson *et al.* 2016). Importantly, androgens can also regulate the endometrial function by acting as precursors to oestrogens, which promote spatio-temporal changes within the tissue. Thus, as androgens can act both directly and indirectly to regulate the endometrial function, the expression and activity of androgen-metabolising enzymes within the endometrium

Figure 1

Androgen:oestrogen balance and regulation of the endometrial microenvironment. Decidualisation is associated with changes in local metabolism and synthesis of bioactive androgens, which may be affected by changes in bioavailability of circulating steroid precursors. (A) Increased expression and activity of AKR1C3 early in decidualisation is associated with biosynthesis of testosterone, which is converted to potent androgen DHT by the action of SRD5A1 (Gibson *et al.* 2016). Testosterone and DHT produced by ESCs during decidualisation signal via an intracrine mechanism to regulate the early decidual response and impact on endometrial transcription of decidualisation and endometrial receptivity factors. (B) Local androgens promote time-sensitive changes in gene transcription during endometrial remodelling, including expression of the decidualisation marker IGFBP1 and the receptivity marker SPP1 (Gibson *et al.* 2016). Insufficient androgen signalling at this time may promote a transcriptional profile that is 'out of phase', which could impact on endometrial receptivity, blastocyst implantation and the establishment of pregnancy. (C) Regulation of testosterone bioavailability may also impact on the regulation of the endometrium by acting as a precursor to oestrogens. Following induction of aromatase expression in decidualised ESCs (Gibson *et al.* 2013), local oestrogens regulate immune-mediated vascular remodelling by altering the function of uNK cells in early pregnancy (Gibson *et al.* 2015).

may represent a key mechanism for controlling steroid bioavailability within the tissue and regulation of endometrial functions (summarised in Fig. 1).

Androgen biosynthesis and expression of steroid-metabolising enzymes in the endometrium

In androgen-target tissues, peripheral precursors are activated within cells that express the enzymes required for the biosynthesis of androgens. This phenomenon, termed intracrinology, is critical for mediating local responses within tissues without inducing body-wide effects (Labrie 1991). We have recently discovered evidence that supports a role for active steroidogenesis within the endometrium, which significantly extends that in previous reports (summarised in Table 1).

Steroidogenesis involves the formation of active sex steroids via the sequential enzymatic conversion of steroid precursors (for a comprehensive review of steroidogenesis,

see Payne & Hales 2004). Analysis of endometrial tissue homogenates and cells suggests that the endometrium has the capacity for steroidogenesis. Expression of the key steroidogenic enzymes, steroidogenic acute regulatory protein (STAR), cytochrome P450 family 11 subfamily A member 1 (CYP11A1) and cytochrome P450 family 17 subfamily A member 1 (CYP17A1) that convert cholesterol to the common sex steroid hormone precursor DHEA have been reported in the endometrium (Aghajanova *et al.* 2009, Chen *et al.* 2011, Huhtinen *et al.* 2014). STAR mRNA is reported to be expressed at low levels in ESCs but not in epithelial cells (Hukkanen *et al.* 1998, Rhee *et al.* 2003, Aghajanova *et al.* 2009). However, STAR protein levels in total endometrial tissue homogenates detected by western blotting analysis were reported to be low to undetectable (Tsai *et al.* 2001, Aghajanova *et al.* 2009, Attar *et al.* 2009), and to our knowledge, there are no studies reporting CYP11A1 or CYP17A1 protein expression or enzymatic activity in the endometrium.

Table 1 Expression of steroid biosynthesis enzymes within human endometrium.

	Protein		mRNA	References
	Stromal cells	Epithelial cells	Tissue homogenates	
<i>STAR</i>	+	–	+	Tsai et al. (2001)
<i>CYP11A1</i>	+	+	+	Huhtinen et al. (2014) , Hukkanen et al. (1998)
<i>CYP17A1</i>	ND	ND	+	Aghajanova et al. (2009) , Huhtinen et al. (2014)
<i>HSD3B</i>	Protein detected in isolated ESCs	Weak in the proliferative phase and moderate in the secretory phase	+	Rhee et al. (2003) , Gibson et al. (2013)
<i>HSD17B1</i>	+	+	+	Dassen et al. (2007) , Huhtinen et al. (2012)
<i>HSD17B2</i>	Highest in the secretory phase	Highest in the secretory phase, stronger than stromal	+	Casey et al. (1994) , Dassen et al. (2007) , Huhtinen et al. (2012)
<i>AKR1C3</i>	Induced upon decidualisation	Highest in the early secretory phase	+	Catalano et al. (2011) , Hevir et al. (2011) , Gibson et al. (2016)
<i>CYP19A1</i>	Induced upon decidualisation	Low/absent	+	Bulun et al. (1993) , Dassen et al. (2007) , Aghajanova et al. (2009) , Gibson et al. (2013) , Huhtinen et al. (2014)
<i>SRD5A1</i>	+	All phases of menstrual cycle	+	Carneiro et al. (2008) , Ito et al. (2002) , Huhtinen et al. (2014) , Gibson et al. (2016)

Summary of expression of steroid biosynthesis enzymes within the normal endometrium. For abbreviations, see text. Protein detection as reported by immunohistochemistry or western immunoblot. ND, not described.

Rhee and coworkers reported the expression of *HSD3B* mRNA in the human endometrial tissue homogenates and described 3BHSD protein expression in total endometrial tissue lysates ([Rhee et al. 2003](#)). In the same study, glandular epithelial cells immunopositive for 3BHSD were detected in proliferative and secretory phase endometrial samples ([Rhee et al. 2003](#)). Immunoexpression was reported to be strongest in the first-trimester decidua and the decidua of ectopic pregnancy consistent with a potential role for this enzyme in decidual steroidogenesis and regulation of early pregnancy. In agreement with these findings, we have detected 3BHSD in isolated proliferative-phase human ESCs by Western blot and immunofluorescence, although expression appears to be constitutive irrespective of whether ESCs were decidualised or not ([Gibson et al. 2013](#)). These expression studies are

consistent with a capacity for local activation of steroid precursors within the endometrium.

Although expression of many isozymes of the 17BHSD enzyme family has been described in the endometrium, aldo-keto reductase family 1 member C3 (*AKR1C3*; also known as 17BHSD5) is the most efficient enzyme for the conversion of A4 to testosterone (reviewed in ([Rizner & Penning 2014](#))). Expression of *AKR1C3* in the endometrium was described by Catalano and coworkers who reported peak mRNA expression in endometrial tissue homogenates from the early-secretory phase at a time when early stages of post-ovulatory remodelling and initiation of decidualisation are occurring in the endometrium ([Catalano et al. 2011](#)). The same study reported that *AKR1C3* was immunolocalised to glandular and luminal epithelial cells throughout the menstrual cycle ([Catalano et al. 2011](#)). Reports of

protein expression in the endometrial stroma have been contradictory, with expression limited to glandular and luminal epithelium as reported by Catalano and coworkers (Catalano *et al.* 2011); however, Hevir and coworkers demonstrated immunopositive staining in both stromal and epithelial cells (Hevir *et al.* 2011). Our own studies showed conclusively that AKR1C3 mRNA and protein are both detectable in isolated human ESCs, and decidualisation results in a marked increase in AKR1C3 expression consistent with peak expression reported in the early-secretory-phase endometrial tissue homogenates (Gibson *et al.* 2016). Interestingly, the mRNA concentration of the oxidative 17BHS isozyme *HSD17B2* (which inactivates testosterone to A4) is reported to be the highest in the endometrial glandular epithelium during the mid-to-late secretory phase, which suggests that active androgens are only bioavailable over a defined window during the secretory phase in the endometrium (Casey *et al.* 1994). Assessment of androgen biosynthesis during decidualisation of ESCs demonstrated a significant secretion of testosterone detected in cell culture supernatants consistent with a potential role for activation of androgens at this time (Gibson *et al.* 2016).

A4 is a weak androgen with low affinity for AR, and although testosterone is a potent AR agonist, either androgen can be converted to oestrogens by the action of the enzyme aromatase. Thus, aromatisable androgens can exhibit dual functionality in controlling androgen:oestrogen balance within the endometrium by acting as activators of AR and/or as precursors to oestrogens. Although some studies have previously reported that aromatase is not detected in the normal cycling endometrium (Labrie 2015), we have demonstrated conclusively that aromatase can be temporally and spatially regulated during decidualisation (Gibson *et al.* 2013). Specifically, we have shown that mRNA and protein expression of aromatase is significantly and uniquely upregulated in isolated primary human ESCs resulting in increased aromatase activity and biosynthesis of oestrone and oestradiol (Gibson *et al.* 2013). Interestingly, Bukulmez and coworkers reported that A4 upregulates aromatase mRNA expression in human ESC and endometrial tissue explants (Bukulmez *et al.* 2008), underlining the importance of androgens in modulating the steroid microenvironment in the endometrium.

Importantly, testosterone can be also be metabolised to the potent androgen DHT by the enzyme 5 α -reductase. Notably, DHT exhibits greater affinity for AR than testosterone and cannot be directly converted to oestrogens (Askew *et al.* 2007). Reports of 5 α -reductase protein

expression in the endometrium have been contradictory; immunohistochemistry studies suggest that expression is limited to epithelial cells (Ito *et al.* 2002), whereas others have reported immunopositive staining in both stromal and epithelial cells (Carneiro *et al.* 2008). Metabolism studies using radiolabelled testosterone demonstrated that testosterone can be converted to DHT in human endometrial tissue homogenates (Rose *et al.* 1978). We have recently investigated the expression and activity of 5 α -reductase in human ESCs. In this study, we found that 5 α -reductase was immunolocalised to isolated ESCs and can be detectable in cell lysates by Western blot (Gibson *et al.* 2016). Notably, although 5 α -reductase protein expression was detected in both undecidualised and decidualised stromal cells, 5 α -reductase protein expression decreased in a time-dependent manner in decidualised ESCs (Gibson *et al.* 2016). In the same study, secretion of DHT was only detected in ESCs that had been stimulated to decidualise; however, the amount of DHT released by the cells declined over time concomitant with decreased 5 α -reductase protein (Gibson *et al.* 2016).

These data suggest an extensive capacity for androgen metabolism in the human endometrium, which has received very little attention to date. The endometrium expresses the enzymes required for biosynthesis of androgens and has a capacity for both biosynthesis of testosterone and DHT and metabolic activation of androgen precursors. Notably, activation of androgens appears to be temporally and spatially regulated, and the evidence supports a role for ESCs during the early-secretory-phase producing active androgens within the tissue. Taken together, these studies highlight that during preparation for pregnancy, steroid metabolism in the endometrium promotes a unique hormonal microenvironment, distinct from that of the circulation, which regulates development of a receptive environment for implantation.

Impact of androgens on endometrial function

Proliferation

Androgens are reported to have an anti-proliferative effect in the human endometrium. In female-to-male transsexuals, testosterone administration was associated with a reduction in the proliferation indices of glands and stroma, accompanied by endometrial histology similar to that of the atrophic menopausal endometrium (Perrone *et al.* 2009) and increased immunostaining of AR in the stroma (Chadha *et al.* 1994). *In silico* analysis

and validation of putative androgen target genes in human proliferative-phase ESCs identified that androgens regulate cellular proliferation and motility in the human endometrium (Marshall *et al.* 2011). Functional studies confirmed that treatment of human primary ESCs *in vitro* with the potent androgen DHT significantly decreased cell proliferation and migration as well as AR-dependent inhibition of staurosporin-induced apoptosis (Marshall *et al.* 2011). Similar effects on proliferation were observed *in vitro* with human primary endometrial epithelial cells treated with the weak androgen A4; A4 induced a dose-dependent decrease in proliferation evident by decreased uptake of ³H-thymidine and MKi67 expression, an effect that was reversed after co-incubation with the AR antagonist cyproterone acetate (Tuckerman *et al.* 2000). However, no similar effect was observed in epithelial cells treated with testosterone or DHT, which may suggest that A4 effects were a result of local metabolism within human endometrial epithelial cells (Tuckerman *et al.* 2000). Anti-progestin administration in women has been shown to exert anti-proliferative effects on the endometrium, and interestingly, this is reported to be accompanied by an elevated expression of AR in the stroma and the epithelium (Slayden *et al.* 2001, Narvekar *et al.* 2004). As treatment with the anti-androgen flutamide reverses the anti-proliferative effect of anti-progestins in the primate endometrium, it has been suggested that the effects of anti-progestins on proliferation may be mediated via AR action (Slayden & Brenner 2003). *In vivo* mouse models support a role of androgens in regulating endometrial growth via AR-dependent actions. A mouse model expressing a transgenic luciferase reporter gene under the control of an AR-specific response element (ARE) demonstrated AR expression and activity in the uterus, whereas treatment with the antiandrogen bicalutamide resulted in a significant reduction of detected bioluminescence (Dart *et al.* 2013). Several global Ar-knockout mouse models have been generated, targeting different exons of the receptor resulting in either partial or complete loss of the AR protein (Yeh *et al.* 2002, Shiina *et al.* 2006, Walters *et al.* 2007, 2009). However, in global Ar knockout mice, deficits in ovarian function and subfertility phenotypes due to defective folliculogenesis or neuroendocrine defects have precluded extensive insights into the role of AR in uterine function. Notably, Ar^{-/-} mice display abnormal uterine growth as evident by a significant reduction in uterine and endometrial surface area at dioestrus and oestrus compared with wild-type (WT) mouse uteri (Walters *et al.* 2009). In addition, reciprocal ovarian transplantation experiments between

WT and Ar^{-/-} mice showed that ovariectomised Ar^{-/-} hosts implanted with a WT ovary exhibited more pronounced defects in uterine growth than WT hosts with Ar^{-/-} ovaries, suggesting a direct role of intrauterine AR action in regulating uterine growth (Walters *et al.* 2009). Cell-selective ablation of AR has provided further evidence that AR-dependent signalling regulates uterine growth as ovariectomised female mice with targeted Ar-knockout in the uterine glandular epithelium demonstrate only partial restoration of uterine size after following treatment with testosterone (Choi *et al.* 2015).

Decidualisation

Although androgens are known to regulate many processes in female physiology, the potential role(s) in the regulation of the establishment of pregnancy is poorly understood. Decidualisation is the differentiation and proliferation of ESC that is initiated in the secretory phase of the menstrual cycle in response to rising levels of progesterone (reviewed in (Gellersen & Brosens 2014)). Decidualisation is accompanied by increased angiogenesis and leukocyte infiltration (Plaisier 2011) and is unique to species that undergo haemochorial placentation (Vogiagis & Salamonsen 1999). Decidualisation is characterised by the coordinated expression of a specific sets of genes, including those encoding growth factors such as prolactin (PRL) and IGF-binding protein 1 (IGFBP1) and is characterised by the morphological transformation of fibroblastic stromal cells into 'epithelioid' decidual cells during the late secretory phase (Dunn *et al.* 2003). Decidualised stromal cells are much larger than stromal fibroblasts with rounded nuclei, increased numbers of nucleoli, an expanded secretory apparatus, dilated rough endoplasmic reticulum, and Golgi apparatus and prominent cytoplasmic accumulation of glycogen (Cornillie *et al.* 1985). In humans, unlike in mice, decidualisation occurs spontaneously in each menstrual cycle and precedes implantation. Transcriptional changes that occur as a result of decidualisation may therefore also impact on endometrial receptivity and implantation. Decidual cells persist in pregnancy and form the maternal component of the placenta, the decidua basalis (Moffett & Loke 2006). Embryo implantation, placentation and establishment of pregnancy are dependent on adequate decidualisation. Consequently, failure of decidualisation is associated with sub/infertility and implicated in the aetiology of pregnancy disorders, including recurrent pregnancy loss, preeclampsia and foetal growth restriction (Robb *et al.* 1998, Karpovich *et al.* 2005).

Exogenous addition of androgens has been shown to impact on the decidualisation of ESCs. Secretion of the decidualisation marker PRL is significantly increased in human ESCs treated with testosterone or DHT during *in vitro* decidualisation compared with control treatment, with androgens reported to increase PRL secretion in both a time- and a dose-dependent manner (Narukawa *et al.* 1994). Both the progesterone receptor (PR) and AR have partially overlapping functions by being able to bind the same response elements and regulate subsets of the same genes (Yen *et al.* 1997). *In vitro* decidualisation of primary human ESCs with targeted siRNA knockdown of either PR or AR identified a distinct set of genes regulated only by AR involved in cytoskeletal organisation, cell motility and cell cycle regulation consistent with gene sets identified by *in silico* analysis (Cloke *et al.* 2008, Marshall *et al.* 2011). AR-dependent signalling is reported to regulate the F-actin polymerisation and stress fibre formation, necessary for the acquisition of the motile phenotype of decidual cells (Cloke *et al.* 2008). Furthermore, DHT is reported to increase resistance to oxidative stress in a dose-dependent manner during *in vitro* decidualisation of ESCs through regulation of superoxide dismutase 2 (SOD2) expression, which was associated with decreased hydrogen peroxide-stimulated apoptosis (Kajihara *et al.* 2012). Regulation of mitochondrial SOD2 expression may in part be controlled by forkhead box protein O1 (FOXO1), an upstream transcription factor reported to be upregulated in ESCs decidualised in the presence of DHT (Kajihara *et al.* 2012). In the decidualised endometrium, cells form gap junctions for the exchange of small molecules, and disruption of gap junction formation severely impacts on decidualisation and pregnancy outcomes (Laws *et al.* 2008, Yu *et al.* 2011). Kajihara and coworkers reported that treatment with DHT increases connexin-43 protein expression, a structural component of gap junctions, in decidualised human ESCs, which was accompanied by alteration of cellular ultrastructural features, including expansion of intracellular organelles and lipid droplet accumulation, all common features of decidualised cells (Kajihara *et al.* 2014). Consistent with the roles for exogenous androgens described in human ESCs, in a murine decidualisation model, mice induced to decidualise in the presence of either testosterone or DHT exhibit enhanced responses to decidualisation characterised by increasing deciduoma weight and induction of alkaline phosphatase activity, a marker of decidualisation (Zhang & Croy 1996). Notably, these effects were reported to be reversed by co-treatment with the AR antagonist hydroxyflutamide consistent with an AR-dependent impact on decidualisation (Zhang & Croy

1996). Thus, evidence from studies directly manipulating AR expression or activity from the addition of exogenous androgens in human and rodent models has demonstrated a role for androgens in the regulation of decidualisation. The recent description of local pro-androgenic microenvironment that is formed during decidualisation of ESC suggests that intracrine androgens could be the important physiological regulators of decidualisation. In human ESCs, *in vitro* decidualisation is associated with time-dependent changes in the expression and activity of AKR1C3 and SRD5A1 together with active biosynthesis of the androgens testosterone and DHT (Gibson *et al.* 2016). Blocking intracrine androgen signalling at the receptor level induced significant changes in the transcriptional profile of ESCs and led to a significant decrease in the expression of key markers of decidualisation PRL and IGFBP1 (Gibson *et al.* 2016) consistent with an essential role for intracrine androgen signalling during decidualisation.

Implantation

Establishment of pregnancy involves the attachment and implantation of a competent blastocyst into a receptive endometrium which provides physical and nutritional support during pregnancy (Psychoyos 1973, Aplin 1996). The uterine epithelium is normally hostile to the blastocyst and is required to undergo transition to a receptive state in order for implantation to occur. Uterine receptivity occurs over a restricted period of time during each normal cycle and requires strict spatial and temporal regulation of gene transcription (Koot *et al.* 2012, Macklon & Brosns 2014). The receptive phase lasts approximately 4 days in women usually falling on days 20–24 of a standard 28-day cycle (Bergh & Navot 1992). Following the receptive phase and in the absence of implantation, the uterine epithelium enters a refractory phase and implantation can no longer occur. Transcriptional profiling of endometrial biopsies from across the menstrual cycle and across the proposed window of implantation has identified distinct gene expression patterns associated with different phases of the menstrual cycle and various endometrial pathologies (reviewed in Garrido-Gomez *et al.* 2013). These studies have been used to generate so-called endometrial receptivity arrays (ERAs) that classify endometrial receptivity according to transcriptomic signature. Reported use of ERAs has included directing personalised embryo transfer (pET) in women with repeated implantation failure (Ruiz-Alonso *et al.* 2013). Notably, in a pilot study of 17 patients, a greater proportion of clinical and ongoing pregnancies was reported in women following

pET compared with routine embryo transfer (Ruiz-Alonso *et al.* 2014). Interestingly, ERA assessment in this pilot study demonstrated that the majority of endometrial biopsies had a transcriptional profile that was assessed as 'pre-receptive' (Ruiz-Alonso *et al.* 2014). These data indicate that the endometrial profile of these women may be 'out of phase', suggestive of delayed maturation. Although these studies suggest that pET can improve implantation rates in women with repeated implantation failure, timing of implantation itself also has a major impact on the likely success of the pregnancy and the future health of offspring. The majority of pregnancies occur between days 22 and 24 of the menstrual cycle, and delayed implantation is associated with increased rates of early pregnancy loss (Mahendru *et al.* 2012, Jukic *et al.* 2013, Macklon & Brosens 2014). Understanding the mechanisms that may promote endometrial maturation may provide new ways of improving implantation rates in natural as well as assisted reproductive cycles.

Implantation depends on sequential actions of the ovarian sex steroid hormones oestrogen and progesterone. Although progesterone drives priming of the uterus, evidence from studies in rodents suggests that it is oestrogen that is essential for implantation. Oestrogen acting via oestrogen receptor alpha (ER α (ESR1)) transforms the progesterone-primed uterus into a receptive state, and in mice, the uterine oestrogen metabolite catecholesterone is reported to activate the blastocyst in a paracrine manner (Paria *et al.* 1998). In mice, bioavailability of oestrogens is a critical determinant of endometrial receptivity; higher levels of oestrogen are associated with aberrant expression of genes normally conducive to implantation, whereas suboptimal levels do not promote progression from the pre-receptive state (Ma *et al.* 2003). Furthermore, decidualisation is regulated by local *de novo* oestrogen biosynthesis in mouse uterus. In their study, Das and coworkers demonstrated that inhibiting the action of the enzyme aromatase, which converts androgens to oestrogens, blocks decidualisation and neovascularisation in a model of induced decidualisation (Das *et al.* 2009). Interestingly, recent studies using isolated human endometrial cells indicate that oestrogens are essential for the regulation of immune cell function and vascular remodelling in early pregnancy (Gibson *et al.* 2015) but appear to be dispensable for regulation of decidualisation in human ESCs (D A Gibson and P T K Saunders, unpublished observations). Notably, as androgens are precursors to oestrogens, the reported effects of aromatase inhibitors in mouse models could be partly attributed to

changes in intrauterine androgen:oestrogen balance. For example, in the model reported by Das and coworkers, the decidualised mouse uterus was reported to express the steroidogenic enzymes necessary for the formation of testosterone (*Star*, *Cyp11a1*, *Cyp17a1*, *Hsd3b*, *Hsd17b1*) (Das *et al.* 2009). Furthermore, 5 α -reductase activity, as measured by the formation of DHT from radiolabelled testosterone, is significantly increased in the gravid uterine horn of pregnant mice compared with the non-gravid horn (Minjarez *et al.* 2001). In support of a role for androgens in regulation of establishment of pregnancy, it has been reported that androgens can have a direct impact on implantation in mice. Insufficient androgens may delay embryo implantation, whereas excess androgens lead to an aberrant gene expression in implantation sites in a mouse delayed-implantation model (Diao *et al.* 2008). Similar mechanisms have only recently been explored in human cell systems. For example, we have recently reported that intrauterine androgens promote progesterone-driven decidualisation and regulate the expression of endometrial receptivity markers in human ESCs. Locally produced androgens regulate the expression of secreted phosphoprotein 1 (SPP1), endothelin receptor type B (EDNRB) and monoamine oxidase A (MAOA) (Gibson *et al.* 2016). SPP1 and EDNRB were significantly reduced when local androgen action was inhibited by the antiandrogen flutamide consistent with a role for androgens in regulating endometrial receptivity in women.

Clinical implications

Taken together, the data describing the potential role for local androgen signalling in the endometrium suggest that altering the bioavailability of androgen precursors could impact on endometrial function. Notably, adrenal androgen production declines with age, and circulating concentrations of DHEA at age 40 are half than those detected at age 21 (Zumoff *et al.* 1995). An age-related decline in fertility has often been attributed solely to a decline in ovarian function extrapolating from data on conditions such as premature ovarian failure (POF) or diminished ovarian reserve (DOR). Hypoandrogenism is reported to be associated with DOR and lower pregnancy rates (Gleicher *et al.* 2013a), and thus, treatment strategies have often focussed on supplementing androgens to improve the ovarian function. The apparent decline in ovarian function is also associated with endometrial senescence, and although this was previously considered secondary to the ovarian function, new data are emerging,

highlighting the role of the endometrium itself. Notably, the midcycle peak in androgens is absent in women of advanced reproductive age, and transfer of fertilised oocytes donated by young fertile women to older recipients (>40 years) is associated with reduced implantation rates and higher miscarriage rates than recipients less than 40 years of age (Mushayandebvu *et al.* 1996, Gupta *et al.* 2012).

Implantation requires a coordinated regulation of complex hormone-, immune- and growth factor-dependent signalling processes, and despite advances in *in vitro* fertilisation (IVF), endometrial receptivity and the subsequent embryo implantation remain the rate-limiting steps. Changes in the availability of circulating androgen precursors may impact on the regulation of implantation by altering intracrine signalling within the endometrium. Supplementation with androgens or androgen precursors has been proposed as a treatment to improve the ovarian function and thus implantation rates. However, there has been a lack of robust clinical evidence to support these approaches, and a number of conflicting studies have been reported. Barad and coworkers reported that in 89 patients with DOR, DHEA supplementation significantly improved clinical pregnancy rates compared with controls, concluding that DHEA supplementation was beneficial in women with DOR (Barad *et al.* 2007). However, Weissman and coworkers reported that the number of retrieved and fertilised oocytes in women with DOR was similar in women who had or who had not received DHEA supplementation (Weissman *et al.* 2011). A recent randomised controlled trial (RCT) that investigated the impact of DHEA supplementation in women with poor ovarian reserve undergoing either IVF or intracytoplasmic sperm injection (ICSI) found that pregnancy rates were higher in control groups than in DHEA-treated women (Kara *et al.* 2014). Notably, despite DHEA supplementation being intended to support the ovarian function in each of these trials, it did not appear to improve the ovarian function measured by either the ovulation rate or the number of fertilised embryos produced. In fact, in the Barad study, the DHEA treatment group produced fewer oocytes, fewer normal day 3 embryos and fewer transferred embryos than the control group, although this is possibly reflected in the greater proportion of DOR in the DHEA group (Barad *et al.* 2007). Remarkably, almost half of the pregnancies reported in the DHEA group occurred before IVF, i.e. occurred spontaneously, and the DHEA group had a lower rate of miscarriage (Barad *et al.* 2007). Thus, although not reported by the authors, the positive impacts of DHEA on pregnancy rates described in this study may be due to an impact of DHEA on the endometrium. An

important consideration, highlighted by Prizant and coworkers in a recent review, is that not all women respond equally well to DHEA supplementation due to inadequate conversion of DHEA to testosterone (Prizant *et al.* 2014). Thus, 'non-responders' may not benefit from DHEA supplementation but require direct supplementation with testosterone; however, whether this would be effective in improving pregnancy outcomes in such patients remains to be determined. An increased capacity to convert supplemented DHEA to testosterone was associated with improved IVF pregnancy rates in a prospective study of patients with DOR who received DHEA supplementation for 6 weeks before IVF (Gleicher *et al.* 2013b) consistent with a role for utilisation and conversion of adrenal precursors in successful pregnancies. Furthermore, a recent meta-analysis of DHEA supplementation in women with DOR undergoing IVF or ICSI assessed evidence from eight studies and concluded that DHEA supplementation improved pregnancy outcomes. DHEA treatment was associated with a significant increase in clinical pregnancy rates (Risk Ratio 2.13); however, there was insufficient power to detect an impact on oocyte retrieval, implantation or miscarriage rates (Li *et al.* 2015). Thus, although it has been suggested that peripheral androgen supplementation may improve reproductive outcomes in women undergoing IVF, further evidence is required to elucidate the mechanisms by which this may impact on the establishment of pregnancy and specifically on endometrial function.

Summary

The human endometrium has the capacity for local sex steroid metabolism and synthesis. The relative expression and activities of steroid-metabolising enzymes can alter the local bioavailability of steroids temporally and spatially within the endometrium and thus regulate the endometrial function through activation of steroid hormone receptors. It is clear from studies in rodents as well as those using human cells/tissues that modulation of endocrine and intracrine androgen signalling affects a number of key functional process in the endometrium, including cell proliferation, apoptosis, resistance to oxidative stress and cell motility. Altering the bioavailability of androgens has an impact on the regulation of establishment of pregnancy through regulation of decidualisation and by directly affecting the expression of endometrial receptivity markers. Furthermore, dysregulation of enzymes that mediate steroid metabolism is an important feature of the pathophysiology of reproductive malignancies

and is associated with fertility- and pregnancy-related disorders such as polycystic ovarian syndrome (PCOS) (Apparao *et al.* 2002), endometriosis (Huhtinen *et al.* 2012) and recurrent pregnancy loss (Okon *et al.* 1998).

Given the recently described roles for locally activated oestrogens (Gibson *et al.* 2013, 2015) and androgens (Gibson *et al.* 2016) in promoting an optimal microenvironment for pregnancy, regulation of androgen:oestrogen balance has emerged as a key pathway that merits further investigation. The importance of intracrine signalling, particularly through AR, in the uterus should also be taken into account when considering the impact(s) of current therapies that target local steroid metabolism, e.g. aromatase inhibitors, on the uterine function. Evidence using androgen supplementation in IVF suggests a potential benefit to pregnancy rates, but more studies are needed to better understand the impact on ovarian and endometrial function. Although androgen therapies have not been well tolerated in women due to virilising side effects, the generation of selective androgen receptor modulators (SARMs) and novel selective modulators of enzymes such as AKR1C3 may provide a more targeted therapeutic approach without unwanted side effects. Furthermore, androgen therapies in the uterus may also benefit from targeted delivery methods, such as intrauterine devices, which may help to minimise off-target effects and provide effective future therapeutics for modulating the uterine function in health and disease. In the future, greater understanding of local androgen signalling within the endometrium could lead to better understanding of fertility and improved procedures for IVF.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

Funding

Studies undertaken in the author's laboratory were supported by MRC Programme Grant G1100356/1 to PTKS.

Acknowledgements

The author thank Ronnie Grant for preparation of figures and members of PTKS group for technical support.

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Received in final form 4 April 2016

Accepted 11 April 2016

Accepted Preprint published online 11 April 2016