Androgens, oestrogens and endometrium: a fine balance between perfection and pathology

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Abstract

The endometrium is a complex multicellular tissue that is exquisitely sensitive to the actions of sex steroids synthesised in the ovary (endocrine system). Recent studies have highlighted a previously under-appreciated role for local (intracrine) metabolism in fine-tuning tissue function in both health and disease. In this review we have focused on the impact of oestrogens and androgens on endometrial function summarising data from studies on normal endometrial physiology and disorders including infertility, endometriosis and cancer. We consider the evidence that expression of enzymes including aromatase, sulphatase and AKR1C3 by endometrial cells plays an important role in tissue function and malfunction and discuss results from studies using drugs targeting intracrine pathways to treat endometrial disorders. We summarise studies exploring the spatial and temporal expression of oestrogen receptors (ERalpha/ESR1, ERbeta/ESR2 and GPER) and their role in mediating the impact of endogenous and synthetic ligands on cross-talk between vascular, immune, epithelial and stromal cells. There is a single androgen receptor gene and androgens play a key role in stromal-epithelial cross-talk, scar-free healing of endometrium during menstruation and regulation of cell proliferation. The development of new receptor-selective drugs (SERMs, SARMs, SARDs) has reinvigorated interest in targeting receptor subtypes in treatment of disorders including endometriosis and endometrial cancer and some show promise as novel therapies. In summary, understanding the mechanisms regulated by sex steroids provides the platform for improved personalised treatment of endometrial disorders as well as novel insights into the impact of steroids on processes such as tissue repair and regeneration.

Introduction

In women, the endometrium is divided into an inner/luminal functional layer (‘functionalis’) and a basal layer (‘basalis’). On its inner (luminal) aspect, columnar epithelial cells form a boundary between the fluid-filled uterine lumen and endometrial tissue containing glands, a well-developed vasculature, stromal mesenchyme (fibroblasts, perivascular cells) and a diverse population of immune cells. Between menarche and menopause, the endometrium responds to fluctuating levels of blood borne ovarian sex-steroid hormones (primarily 17β-oestradiol (E2) and progesterone (P)), with cyclical proliferation and differentiation ready to support a prospective pregnancy. In a non-pregnant cycle, the functional layer is shed during menstruation, but within a few days the luminal surface is healed and tissue integrity restored ready to resume the next cycle (Garry et al. 2009).
While sex-steroid hormones are essential for the maintenance of normal uterine function and fertility, they may also contribute to the development of hormone-dependent endometrial disorders that affect millions of women (Table 1). In this review we have focused on the impact of oestrogens and androgens on the function and malfunction of the endometrium, considering evidence for expression of receptors that can mediate their function as well as enzymes that modulate local bioavailability of steroids. The emergence of new classes of drugs that target receptors or enzymes and offer some potential as novel treatments for endometrial disorders is summarised.

### Oestrogen and androgen receptors and their expression in endometrial tissues

**Overview of changes in tissue function during the menstrual cycle**

Based on evaluation of 8000 endometrial biopsies, Noyes et al. (1975) published a classification of the different stages of the menstrual cycle which is still considered the gold standard for histological staging. Although cycle length can vary between individuals, staging is typically based on an average menstrual cycle of 28 days: menstruation (day 1), proliferative phase (day 4 to 14) and secretory phase (days 16 to 28). Histologically, the functional layer thickens from about 2 mm recorded immediately after the menstrual phase to 14 mm prior to ovulation on day 14. Following ovulation and formation of the corpus luteum (CL), there is a rapid rise in circulating concentrations of P, which stimulates functional transformation of the stromal fibroblasts (decidualisation) resulting in shape change and reprogramming of gene expression leading to secretion of factors that regulate immune cell recruitment and receptivity (see comprehensive review by Gellersen et al. 2007). In the absence of a healthy blastocyst, the regression of the CL results in a rapid decrease in the circulating concentrations of ovarian-derived steroid hormones (progesterone withdrawal) and triggers a cascade of changes in endometrial tissue that results in tissue breakdown, piecemeal shedding and synchronous healing during menstruation (Garry et al. 2009).

### Structural and functional features of oestrogen and androgen receptors: genomic and non-genomic signalling

Changes in expression of oestrogen- and androgen-dependent genes are orchestrated by interaction of their receptors with DNA-binding domains within gene promoters/enhancers as well as non-genomic signalling.

<table>
<thead>
<tr>
<th>Endometrial pathology</th>
<th>Incidence</th>
<th>Features</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Implantation failure, recurrent pregnancy loss</td>
<td>One in six couples have infertile rates of implantation failure difficult to determine other than in IVF, RPL 1–2%</td>
<td>Poor/out-of-phase decidual response. Changes in immune cell cohorts (uNK). Stromal cell senescence with age?</td>
<td>(Quenby et al. 2009, Lucas et al. 2020)</td>
</tr>
<tr>
<td>Heavy menstrual bleeding (HMB)</td>
<td>20–30% of women; may be worse during perimenopause; associated with fibroids</td>
<td>Acute or chronic; FIGO classification of causes (Palm-Coen)</td>
<td>(Whitaker &amp; Critchley 2016)</td>
</tr>
<tr>
<td>Endometriosis</td>
<td>~10% women of reproductive age; may be asymptomatic. 40% of infertile patients may have endometriosis</td>
<td>Three subtypes – aetiology may be different. Neuroinflammation and chronic pain. Changes in peritoneal environment.</td>
<td>(Horne et al. 2017, Horne &amp; Saunders 2019)</td>
</tr>
<tr>
<td>Adenomyosis</td>
<td>~20% in women in gynaecology clinics (higher in older women)</td>
<td>Growth of endometrial fragments within myometrial wall. Myometrial thickening on ultrasound. Association with endometriosis.</td>
<td>(Naftalin et al. 2012)</td>
</tr>
<tr>
<td>Asherman's syndrome</td>
<td>Estimates of incidence vary widely: 3–45% in infertile population?</td>
<td>Adhesions within uterine cavity; risk increased by endometrial ablation/surgery Increase in gland to stroma ratio when compared with proliferative endometrium. Some types may progress to endoCa</td>
<td>(Dreisler &amp; Kjer 2019)</td>
</tr>
<tr>
<td>Endometrial hyperplasia</td>
<td></td>
<td>Risk increased by high BMI and Lynch syndrome. Classifications based on histology or genetics with 'unopposed' oestrogen key risk factor for some subtypes.</td>
<td>(Sanderson et al. 2017, Ryan et al. 2019)</td>
</tr>
<tr>
<td>Endometrial cancer</td>
<td>Fourth most common cancer in UK women; rates rising</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For each pathology, an estimate of incidence, hallmark features and one or two key references are summarised.

**Table 1** Hormone-dependent endometrial pathologies in women.
pathways initiated at the membrane. Steroid receptors contain three key structure-function domains: a variable amino-terminal domain, a highly conserved DNA-binding domain (DBD), and a less conserved carboxyl-terminal ligand binding domain (LBD). Differences in the sequence of amino acids located within a C-terminal ligand binding pocket play a critical role in ligand selectivity (Shiau et al. 1998, Nadal et al. 2017). A linker region situated between the DBD and the LBD functions as a flexible hinge with a nuclear localization signal: the proteins also contain multiple sites for phosphorylation (Lannigan 2003). There are two oestrogen receptors (alpha and beta) encoded by separate genes, ESR1 and ESR2, respectively: the full-length WT proteins they encode (hERα and hERβ1 respectively) bind a range of oestrogenic ligands with high affinity and specificity. Notably, analysis of natural ligand reported that, while 17β-oestradiol (E2) bound both receptors with high and equal affinity, oestrone (E1) had higher affinity for WT ERβ(1) (Zhu et al. 2006). Multiple splice isoforms of both genes have been identified (reviewed in Gibson & Saunders 2012). ERα6 was the first splice variant of human ESR1 described (initially designated hERα-46; Fournier et al. 2000). ESR2 splice variants including ERβ2/bcx and ERβ5 are co-expressed in multiple reproductive tissues and reproductive cancers (Crichtley et al. 2002, Saunders et al. 2002, Shaaban et al. 2008, Collins et al. 2009). In addition to ESR1 and ESR2, a family of closely related genes have been identified as encoding ‘oestrogen receptor related’ proteins (ESRR1, ESRR2, ESRR3) which do not bind directly to E1 or E2 as they lack a proper binding pocket at their C-terminus but which may be activated by co-factors or other lipids (reviewed in Horard & Vanacker 2003, Gibson & Saunders 2012).

There is a single androgen receptor gene (AR) located on the X chromosome. Elegant studies, including those using surface plasmon resonance, have revealed that the long AR N-terminal domain (NTD) is structurally important for receptor-dependent gene expression (Lavery & McEwan 2008) and is a promising drug target (Ponnusamy et al. 2019). Several splice variant isoforms of AR have been identified with particular attention paid to their role in ligand-independent gene activation in advanced prostate cancers (Dehm & Tindall 2011). Expression of AR variants including AR-V7 (exons 1/2/3/CE3) has also been reported in primary breast cancers and breast cancer cell lines (Hickey et al. 2015), but a literature search did not identify any data related to their expression in endometrium or endometrial disorders.

There have been extensive studies on the functional consequences of steroid ligand binding to ERs and AR that have been well-reviewed elsewhere (McKenna et al. 1999, Gronemeyer et al. 2004). Briefly, ligand binding induces a conformational change in the ligand binding domain, dimerization and recruitment of co-regulators that play a critical role in regulating the hormonal response. Ligand-activated receptors bind directly to DNA sequences within regulatory regions of genes: sequences that are recognised by oestrogen (ERE – oestrogen response elements) or androgen (ARE – androgen response elements) receptors have been described (Brodie & McEwan 2005, Carroll et al. 2006). Binding studies have also identified a number of so called ‘pioneer’ factors such as FOXA1 and GATA2 that can enhance direct binding of ER or AR to DNA (Carroll et al. 2005, He et al. 2014). ERs also regulate gene expression through protein-protein interactions with other transcription factors already bound on DNA (‘tethering’) – examples of tethering mechanisms include binding to the transcription factor Sp1 which has been implicated in regulation of the progesterone receptor gene (Petz et al. 2004) and ERβ-dependent induction of gene expression in human endometrial endothelial cells (Greaves et al. 2013)

Oestrogens and androgens can also induce changes in cell function following binding to ERs or ARs localised in the cell membrane. These ‘non-genomic’ signalling cascades can be initiated through the membrane localization of the classical receptors following palmitoylation and interaction with scaffolding proteins or by hormone-responsive G protein-coupled transmembrane receptors (GPCRs) (Hammes & Levin 2007). One of the most extensively investigated GPCRs is GPER (originally named GPR 30, also known as GPER1), which was cloned from breast cancer cells in 1997 and binds oestrogens with nanomolar affinity (Carmeci et al. 1997). Information on GPCRs that bind to androgens is less comprehensive, but several candidates including GPRC6A have been identified in cancer cells (Ye et al. 2019).

A recent review provided a useful summary of the wide range of different non-genomic signalling pathways and how the different genomic and non-genomic pathways may interact (Wilkenfeld et al. 2018).

Expression and functional impact of oestrogen receptors during the menstrual cycle

We, and others, have used highly specific antibodies to explore temporal and cell-specific patterns of expression of ERα, ERβ, ERRs and AR in endometrium during the normal cycle (Crichtley & Saunders 2009, Young 2013). We have documented cell-specific and temporal

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immunoexpression of full-length ERα (ER66) in both normal endometrium and in endometrial pathologies including cancer (Critchley et al. 2002, Collins et al. 2009). In full-thickness sections of endometrium (Fig. 1), immunoexpression of ERα is intense in the epithelial glands and in the stroma of both the functional and basal layers: endothelial cells lining the blood vessels appear immuno-negative (Critchley et al. 2001). Expression is downregulated in the functional layer during the secretory phase in response to the rising levels of progesterone (Fig. 1) (Lessey et al. 1988, Young 2013). We have recently explored expression of ER46 in the endometrium using a combination of immunohistochemistry and Western blotting (Gibson et al. 2020). Notably, the variant protein was co-localised with ER66 in cell nuclei during the proliferative phase with striking expression in a population of uterine natural killer cells (uNK) implicated in vascular remodelling (Quenby et al. 2009, Gibson et al. 2015).

Studies in mice suggest a complex role for ERα in epithelial and stromal compartments of the endometrium. For example, the role of epithelial ERα was studied using a conditional knockout mouse which was ovariectomised and then treated with a single intraperitoneal injection of 0.25 μg 17β-estradiol (E2) in 100 μL sesame oil. Analysis of samples recovered 2, 24 or 72 h after E2 injection revealed that epithelial ERα was dispensable for the proliferative response observed at 2 h but essential for responses at 24 and 72 h (Winuthayanon et al. 2014). Similar studies also revealed a critical role for ERα in paracrine regulation of stromal decidualization in this species (Pawar et al. 2015). The pattern of expression of ERβ is distinct from that of ERα, with highest concentrations of mRNA encoding full-length ERβ1 in the secretory phase and immunoexpression in epithelial, stromal, endothelial cells and immune cells (Critchley et al. 2001): ERβ1 is not downregulated in the functional layer during the secretory phase (Bomball et al. 2008). Studies in mice with Esr2 knockout have suggested a less striking phenotype than in the Esr1 knockout, although a re-evaluation of the evidence by Hapangama et al. (2015) concluded that sustained E2 stimulation of endometrial epithelial cells via ERβ might induce apoptosis. There has been some disagreement about the cyclical expression (or otherwise) of ERβ in endometrial endothelial cells (Critchley et al. 2001, Lecce et al. 2001). Our own study using endothelial cells from different vascular beds demonstrated those originally isolated from endometrium or myometrium were ERβ+/ERα− and revealed cell-specific impacts of an ERβ-selective agonist on gene expression (Greaves et al. 2013). In contrast, studies using isolated human uNK cells suggest their response to oestrogens may be complex involving rapid membrane-initiated signalling via ER46 (Gibson et al. 2020) and/or binding to ERβ (Gibson et al. 2015). Treatment of isolated uNK cells with either oestrone (E1) or E2 promotes cell migration and secretion of chemokine (C-C motif) ligand 2 (CCL2) (Gibson et al. 2015). These studies highlight the importance of endogenous oestrogens in the dynamic interplay between different endometrial cell types that play a critical role in preparation for pregnancy.

Figure 1
Expression of oestrogen receptor alpha (ERα) and androgen receptor (AR) in full-thickness samples from the human uterus. The tissue is divided into functional and basal layers supported on the myometrium below and bounded on upper surface by the luminal epithelium. ERα (red stain) is abundant in epithelial cells in the proliferative phase but downregulated in the secretory phase. AR (green) is localised to stromal cells in the basal and functional layers during the proliferative phase but only expressed in the basal stromal cells in the secretory phase when its expression is upregulated in epithelial cells. P, proliferative phase; S, secretory phase; M, menstrual phase.
Expression of proteins encoded by human ESR2 splice variant mRNAs (ERβ2, ER65) has been detected in human endometrial cells (Critchley et al. 2002, Collins et al. 2009, 2019). Notably, these variants may also be present in primates (Sierens et al. 2004) but are not expressed in rodents. In vitro studies have demonstrated that the variants can have a functional impact on endometrial cell function by forming heterodimers with full-length isoforms (Collins et al. 2019). Expression of ERRs has also been detected in human endometrium with cell-based studies, highlighting the potential for them to alter cell metabolism or ERα-dependent cell functions (Bombail et al. 2010a,b).

Plante et al. (2012) examined expression of GPER in endometrium using RT-qPCR and immunohistochemistry reporting maximal expression in the proliferative phase. An earlier study by Kolkova et al. (2010) claimed protein expression was less variable than the mRNA and immunostaining was more intense in the epithelial cells than stroma throughout the cycle. GPER may be involved in neoplastic transformation of endometrium (Jacenik et al. 2016) or in promotion of HIF1α-induced expression of MMPs in endometrial stromal cells in women with endometriosis (Zhang et al. 2017). A number of GPER knockout mice have been generated using different targeting strategies: females are fertile with no obvious reproductive defects, although impacts on obesity and vasculature have been claimed (Prossnitz & Hathaway 2015).

Expression and functional impact of androgen receptors during the menstrual cycle

Immunostaining for AR in full-thickness endometrial tissue sections (Fig. 1) (Marshall et al. 2011) detected intense staining in stromal fibroblasts which exhibited cyclical variation in the functional layer but remains unchanged within the basal compartment across the cycle. How this difference in expression within closely adjacent cells is regulated remains unknown. Epithelial cells in the functional layer upregulate expression of AR in response to falling levels of progesterone in a normal cycle or following administration of anti-progestins and this is associated with reduced proliferation (Narvekar et al. 2004, Marshall et al. 2011). We have identified androgen-regulated genes in primary human endometrial stromal cells, several of which (e.g. CITED2, HIF1α, CD44) are implicated in networks that protect cells against stress and apoptosis (Marshall et al. 2011). These data coupled with the observation that AR expression remains unchanged in the stromal cells of basal compartment at time of menses (Garry et al. 2009) prompted us to investigate whether androgens might also play a role in regulating endometrial breakdown and repair using a mouse model that recapitulates key features of menstruation in women (Cousins et al. 2014, 2016a,b). In this model, administration of a single injection of DHT at the time of progesterone withdrawal to induce menstruation had a striking impact on both tissue breakdown and restoration of tissue homeostasis. Although our understanding of the role of androgens in endometrial tissue function is still incomplete, we identified changes in expression of matrix metalloproteinases (MMP3, 9) which are implicated in breakdown of human endometrium (Cousins et al. 2016a).

Expression of enzymes implicated in biosynthesis and metabolism of oestrogens and androgens in endometrial tissue

In recent years there has been a rapid increase in evidence to support a role for local tissue (‘intracrine’) regulation of endometrial steroids (Gibson et al. 2013, 2016a, 2018b). Key findings have included direct measurement of steroids in endometrial tissue homogenates recovered during the menstrual cycle: notably Huhtinen and colleagues reported they did not parallel those in blood (Huhtinen et al. 2012, 2014). In women (but not in mice), the adrenals are an important source of sulphated steroids that circulate at high concentrations in the blood but are unable to bind directly to the steroid receptors. A brief summary of enzymes detected in endometrial tissue and their substrates is provided in Fig. 2 with a few complementary references discussed subsequently. Readers interested in the topic of intracrine steroids are recommended to read the comprehensive review by Konings et al. which includes a systematic search for papers reporting expression of steroidogenic enzymes in pre- and postmenopausal endometrium (Konings et al. 2018).

Briefly, a strong case has been made that the ‘inactive’ adrenal steroid dehydroepiandrosterone (DHEA) is an important precursor of bioactive androgens in women (Labrie et al. 2005), a proposal which has been supported by detection of all the enzymes that regulate conversion of DHEA via intermediates to testosterone, DHT or oestrogens (Gibson et al. 2013, 2016a, 2018c). Catalano and colleagues reported increased expression of AKR1C3 in the early secretory phase (Catalano et al. 2011), consistent with results obtained using an in vitro model of stromal decidualisation (Gibson et al. 2016a).
Inter-conversion of active/inactive oestrogens and androgens is mediated via 17β-hydroxysteroid dehydrogenase isozymes, of which several isoforms are expressed in endometrium. For example, 17βHSD type 1 is responsible for production of testosterone and E2, from A4 and E1, respectively, whereas 17βHSD2 catalyses the opposite reaction. HSD17B2, expressed in glandular epithelial cells, is markedly increased in the secretory phase (Maentausta et al. 1991), and reported overexpression of 17βHSD2 is a feature of endometrium in women with disorders such as endometriosis, adenomyosis, and/or leiomyomas (fibroids) rather than those who are disease-free (Kitawaki et al. 2000).

Expression of steroid sulphatase (STS) in endometrial tissue can catalyse conversion of DHEAS to DHEA (Fig. 2) but can also increase the concentration of E1 by removal of sulphate moieties from E1S. Using an in vitro model of decidualisation, we have confirmed expression of both STS and aromatase (CYP19A1) in endometrial stromal cells with evidence that both enzymes contribute to production of oestrogens during decidualisation (Gibson et al. 2013, 2018a).

Endometrial disorders: altered expression of enzymes and receptors implicated in disease aetiology

Implantation failure and recurrent miscarriage

Timely and efficient decidualisation of endometrial stromal cells in response to ovarian-derived progesterone is essential for the generation of an endometrial microenvironment that can support and nurture the implanting blastocyst. Disruption of decidualization is implicated in implantation failure and miscarriage. Studies in mice using aromatase inhibitors (AI) demonstrated local intra-uterine production of E2 is critical for establishment of pregnancy (Das et al. 2009). In women, E2 is produced during decidualisation of endometrial stromal cells which regulates uNK cell migration (Gibson et al. 2015, 2020). Given the evidence that disturbances in the numbers/location of uNK cells can predispose women to experiencing a miscarriage (Lash et al. 2016), these data are consistent with a role for E2 in regulating the endometrial microenvironment during the establishment of pregnancy.

We have demonstrated that during in vitro decidualisation of primary human endometrial stromal cells there is a significant increase in the expression of AKR1C3, the enzyme responsible for the conversion of androstenedione to testosterone, which is also accompanied by increased secretion of testosterone into the culture medium (Gibson et al. 2016a). In addition, blocking AR action using flutamide during in vitro decidualisation revealed a role for AR-mediated gene expression of osteopontin, a protein implicated in receptivity (Gibson et al. 2016a). Further studies using primary human endometrial stromal cells from women of advanced reproductive age suggested that the age-related decline in adrenal steroids may have an impact on the ability of the endometrium to support a pregnancy and that increased availability of adrenal precursors enhanced androgen production and secretion of decidualisation markers (Gibson et al. 2018c). Intravaginal supplementation with DHEA has shown promising results in alleviating postmenopausal vaginal dryness and atrophy in clinical trials without any adverse effects (Labrie 2019), but delivery into the endometrium of premenopausal women has not been tested. Other studies have reported a positive impact of DHT on stromal cell
decidualisation and resistance to oxidative stress (using hydrogen peroxide) (Kajihara et al. 2012), expanding our understanding of the potentially beneficial role of androgens as direct modulators of endometrial function as well as precursors of oestrogen biosynthesis (see review by Gibson et al. 2016b).

Failure to downregulate ERα during the secretory phase (see Fig. 1 and discussion) has been reported in women with defects in uterine receptivity (Lessey et al. 2006). A complementary study using samples from women with unexplained infertility also showed that in these patients elevated expression of ERα in the mid-luteal phase was associated with reduced expression of glycodegin-A, low levels of which have been implicated in recurrent implantation failure (Dorostghoal et al. 2018). There is no information about dysregulation of ERβ in implantation failure. Fertility problems in women with polycystic ovaries and excess androgens might relate to overstimulation of AR signalling pathways, but currently the evidence is quite limited (Schulte et al. 2015).

Endometrial cancer

The majority of endometrial cancers (EC) present with abnormal endometrial bleeding in postmenopausal women: rates are rising particularly in younger women, with obesity considered a significant contributing factor (Table 1, reviewed by Sanderson et al. 2017). EC are historically classified as type 1 or type 2; type 1 is the most commonly diagnosed form (about 80% of the cases), is considered oestrogen-dependent and characterised by hyperplastic proliferation of the endometrial glands. A large number of studies have investigated the source and impact of oestrogens in endometrial cancer with landmark papers including those by Sasano and collaborators who reported evidence of increased immunoexpression of aromatase, STS and 17βHSD enzymes in both endometrial hyperplasia and EC (Sasano et al. 1996, Utsunomiya et al. 2001, 2004). A recent comprehensive systematic review considered the evidence that intracrine metabolism contributes to EC (Cornel et al. 2019). The authors highlighted the importance of sulphatase and aromatase enzymes in the generation of E1 and E2 within endometrial cancer tissue in promoting a pro-oestrogenic environment favouring proliferation of epithelial cells (Cornel et al. 2019). The authors sounded a note of caution by highlighting the variability between individuals and methodologies which may explain some variations in drug responses (discussed subsequently).

The best evidence for an impact of androgens on EC risk has come from studies in women with polycystic ovarian disease, where the risk of type 1 cancers is higher in women with symptoms of androgen excess such as hirsutism and irregular periods (Fearnley et al. 2010). Tanaka et al. reported DHT was elevated in endometrioid endometrial adenocarcinoma tissues compared with that in normal endometrial tissues (8.0 fold) in a group of 41 patients (Tanaka et al. 2015). These results have been complemented by reports that AKR1C3 (conversion from A4 to testosterone) and 5α-reductase (reduction of testosterone to DHT) are both expressed in EC (Ito et al. 2016, Gibson et al. 2018a).

Expression of ERα, ERβ1 and splice variant isoforms of ERβ (ERβ2, ERβ5) in EC have been documented (Collins et al. 2009, 2019). In a recent paper we highlighted the potential that ERβ5, a variant unable to bind directly to E2, may still influence the response of EC to oestrogens by forming heterodimers with ERα (Collins et al. 2019). High GPER expression is predictive of poor survival in endometrial cancers (Smith et al. 2007). Prossnitz and colleagues have reported interesting results using ERα-negative/GPER-positive cells which suggest activation of downstream signalling in response to SERMs such as tamoxifen may explain why women treated with this drug are at higher risk of EC (Petrie et al. 2013). We, and others, have reported widespread expression of AR in EC (reviewed in Gibson et al. 2014). Evidence that loss of AR is associated with poorer prognosis, reports that AR was elevated in metastases (Kamal et al. 2016), and that androgens may be anti-proliferative in EC cells have raised the prospect that SARMs should be explored for this cancer as well as those of breast (see subsequent section). There are no reports of AR variants being expressed in EC.

Endometriosis

Endometriosis is an oestrogen-dependent neuroinflammatory pain disorder characterised by the presence of ‘lesions’ of endometrial-like tissue in sites outside the uterus (Horne & Saunders 2019). Endometriosis and adenomyosis are often found in the same patient and may share a common aetiology (Yovich et al. 2019). Infertility is a common co-morbidity of endometriosis and differences between expression profiles of miRNAs, mRNA and proteins in endometrial biopsies from controls and women with endometriosis have been reported (Burney et al. 2007, 2009) and have recently been reviewed (Bulun et al. 2019). Notably, there remain differing views as to whether receptivity is or is not affected (Lessey & Kim 2017,
Miravet-Valenciano et al. 2017). Studies comparing the impact of a decidualisation stimulus on isolated endometrial stromal cells have reported alterations in the expression of steroidogenic enzymes in cells from women with endometriosis (Aghajanova et al. 2009). Blunted responses to progesterone, often termed ‘progesterone resistance’, are considered a hallmark of the disorder (Aghajanova et al. 2010, Bulun et al. 2010). Some have questioned whether this property is an innate feature of the eutopic endometrial cells or acquired when they grow in ectopic sites (McKinnon et al. 2018).

Some of the best evidence for the importance of intracrine action of steroids has come from studies comparing concentrations of steroids in lesions and eutopic endometrium in women with endometriosis (Huhtinen et al. 2012, 2014). To complement mass spectrometry data, expression of enzymes in lesions such as aromatase, AKR1C3 and STS has been measured with evidence that their overexpression is responsible for generation of a lesion tissue environment rich in oestrogens that can bind ERs or GPER (Rizner 2009, 2016). Notably, aromatase appears to be involved in local biosynthesis of both E2 and the pro-inflammatory regulator prostaglandin E2 (Attar & Bulun 2006). Upregulation of ERβ is also considered a hallmark of the altered microenvironment of lesions, which may promote the impact of oestrogens on inflammation, angiogenesis or pain pathways (Bulun et al. 2012, Greaves et al. 2014a,b).

Adenomyosis

Adenomyosis is a condition characterised by the presence of heterotopic endometrial glands and stroma within the myometrium and has traditionally been difficult to diagnose as it can present with symptoms such as infertility, pain and heavy menstrual bleeding, which are also characteristics of other conditions, including endometriosis and fibroids (Pontis et al. 2016). Recent advances in imaging offer hope for improved understanding of its presentation and pathogenesis (Chapron et al. 2020). Altered gene expression in the endometrium of women with adenomyosis has been reported, although results have been based on small numbers of samples (Herndon et al. 2016, Xiang et al. 2019). It has been suggested that development of adenomyosis may involve mechanisms activated but not resolved during endometrial tissue injury with a common aetiology to some forms of endometriosis (Donnez et al. 2018, 2019). Studies using tissue recovered from women with adenomyosis have identified increased expression of GPER and some association between GPER polymorphisms with the disease; however, it must be noted that study populations have been small (Li et al. 2017, Hong et al. 2019). In vitro studies have identified pathways promoting E2-induced overproliferation of uterine smooth muscle cells from women with adenomyosis (Sun et al. 2015). Immunostaining of tissue sections from adenomyosis uteri have detected changes in ERα, reduced PR and elevated expression of ERβ (Mehasseb et al. 2011) and aromatase (Barcena de Arellano et al. 2013), all consistent with an oestrogen-dependent disease. In older papers, expression of AR has been reported (Horie et al. 1992).

Drugs targeting sex steroid metabolism

Aromatase inhibitors

An excellent historical summary of the discovery of aromatase, identification of increased expression in quadrants of breast containing a tumour, and the development and refinement of aromatase inhibitors (AIs) has been published by leaders in the field (Santen et al. 2009). The development of highly effective 3rd generation AIs (anastrozole, letrozole, exemestane) led to clinical trials for a number of indications including postmenopausal breast cancer, gynaecomastia in men and ovarian cancer (Miller et al. 2001, Santen et al. 2009, Langdon et al. 2017). One key reproducible finding has been a lower rate of EC and venous thrombosis in women treated with AIs compared with those treated with tamoxifen (Chlebowski et al. 2015). The ClinicalTrials.gov website lists 22 trials with search terms endometrial cancer+aromatase inhibitor with the main focus being on women with more advanced disease. Many trials are not yet completed but evidence of benefit in some ER+ cancers has been reported. For example, in 40 women treated with exemestane, there was remission in 10% and lack of progression after 6 months in 35% of the patients (Lindemann et al. 2014). The PARAGON trial, a phase 2 open label study using anastazole in 82 patients with ER and/or PR positive hormonal therapy naïve metastatic endometrial cancer, reported clinical benefit in 44% of patients (Mileshkin et al. 2019), although results from other trials have been disappointing and may have been influenced by obesity in the target population (van Weelden et al. 2019). Some promising results have been reported following treatment of women with the rarer cancer low grade endometrial sarcoma with AIs (reviewed in Pannier et al. 2019).
Letrozole and anastrozole have also been evaluated in both pre- and postmenopausal women with endometriosis (Pavone & Bulun 2012). These authors propose that AIs appear to be a suitable therapy for endometriosis-associated pain in women who are postmenopausal by targeting the intracrine oestrogen biosynthesis that contributes to sustained symptoms in this age group. Recent advances have included development of vaginal ring delivery systems for co-administration of anastrozole and the androgenic progestin levonorgestrel (LNG) as a potential therapy for endometriosis-associated pain: a phase I trial reported promising findings (Schultze-Mosgau et al. 2016). While these results seem promising, a recent ESHRE guideline that considered whether AIs should be given in combination with contraceptives or other therapies concluded that due to side effects they should only be prescribed to women after all other options for medical or surgical treatment are exhausted (Dunselman et al. 2014). AIs have also been suggested as therapies for adenomyosis but with the caveat that further studies are required (Vannuccini et al. 2018).

**Sulphatase inhibitors**

A number of potent STS inhibitors have been developed with the primary indication being treatment of hormone-dependent cancers (Day et al. 2009, Purohit & Foster 2012). The compound STX64 (Irosustat) was effective in blocking oestrogen synthesis in endometrial cancer cells in vitro and was tested as a therapy for advanced endometrial cancer before being discontinued as a mono-therapy by Ipsen (Pautier et al. 2017). Irosustat has recently been used as an addition to aromatase inhibitors in women with advanced ER+ breast cancer and reported as having a positive clinical impact (Palmieri et al. 2017). Another inhibitor, estradiol-3-O-sulfamate (E2MATE), has been reported which deceased STS activity in human endometrial explants and decreased lesion weight and size but did not alter systemic oestrogens in a mouse model of endometriosis (Colette et al. 2011). E2MATE, under the trade name PGL2001, has been shown to reduce STS activity in endometrium when given once a week for 4 weeks (Pohl et al. 2014); the same drug was used in a trial for treatment of endometriosis-associated pain (NCT01631981) but results have not been reported.

**Hydroxysteroid dehydrogenase inhibitors**

17βHSD1 inhibitors were originally developed to target the biosynthesis of bioactive E2 in hormone-dependent breast cancer (Day et al. 2008). Recently, with evidence for expression of 17βHSD1 in endometriosis lesions, their use has been expanded to treatment of endometriosis with promising results reported (Delvoux et al. 2014). The role of 17βHSD5/AR1C3 in metabolism of steroids and prostaglandins, both of which are implicated in endometriosis-associated pain, make it an attractive target as a novel therapy for this disorder. A number of inhibitors have been developed with the Bayer compound BAY1128688 showing sufficient promise for it to be used in a phase 2 randomised clinical trial to assess efficacy of different doses in 121 women with symptomatic endometriosis. The trial (NCT03373422) was terminated after 8 months due to an increased incidence of liver toxicity highlighting the challenge of developing drugs that may target enzymes present in multiple tissues (van Weelden et al. 2019). In their recent review, Rizner & Penning (2020) concluded that the ‘hepatotoxicity effect was probably compound related which does not preclude AR1C3 as a target’ and that development of other drugs targeting this enzyme alone or in combination with other targets is continuing (Wangtrakuldee et al. 2019).

**Dual/combined targeting**

While initial studies have focused on mono-therapies, a new generation of drugs with dual actions has also been developed – examples include those that target aromatase and STS (DASI, Purohit & Foster 2012) or STS and 17βHSD1. While some in vitro studies seem promising, clinical trials are yet to be completed (reviewed in Potter 2018).

**Drugs targeting oestrogen and androgen receptors and their potential to treat endometrial disorders**

The solving of the crystal structure of nuclear ERs as well as detailed modelling of the impact of ligand binding on conformation, recruitment of co-factors and gene expression laid the foundation for the development of synthetic ligands that exhibit selectivity, tissue-specific agonism, antagonism or induce receptor degradation; a comprehensive perspective and background is provided by Burris et al. (2013). Table 2 summarises the specificities and properties of some of the novel non-steroidal ligands developed to target ERs and AR, a number of which have been investigated in the context of endometrial disorders and are discussed subsequently.
Oestrogen receptors

Agonists and antagonists with selectivity for ERα, ERβ and GPER have been validated using a range of cell based and animal models (Table 2). When Frasor et al. (2003) compared the effect of 4 × daily injections of 4,4′,4″-(4-propyl-1H)-pyrazole-1,3,5-triyl)trisphenol (PPT) or 2,3-bis(4-hydroxyphenyl)-propionitrile (DPN) to immature (d21) female mice, they noted differences in tissue response which they attributed to activation of ERα or ERβ respectively. PPT caused epithelial cell proliferation, increased uterine weight and expression of lactoferrin but decreased Ar mRNA. In contrast, DPN did not increase uterine weight or luminal epithelial cell proliferation but appeared able to reduce stimulation by PPT. These findings are consistent with a large body of work that implicates ERα as the major regulator of oestrogen-dependent proliferation in the uterus (Hewitt & Korach 2003, Winuthayanon et al. 2017). In contrast, it appears that ERβ may have other functions including specific roles in inflammation and angiogenesis (Critchley et al. 2001, Gibson & Saunders 2012, Greaves et al. 2013, Gibson et al. 2015). There have been fewer studies focused on GPER, but when Zhang et al. (2017) treated primary endometrial stromal cells with E2, G1 ((±)-1-((3aR*,4S*,9bS*)-4-(6-bromo-1,3-benzodioxol-5-yl)-3a,4,5,9b-tetrahydro-3H-cyclopenta[c]quinoline; GPER, G protein-coupled oestrogen receptor 1; PPT, 4,4′-(4-propyl-1H)-pyrazole-1,3,5-triyl)trisphenol; SARM, selective androgen receptor modulator; SERD, selective oestrogen receptor degrader; SERM, selective oestrogen receptor modulator.

Selective oestrogen receptor modulators (SERMs)

Selective oestrogen receptor modulators (SERMs) were developed to treat ERα-positive breast cancers with the ideal SERM being one that acts as an antagonist in breast but an agonist in bone (Burris et al. 2013). The evolution in our understanding of tissue selective activities of ligand-activated receptors coupled with the
discovery of different ER subtypes and splice variants has resulted in several generations of SERMs. Tamoxifen is a first generation SERM that displays agonism in the endometrium, increasing EC risk; second generation SERMs such asRaloxifene do not agonize endometrial growth and are associated with lower risk of EC and may have additional positive effects on cognition and the cardiovascular system (Muchmore 2000). Other SERMs have a mixture of agonist/antagonist activity in endometrium, agonist activity in bone and antagonism in breast (Pickar et al. 2018).

**Selective oestrogen receptor degraders (SERDs)**

Selective oestrogen receptor degraders (SERDs) antagonize ERα and induce its degradation, resulting in a decrease in ERα protein levels: they do not show agonist properties in other tissues (Kieser et al. 2010). Fulvestrant was the first SERD to be approved as a therapeutic and is commonly used as a treatment for advanced breast cancer (Blackburn et al. 2018). Although originally marketed under the trade name Faslodex by AstraZeneca, manufacture of generic versions has been approved by the US Federal Drugs Administration. A number of new generation SERDs are in development (Pepermans & Prossnitz 2019), one of which is bazedoxifene (BZA), a compound which exhibits SERD properties in breast cancer with beneficial properties in bone and no adverse impact on endometrium leading to its approval for hormone replacement therapies (Pickar et al. 2018). Recent mechanistic studies suggest BZA may be useful in treating cancers which contain ERα mutants (Fanning et al. 2018). In addition to activation by endogenous oestrogens, there is evidence that GPER may also be activated by SERMs/SERDs developed to target ERα which may explain some apparent discordant results in ERα negative cancers (see review by Meyer et al. 2011).

**Androgen receptors**

Selective androgen receptor modulators (SARMs) have been developed to support the beneficial impacts of AR-mediated cell function in bone and muscle without the adverse side effects seen with high doses of testosterone or DHT (gynaecomastia, aggression, prostate hyperplasia) (Burris et al. 2013, McEwan 2013) (Table 2). New generation SARMs have been proposed as therapeutics for women suffering from breast cancer, muscle wasting or urinary incontinence and a number of clinical trials have been undertaken to evaluate their use for these indications (Brodie & McEwan 2005, Dalton et al. 2011).

**Targeting oestrogen receptors in endometrial disorders**

A high proportion of low grade EC express ERα as well as progesterone receptors. In a recent systematic review, van Weelden and colleagues highlighted the progestins as a first-line hormonal therapy and use of anti-oestrogens as an alternative therapy option, highlighting results from ten trials using SERMs or SERDs as monotherapies between 1981 and 2013 (van Weelden et al. 2019). All studies showed some beneficial response to therapy, although results were variable and the authors concluded that tamoxifen or a combination of tamoxifen and progestin might be the best choice when selecting second-line hormonal treatment. In subsequent studies, the SERM Ospemifene has been shown as an effective in treatment of vaginal symptoms in postmenopausal women (Archer et al. 2019) and only acts as an agonist in endometrium in high doses. The SERD fulvestrant/faslodex (Table 2) has been investigated as a treatment for endometrial cancer in phase I/II trials, although well-tolerated, it has low oral bioavailability and further trials are needed (Bogliolo et al. 2017). Another recent study suggested dual targeting of ERα with tamoxifen and ERRα with XCT790 may be beneficial for EC treatment, but this requires further validation (Mao et al. 2019).

While administration of SERMs/SERDs may be appropriate for postmenopausal women with cancer, their use in younger women with non-malignant endometrial disorders such as endometriosis is more challenging with data limited to promising results in preclinical models (Kulak et al. 2011, Khine et al. 2018). The observation that ERRβ is highly expressed in endometriosis lesions and the development of ERRβ-selective agonists such as ERRβ-041 with apparent anti-inflammatory properties provided a rationale for testing them as therapies for endometriosis with promising results obtained in preclinical models (Harris 2006). Several clinical trials were conducted with ERRβ-041, but no positive outcomes were reported. In a recent review, Guo and Groothuis highlighted a number of reasons why drugs targeting ERRβ including the SERM Fulvestrant and ERRβ-041 failed to deliver the patient benefit in clinical trials. The reasons highlighted included, but were not limited to, animal models that did not recapitulate long-established disease, translation of dose from rodent to women and incomplete understanding of the role of ERβ antagonism in pain mechanisms (Guo & Groothuis 2018). SERMs are not considered suitable therapies for adenomyosis (Pontis et al. 2016). The SERM Ormeloxifene, developed for use as a contraceptive,
has also shown promising results in treating heavy menstrual bleeding (HMB) in perimenopausal women in India (Pati et al. 2017).

GPER has also been investigated as a target for treatment of endometriosis with reports that the GPER agonist G-1 induced cell cycle arrest and apoptosis of stromal cells derived from ovarian endometriosis cysts (Mori et al. 2015). GPER has been implicated in E2-stimulated nociceptive pain in endometriosis, with results in a mouse model showing administration of the selective GPER antagonist G36 inhibited the pain response (Alvarez et al. 2014). Properly designed clinical trials are needed to explore GPER as a target for relief of painful symptoms in endometriosis in women.

**Targeting androgen receptors in endometrial disorders**

The development of SARMs has prompted renewed interest in targeting of AR in reproductive disorders while also raising concerns related to side effects including hirsutism that are a hallmark of excess androgens in PCOS. Transgender individuals may be one group who might benefit from SARMs, as administration of high concentrations of testosterone can result in abnormal uterine bleeding and metabolism to oestrogen may explain increased rates of endometrial cancer (Grimstad et al. 2019), but there are no registered clinical trials.

Danazol is a synthetic androgen first used as a treatment in the 1970s: it binds AR with high affinity and is also reported to reduce the activity of a number of enzymes including steroid sulphatase (Carlstrom et al. 1984). Danazol has anti-proliferative effects on uterine cells (Kauppila et al. 1985). A systematic review of RCTs using Danazol to treat endometriosis concluded that treatment was associated with reduced lesion size and relief of pain symptoms and that women who took Danazol were more satisfied with their treatment compared with women who had placebo treatment (Farquhar et al. 2007). The anti-proliferative and hormone-suppressive activities of Danazol has formed the basis of treatments for adenomyosis (Vannuccini et al. 2018) and heavy menstrual bleeding (Beaumont et al. 2007) with efficacy being demonstrated. The androgenic activity of Danazol is associated with side effects including hirsutism and deepening of the voice and it is contraindicated for women at risk of pregnancy because of the risk of virilisation of the fetus (Farquhar et al. 2007). These side effects have limited its use and prompted efforts to develop therapies that are less virilising.

Using a mouse model, we have compared the impact of DHT with Danazol and new generation SARMs GTx-024 and GTX-007 (Table 2) and found that both Danazol and GTX-024 restored uterine weight of ovariectomised female mice to that of intact animals, while GTX-007 had no similar effect (Simitsidellas et al. 2019). These preclinical studies highlight the importance of considering impacts on the endometrium when women are included in clinical trials using SARMs (Dalton et al. 2011, Neil et al. 2018). While SARMs have been used in clinical trials for treatment of breast cancer, they have not as yet been tested as treatments for endometrial cancer or endometriosis (Narayanan et al. 2018). Standard medical treatment for HMB involves targeting of the progesterone receptor either with the androgenic progestagen levonorgestrel delivered in an intra-uterine device or with newly developed selective progestrone receptor modulators (SPRMs; Maybin & Critchley 2016).

Interestingly, administration of progesterone receptor antagonists or SPRMs such as UPA (ulipristal acetate) as a treatment for heavy menstrual bleeding results in a significant increase in expression of AR (Whitaker et al. 2017) which may, in part, explain their anti-proliferative action. Treatment with new generation SARMs is yet to be investigated.

**Summary and future directions**

The endometrium is a dynamic tissue which, by virtue of its expression of high affinity receptors, is exquisitely sensitive to the actions of oestrogens and androgens. Temporal and spatial changes in tissue function in response to steroids play a critical role in preparation for pregnancy and in breakdown and shedding if pregnancy does not occur. Balanced regulation of sex-steroid action is essential for endometrial function and is controlled via local metabolism and cell- and tissue-specific expression of steroid receptors/isoforms. Drugs targeting steroid metabolising enzyme activity and/or receptor function have reported efficacy in several endometrial disorders, but their use has often been limited due to lack of tissue specificity and undesirable side-effect profiles. Recent development of drugs that selectively target steroid receptors such as next generation SERMs, SERDs, SARMs and SARDs show promise as new therapeutics, but further preclinical studies and clinical trials are needed to determine if these drugs have efficacy specifically for the indication of endometrial disorders.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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