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Review

Endometrial Intracrinology: Oestrogens, Androgens and Endometrial Disorders

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Abstract:

Peripheral tissue metabolism of steroids (intracrinology) is now accepted as a key way in which tissues, such as the endometrium, can utilize inactive steroids present in the blood to respond to local physiological demands and 'fine-tune' the activation or inhibition of steroid hormone receptor-dependent processes. Expression of enzymes that play a critical role in activation and inactivation of bioactive oestrogens (E1, E2) and androgens (A4, T, DHT), as well as expression of steroid hormone receptors, has been detected in endometrial tissues and cells recovered during the menstrual cycle. There is robust evidence that increased expression of aromatase is important for creating a local microenvironment that can support a pregnancy. Measurement of intra-tissue concentrations of steroids using liquid chromatography–tandem mass spectrometry has been important in advancing our understanding of a role for androgens in the endometrium acting both as active ligands for the androgen receptor and as substrates for oestrogen

biosynthesis. The emergence of intracrinology, associated with disordered expression of key enzymes such as aromatase, in the aetiology of common women's health disorders such as endometriosis and endometrial cancer has prompted renewed interest in development of drugs targeting these pathways opening up new opportunities for targeted therapies and precision medicine.

Keywords: decidualisation 1; oestradiol 2; aromatase 3; testosterone 4; DHEA 5; endometriosis 6; endometrial cancer 7; sulfatase

1. What do we mean by 'intracrinology'?

The term 'intracrine' emerged in the 1980s as a new concept in endocrinology based on the ability of cells within non-gonadal tissues to both produce (synthesise) a hormone (peptide, protein or steroid) and to respond to that same factor [1, 2]. For many researchers working in the field of sex steroid hormones the 'At the cutting edge' review by Fernand Labrie published in 1991 and simply titled 'Intracrinology' was the paper that first made them expand their horizons beyond thinking of gonad-derived sex steroids as the only regulators of steroid target tissues such as the endometrium [1]. This review made, what at the time appeared to be a bold claim, that the 'best estimate of the intracrine formation of estrogens in peripheral tissues in women is in the order of 75% before menopause, and close to 100% after menopause'. In recent years, particularly following increasing use of sensitive techniques such as liquid chromatography–tandem mass spectrometry (LC-MS/MS), the number of papers providing evidence for changes in tissue-specific concentrations of steroids that did not necessarily parallel those in blood has accelerated and there has been an increase in the range and number of enzymes and pathways under consideration [3-6]. Peripheral tissue metabolism of steroids is now accepted as a key way in which tissues, such as the endometrium, can respond to local physiological demands and 'fine-tune' the activation or inhibition of steroid hormone receptor-dependent processes. The original concept of 'intracrine' regulation was defined as involving both biosynthesis *and* response by the *same* cell (see Figure 1 in reference [1]) to distinguish it from autocrine or paracrine regulation. However in more recent studies and reviews, 'intracrinology' is now usually discussed on the basis that it is tissue-specific, local production (and inactivation) of sex steroids without significant release of active sex steroids into the

peripheral circulation [1, 5, 6] with less attention being paid to the source and site of action being in the same cell and a greater emphasis on the tissue micro-environment. In this regard, a strong case has been made that the 'inactive' adrenal steroid DHEA is the most important precursor of bioactive androgens in women [7]. There has also been a rapid increase in the number of studies considering the role of locally produced (intracrine) steroids in the aetiology of pathologies including cancers of the breast [8, 9] and endometrium [10-12] as well as in regulation of fertility [13] and the aetiology of the oestrogen-dependent disorder endometriosis [14]. In the current review we have based our discussion on the evidence for 'local' production and/or activation of steroids that may act in an intracrine, autocrine or paracrine manner within the endometrium or associated disorders.

In the following sections we will provide a brief overview of the structure of the endometrium, its regulation by ovarian-derived steroids and expression of steroid receptors as the prelude for a review of the evidence supporting a role for local tissue activation/biosynthesis of bioactive oestrogens (oestrone, E1: oestradiol, E2) and androgens (testosterone, T: dihydrotestosterone, DHT) in the normal endometrium and in some disorders associated with endometrial malfunction. We will also briefly review the evidence that supplementation with inactive steroids or administration of drugs targeting intracrine steroid biosynthesis may offer a new therapeutic opportunity to treat a range of disorders, including infertility.

2. Endometrium - a sex hormone-dependent multicellular tissue.

2.1. Endometrial tissue structure and response to ovarian-derived hormones

The human endometrium is located within the central area of the uterus surrounded by the muscular layers of myometrium with a layer of epithelial cells providing an interface between the tissue and the luminal compartment (Figure 1) [15]. Histologically the human endometrium has two distinct layers: an outer basal compartment and an inner functional compartment. Both layers contain glands bounded by epithelial cells embedded in a multicellular stroma consisting of fibroblasts, resident immune cells and an extensive vascular compartment (endothelial cells, pericytes and vascular smooth muscle). In response to fluctuating changes in the concentrations of oestrogen and progesterone circulating in the blood as a result of changes in ovarian function, the tissue also experiences cyclical episodes of proliferation (oestrogen-dominated proliferative phase), differentiation (progesterone-dominated secretory phase), and in the absence of a pregnancy, breakdown of the inner (functional) layer, shedding and scarless healing (menstruation).

We, and others, have shown that the changes in endometrial tissue function are characterised by changes in the architecture of the glands and differentiation of stromal cells (decidualisation) (Figure 1B) [16]. These are accompanied by changes in both the number and population of resident immune cells that play key roles in regulating differentiation of the tissue vasculature in preparation for implantation and in endometrial tissue repair at the time of menstruation [17-20]. It is notable that many authors have classified menstruation as an 'inflammatory event' highlighting increased concentrations of prostaglandins, which may also be generated by intracrine mechanisms, involving local enzyme expression, as well as increased synthesis of pro-inflammatory cytokines, chemokines and matrix metalloproteinases [21, 22].

2.2. Expression of androgen and oestrogen receptors in endometrium, endometriosis and endometrial cancer

Steroid hormone action is classically mediated by intracellular proteins encoded by members of the nuclear receptor subfamily NR3: there is a single androgen receptor gene (*NR3C4, AR*) found on the X-chromosome and two oestrogen receptor genes *NR3A1 (ESR1*, oestrogen receptor alpha) and *NR3A2* (*ESR2*, oestrogen receptor beta) found on chromosome 6 and chromosome 14 respectively in women [https://www.nursa.org/nursa/index.jsf]. These receptors may act within the nucleus as ligand-activated transcription factors by several different mechanisms: 1) binding directly to hormone 'responsive elements' on target genes for example DNA sequences shown to have specificity for the androgen receptor (androgen response element, ARE) or either of the oestrogen receptors (estrogen response element, ERE), 2) in association with the binding of other transcription factors (AP-1, Sp-1) or 3) acting outside the nucleus via rapid, 'non-genomic' pathways – the evidence for all of these pathways has recently been extensively reviewed and will not be discussed further [23, 24]. Detailed immunohistochemical studies using fixed full-thickness endometrial tissues in combination with validated antibodies [25] have identified nuclear staining for AR, ERalpha and ERbeta in the human endometrium during the normal menstrual cycle [16, 18, 26, 27]. Notably, the tight spatial and temporal localization of these receptors can provide insights into the cells that may be influenced by the actions of intracrine-derived steroids. Notably, key target cells for androgens are endometrial fibroblasts which are AR-positive in the functional layer during the proliferative phase and in the basal compartment throughout the cycle. AR is downregulated in stromal cells in the functional layer during the secretory phase and upregulated in epithelial cells when progesterone levels decline (functional withdrawal with demise of the corpus

luteum) [26] or in response to administration of progesterone receptor antagonists/selective modulators [28].

In the human endometrium, ERalpha and ERbeta exhibit cell-specific patterns of expression during the menstrual cycle [27]. Notably, ERalpha is present in the epithelial cells lining the glands and lumen during the proliferative phase, at a time when circulating oestrogens are rising rapidly due to growth of antral follicles containing granulosa cells expressing aromatase [29], but is down-regulated during the secretory phase [27]. Immunolocalisation of ERbeta protein suggests it does not mirror the dynamic change in expression in stromal or epithelial cells seen with ERalpha and that the protein is present in endothelial cells and multiple populations of immune cells that are ERalpha-negative [17, 18, 27]. As discussed below, the identification of ERbeta in endothelial and immune cells is consistent with evidence for direct actions of E2 on these cell types. Studies using targeted deletion of *Esr1* and *Esr2* in mice have reported E2-dependent signaling via ERalpha is critically important for stromal-epithelial interactions in the endometrium and epithelial cell proliferation. Uterine epithelial ERalpha is dispensable for proliferation but essential for complete biological and biochemical responses; [30]. Global ablation of *Esr2* resulted in a predominant ovarian phenotype [31]. There is also evidence for the expression of variant isoforms of both subtypes in the human endometrium formed by translation of mRNAs generated by alternative splicing of the *ESR1* and *ESR2* genes [23, 27, 32]: these variants are not present in mice and their function is poorly understood.

Comparison between the patterns of expression of receptor proteins in normal endometrium with samples of endometrial cancer and endometriosis lesions has revealed evidence of aberrant expression of both AR and ERs which may result in novel disease-specific targets for the action of steroids generated by intracrine activation/metabolism discussed below. Examples include epithelial cell expression of AR in endometrial cancers [24] and up-regulation of ERbeta in endometriosis [33].

3. Methodology.

On 4th May 2018 searches were conducted of the PUBMED [https://www.ncbi.nlm.nih.gov/pubmed/] and SCOPUS [https://www.scopus.com/search/form.uri?display=basic] databases using a range of terms. A variable number of references some of which overlapped were identified: ‘intracrinology’ (167 references Scopus); intracrinology [and] endometrium (11 references Pubmed/ 21 references Scopus). These basic searches were expanded by considering individual enzymes known to be

implicated in intracrine biosynthesis and pathologies: this yielded a larger number of references probably indicating that local expression of enzymes in tissues is not always tagged as being indicative of 'intracrinology'. Examples from Pubmed searches include aromatase[and]endometrium = 398, aromatase[and]endometrium[and]cancer[and human] = 135; sulfatase[and]endometrium = 84: HSD[and]endometrium = 131.

4. Intracrine steroid biosynthesis in the normal endometrium

Classically, the endometrium was considered as a target for endocrine hormones with early studies focused on the capacity for the tissue to metabolise (inactivate) steroids. Subsequent more detailed investigations have demonstrated that the endometrium expresses enzymes capable of biosynthesis as well as metabolism of steroids, with endometrial cells/tissue having the capacity to enzymatically convert androgens into oestrogens, as well as to utilize adrenal and sulphated steroid precursors [34]. Notably, the capacity to convert different substrates was found to vary with menstrual cycle phase, characterised by increased conversion of DHEA, and formation of T during the secretory phase [34]. Although biosynthesis of active steroids was known to be a feature of endometrial disorders such as endometriosis and endometrial cancer (see section 5), it has only become apparent in the last ten years that intracrine steroid biosynthesis and metabolism plays an important role in the regulation of normal endometrial function and fertility.

4.1. Insights gained from measurement of steroid precursors and metabolites in endometrial tissue.

Aided by improvements in sensitivity of LC/MS-MS for measurement of oestrogens, the Poutanen group and their collaborators compared concentrations of E1 and E2 in blood and in matched endometrial tissue biopsies from individual women. Strikingly, they found that concentrations of oestrogens were higher in endometrial tissue than in the circulation and that concentrations of E2 were increased in secretory phase compared to proliferative phase tissues [3, 35] with the latter finding being consistent with increased expression of enzymes such as aromatase or 17β-HSD in response to a decidualisation stimulus (see section 4.3). In complementary studies, the concentrations of androgens and progestins were also measured in

blood and in matched endometrial tissue. Notably intra-tissue concentrations of the androgen precursor DHEA were significantly increased compared to serum concentrations in samples collected from the secretory phase. In contrast, they found that concentrations of androstenedione (A4) and testosterone (T) were significantly lower in endometrial tissue homogenates than in the serum and that these were not cycle phase dependent [3].

4.2 Androgen activation and metabolism

Androgen biosynthesis within tissues can arise from *de novo* steroidogenesis, such as in the ovary, or via conversion of androgen precursors in extragonadal tissues. *De novo* steroid biosynthesis requires cholesterol, which is shuttled to the inner mitochondrial membrane by steroidogenic acute regulatory protein (StAR), where it undergoes side-chain cleavage by CYP11A1 (Cholesterol side chain-cleavage enzyme) to yield pregnenolone (P5). P5 undergoes two enzymatic conversions mediated via CYP17A1, first to 17α-hydroxypregnenolone (17α-hydroxylase activity) and then following 17,20 lyase action to yield dehydroepiandrosterone (DHEA) [36]. DHEA is produced by the adrenal glands and by the ovary and is abundant in the circulation. Expression of StAR, CYP11A1 and CYP17A1 has been reported in the endometrium [37], however to the best of our knowledge no study has identified definitive *de novo* steroidogenesis from normal endometrial tissue.

DHEA and its sulphated form DHEAS are abundant in the circulation and can be utilised as precursors by endometrial cells. We and others have reported that 3β-hydroxysteroid dehydrogenase (3βHSD) is expressed in endometrial stromal cells [13] and that during decidualisation DHEA can be utilised as an androgen precursor yielding both androstenedione (A4) and testosterone (T) via 3βHSD [3, 38, 39]. Following conversion of DHEA to A4 by 3βHSD, activation of androgen agonists (T and dihydrotestosterone; DHT) is tightly controlled via the action of aldo-keto reductase family 1 member C3 (AKR1C3). Assessment of endometrial tissue samples by immunohistochemistry and qRT-PCR of whole-tissue homogenates performed by Catalano et al demonstrated that AKR1C3 expression is increased in the secretory phase relative to proliferative phase with peak expression reported in the early-

to mid-secretory phase [40]. Our studies using an in vitro time course of decidualisation paralleled this expression pattern with peak expression detected between day 2 and 4 of an 8-day decidualisation protocol [41]. In contrast to AKR1C3, expression of 5α-reductase (SRD5A1) which converts T to the more potent and non-aromatisable androgen DHT, is decreased in endometrial cells as decidualisation progresses [41]. Thus, time-dependent conversion of A4 to T determines substrate availability and hence production of DHT.

Interconversion of active/inactive androgens is mediated via 17β-hydroxysteroid dehydrogenase isozymes. Expression of several 17β-hydroxysteroid dehydrogenase (HSD17B) isozymes have been reported in endometrial tissues/cells [42]. In addition to AKR1C3 (also known as HSD17B5), HSD17B7 and 12 are reported to have reductive 17β-HSD activity, mediating conversion of A4 to T, and mRNA expression of *HSD17B7* and *HSD17B12* has been reported in total tissue homogenates of normal human endometrium but with no significant cycle-dependent change in expression [4]. The predominant isoform with oxidative 17β-HSD activity in the endometrium is HSD17B2, which converts active T to A4. Expression of HSD17B2 is increased by progesterone and elevated in the secretory phase [4, 43]. HSD17B14 also has oxidative 17β-HSD activity and has been immunolocalised to endometrial glandular epithelial cells [44] although relative efficiency for oxidation of E2 or T was much lower than that of HSD17B2 in cell metabolism assays. Although HSD17B2 has oxidative action on androgens and oestrogens, our own metabolism studies suggest that this activity is decreased in endometrial stromal cells during decidualisation even when *HSD17B2* mRNA expression is increased [13, 41]. This may reflect alternative activity of HSD17B2 during the secretory phase, such as by activating 20α-hydroxyprogesterone to increase bioavailability of P4 [45].

4.3 Oestrogen biosynthesis and metabolism

There are two main pathways by which oestrogens are synthesized within endometrial cells: 1) conversion of androgens such as A4 and T to active oestrogens, E1 and E2 respectively, via the action of the aromatase enzyme complex, the key component of which is the aromatase protein encoded by the *CYP19A1* gene; 2) conversion of sulphated oestrogens oestradiol sulphate (E2S)

238 or oestrone sulphate (E1S) into their bioactive metabolites (E2, E1 respectively) via the action
239 of steroid sulphatase encoded by *STS* (discussed below) [13, 46].

240 Landmark studies in mice by the Bagchi group demonstrated that aromatase activity within the
241 endometrium was essential for establishment of pregnancy in that species [47, 48]. In a number
242 of elegant experiments they showed that decidualisation and vascular remodelling were
243 impaired if an aromatase inhibitor was administered in vivo [47]. In women, when assessed as
244 whole tissue homogenates, expression of aromatase is low/undetectable in endometrial samples
245 recovered during the normal cycle. However, using primary human endometrial stromal cells
246 we discovered that aromatase protein and enzyme activity were increased following
247 decidualisation resulting in increased synthesis of E1 and E2 [13]. Critically, these studies
248 demonstrated the temporal regulation of this process, whereby expression of aromatase and
249 secretion of E2 increased in a time-dependent manner while metabolism (inactivation) of E2 to
250 E1 was decreased [13] resulting in an increased E2:E1 ratio. We speculate that the failure to
251 detect altered expression in tissue homogenates may reflect tight temporal and/or spatial
252 regulation which will be missed unless samples are recovered from areas of active
253 decidualisation in the functional layer of the tissue.

254 Expression of oxidative and reductive 17BHSD isoforms may also contribute to generation of
255 E2 from E1: although both E1 and E2 can activate ERs, E2 is generally considered to be a more
256 potent agonist. Activation of E1 to E2 via reductive 17β-HSD activity is primarily mediated by
257 HSD17B1 but also HSD17B4 and 7 in the uterus. Expression of these enzymes has been
258 reported in normal endometrium [4], and as elevated in endometrial disorders (see section 5).

259 ### 4.4 Role of sulphated steroids as a source of endometrial androgens and oestrogens

260 Androgens and oestrogens can be metabolised from the common precursor
261 dehydroepiandrosterone sulphate (DHEAS) via the action of steroid sulfatase (STS) which de-
262 sulphates DHEAS to DHEA. Additionally, STS can metabolise E1S and E2S sulphate to
263 bioactive E1 and E2 respectively. STS is expressed in normal endometrial tissues and in
264 endometrial cancers [49, 50]. STS expression and activity is increased during decidualisation

of endometrial stromal cells in vitro consistent with a detectable increase in oestrogens detected in tissue samples recovered during the secretory phase [3, 46].

Sulfation is a key mechanism for deactivating steroids and is essential for appropriate intracrine regulation within the tissue environment. Sulfation is mediated by sulfotransferase enzymes and requires the action of 3′-phospho-adenosine-5′-phosphosulphate synthase (PAPSS) enzymes to provide a sulphate moiety for conjugation [51]. There is little evidence for expression of DHEA sulphating enzymes/activity in the endometrium, although expression of *SULT1A1* and *SULT2B1a* were reported in endometrial stromal cells together with *PAPSS1* and *PAPSS2* [46]. The main oestrogen sulphotransferase (SULT1E1) is expressed in endometrial epithelial cells and expression is increased in response to progesterone [52] highlighting a potentially complex balance between activation and inactivation of steroids during the fertile phase of the cycle that may involve more than one cell type.

4.5 Intracrine and paracrine impact of tissue biosynthesis of androgens and oestrogens in endometrium

Perivascular decidualisation is associated with accumulation of specialised immune cells known as uterine natural killer cells (uNK) that are critical mediators of vascular remodelling in early pregnancy. In common with other immune cells found in the endometrium (macrophages, mast cells [53, 54]), uNK cells express ERbeta [18]. Strikingly, we have demonstrated that conditioned media from decidualised stromal cells increases uNK migration in an oestrogen-dependent manner: uNK cells treated with E2 also increased secretion of angiogenic factors including CCL2, which had a significant impact on endometrial endothelial cell network formation [55]. The impact of a local oestrogen-rich microenvironment on the function of endometrial macrophages or mast cells has yet to be elucidated. Recent evidence suggests that sulphated oestrogen precursors are also utilised by endometrial stromal cells as intracrine/paracrine regulators, as secretion of E1 and IGFBP1, a prominent decidualisation marker, are decreased in the presence of the STS inhibitor STX64 (Irosustat) [46]. Other studies that have highlighted oestrogen-dependent changes in endometrial endothelial cell gene expression [56] that will also be influenced by local (intacrine) metabolism/biosynthesis of

bioactive oestrogens. In summary, the evidence presented in the studies described above together with those in mice [47], all support the hypothesis that tight temporal-spatial regulation of tissue function by intracrine oestrogens plays a previously underappreciated role in modulating the function of cells (endothelial, uNK, decidual fibroblasts) which play key role(s) in formation of a receptive endometrium capable of supporting a viable pregnancy.

Endometrial biosynthesis of androgens has also emerged as a regulator of fertility and endometrial function. Specifically, human primary stromal cells are AR-positive and treatment of cells from proliferative phase endometrium with DHT has identified a number of androgen-regulated genes [26]. Complementary studies by Gibson et al [41] in which primary cells were stimulated to decidualise, showed local intracrine biosynthesis of androgens may play a critical role in maintenance of a decidual phenotype, with addition of flutamide reducing biosynthesis of the decidualisation marker IGFBP1 [41, 57]. These studies complement and extend those of Cloke et al [58, 59] who showed that AR and PR regulated distinct genomic pathways during decidualisation, consistent with a role for androgens (either local or peripheral) in regulation of fertility. Studies in mice have also identified a potential role for androgens in endometrial repair at the time of menstruation [60] at a time when peripheral oestrogens are low which merits further investigation.

The identification of aromatase expression and production of oestrogens in both mouse tissue and human endometrial cells has highlighted the importance of locally produced androgens acting as precursor steroids for oestrogen biosynthesis. Results from studies reporting that intra-tissue concentrations of androgens T and A4 were *lower* than serum [3] would be consistent with this hypothesis. However, one caveat is that these studies assessed whole endometrial tissue homogenates which do not allow for the contribution of specific cellular compartments to be quantified. We therefore investigated the temporal dynamics of androgen metabolism using an *in vitro* model of isolated primary human endometrial stromal cells. In these studies, treatment of cells with the AR antagonist flutamide, reduced secretion of both decidualisation and endometrial receptivity markers [41]. We further demonstrated that supplementation with the androgen precursor DHEA increases biosynthesis of T and DHT and is associated with dose-dependent increases in expression of the decidualisation markers IGFBP1 and prolactin, as well as the endometrial receptivity marker SPP1 [38]. Taken together, these studies suggest

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both local activation and metabolism of androgens occurs during decidualisation and that temporal regulation of intracrine androgen bioavailability is a critical mediator of endometrial competence during remodelling required for establishment of pregnancy [37, 57]. These studies are of particular relevance to the impact of aging on fertility, as circulating concentrations of androgens precursors, such as DHEA and A4 as well as T and DHT decline with age [36, 61].

5. Evidence for the importance of intracrinology in endometrial disorders

5.1 Endometriosis

Endometriosis is a chronic oestrogen-dependent disorder that is characterized by growth of endometrial cells/tissue fragments (lesions) outside the uterus [62]: there are 3 broad classifications based on location of the lesions, peritoneal, ovarian (endometriomata) and deep infiltrating lesions in the rectovaginal area. A recent review provides an excellent overview of the features of endometriosis as determined by magnetic resonance imaging [63]. Common histologic features of all three manifestations include the presence of endometrial-like cells (either stromal and/or glandular, vascular cells and nerves) as well as evidence of inflammation (immune cell populations). Endometriosis can be associated with debilitating pelvic pain: in most but not all women, symptoms regress after menopause and many of the drug regimes used to treat symptomatic endometriosis are based on suppression of ovarian cyclicity [62]. Examination of ectopic lesions has reported that they are characterized by high aromatase expression levels together with deficiency in 17β-HSD2, the enzyme responsible for the inactivation of E2 to E1 [64]. A recent Cochrane review concluded that ‘for women with pain and endometriosis, suppression of menstrual cycles with gonadotrophin-releasing hormone (GnRH) analogues, the levonorgestrel-releasing intrauterine system (LNG-IUD) or Danazol were beneficial interventions’ [65]. However it is notable that for many women, particularly those wishing to conceive, ovarian suppression is not desirable, the associated menopausal side effects can be severe and intracrine mechanisms are not suppressed. Whilst our literature search terms ‘intracrine and endometriosis’ only identified 5 papers on PUBMED it is notable that the alternative search ‘aromatase and endometriosis’ identified more than 300, reflecting widespread interest in the role of local (intracrine) biosynthesis in the aetiology of this complex disorder and the potential that this might provide a novel therapeutic opportunity [66].

Local oestrogen production, accompanied by intracrine and paracrine signalling via ERβ in endometriotic tissues is believed to contribute to a feed-forward signalling cascade that

maintains an inflammatory state and cell proliferation within the endometriotic lesions (for a comprehensive review on the role of oestrogen production and action in endometriosis see [67]). Peritoneal fluid from women with endometriosis contains high concentrations of pro-inflammatory cytokines, such as TNFα and IL-1β [68, 69], which have the capacity to stimulate expression of the prostaglandin synthesis enzyme COX-2 and increase secretion of prostaglandin E2 (PGE_2) by endometriotic cells and peritoneal macrophages [70, 71]. PGE_2 in turn stimulates production of cyclic AMP (cAMP) which together with steroidogenic factor 1 (SF1), whose expression is aberrantly upregulated in endometriotic tissue compared to endometrial tissue induces expression of mRNAs that encode enzymes that play a critical role in the steroidogenic enzyme machinery, including STAR, 17-hydroxylase/17,20-lyase, 3β-HSD and aromatase which may be consistent with the synthesis of E2 from cholesterol within lesions [70, 72].

The significance of intracrine signaling in the aetiology of endometriosis has been supported by Huhtinen et al. who used LC/MS-MS to interrogate the concentrations of steroids in matched endometrial, endometriotic and serum samples from women with or without endometriosis [3, 4]. A striking finding from these studies was that the concentrations of testosterone in endometriotic lesions (both ovarian and extra-ovarian) far exceeded those in the blood regardless of menstrual cycle stage [3]. This increase was mirrored by elevated expression of *CYP11A1*, *CYP17A1* and *HSD3B2* in endometriotic lesions, especially those associated with the ovary (endometriomatas), compared to intrauterine endometrium. It was also accompanied by significant changes in expression of androgen-regulated genes (*PRUNE2*, *HGD*, *PDGFRL*) [3] providing a readout of the action of androgens binding to AR expressed in the lesions. Thus, increased intra-tissue testosterone synthesis in endometriotic lesions as well as providing a substrate for aromatase and biosynthesis of E2, may also promote the activation of an AR transcriptional network within the lesions.

Apart from the discrepancy between intra-tissue steroid concentrations and those in the circulation, there are also significant variations in the levels of endometrial and circulating steroid hormones that are menstrual cycle-dependent. Using the same experimental approach described above, Huhtinen et al. reported that while endometrial and endometriotic intra-tissue concentrations of E2 were significantly higher compared to those in the serum of women in the proliferative phase of the menstrual cycle, the opposite is the case for the secretory phase [4]. Moreover, expression of *HSD17B2* was significantly lower within lesions compared to endometrial tissue while expression of *HSD17B6* and *CYP19A1* was significantly higher [4]. It

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must be noted that there is a distinct difference both in the local steroid concentrations and the expression of steroid metabolizing enzymes within different types of lesions. For example, in the proliferative phase, E2 concentrations in ovarian lesions (endometriomas) were approximately 3,430pg/ml, while in peritoneal lesions, E2 concentrations were 238pg/ml [4]. This demonstrates a heterogeneity in intracrine steroid action within different types of lesions, which in the case of ovarian lesions could derive from the proximity of the endometriotic cells to ovarian follicles and the constant supply of steroids from the follicles within the ovaries. Further studies are required to explore this possibility.

5.2. Endometrial cancer

Endometrial cancer (EC) is the 4th most common cancer in women with the majority of women being diagnosed at early stages of the disease following a uterine bleed after menopause [cancerresearchuk.org]. Established risk factors for development of EC include obesity and the presence of premalignant lesions associated with endometrial hyperplasia [73, 74] with oestrogen exposure considered a key driver of both endometrial hyperplasia and Type I EC that make up 75% of EC cases [74]. Rizner and colleagues have recently provided a comprehensive overview of the different studies contributing to our current understanding of the mechanisms that contribute to increased bioavailability of oestrogens in EC [50, 75]: a few key studies are described below.

Expression of the *CYP19A1* gene is regulated by tissue specific promoters distributed over a 93kb regulatory region which have been the subject of extensive investigation [76] Notably Bulun and colleagues have found that in cancers of breast, endometrium and ovary, expression is primarily regulated by increased activity of the I.3/II promoter region which can be upregulated by prostaglandins such as PGE2 providing a link between overexpression of PGs that has been reported to occur in EC [77] and intracrine oestrogen biosynthesis [78]. Sasano and collaborators have developed novel antibodies and reported evidence of increased immunoexpression of aromatase [79] and steroid sulphatase [80] and 17βHSD enzymes [81] in endometrial hyperplasia and EC. These finds are all consistent with increased bioavailability of E1/E2 in association with malignant transformation. Notably expression of aromatase and 17βHSD 1 in EC have both been correlated with poor prognosis [82, 83].

We and others have shown that AR are widely expressed in EC and also in EC cell lines such as Ishikawa which have been extensively studied (reviewed in [24]). In a recent paper Kamal et al [84] reported expression of AR in high grade EC was down regulated but was elevated in

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metastases raising the possibility they might be a target for therapy. In a recent review Ito et al summarized the epidemiological data supporting an association between elevated androgens in the circulation and the risk of developing EC [11]. Whilst there has been less interest in the intracrine generation of androgens, other than as substrates for aromatase, it is notable that a study by the same group revealed that EC tissues had an 8-fold elevation in DHT compared with that in normal endometrial tissues [85]. The same study compared AR expression with that of 5 alpha reductase enzymes 1 and 2 (5αR1 and 5αR2) concluding that expression of 5αR1 (65% of samples) was positively correlated with histological grade (but not clinical grade). They found women immunonegative for both AR and 5αR1 had a poorer prognosis [85] consistent with other studies that have suggested androgens can be anti-proliferative for EC cells.

6. Intracrinology and metabolism

Whilst this review has focused on endometrial tissue and its pathologies there is a growing body of evidence showing the importance of intracrine metabolism in non-reproductive tissues. For example, in their recent comprehensive review of intracrine androgen biosynthesis and metabolism, Schiffer and colleagues highlighted the importance of peripheral metabolism of steroids in metabolic target tissues including adipose and skeletal muscle [86]. Studies on the role of AR in skeletal muscle conducted using transgenic mouse models have shown it is expressed in multiple cell types in muscle [87, 88]. The pharmaceutical industries have developed a number of selective androgen receptor modulators to target AR in muscle as a therapy for age-related or cancer-related loss of muscle function [89, 90].

7. New therapeutic approaches for treatment of endometrial disorders based on intracrine targets

Labrie and colleagues have conducted several studies and clinical trials to analyse the impact of intravaginal administration of DHEA (Prasterone, brand name in USA Intrarosa) on adverse symptoms resulting from postmenopausal steroid deprivation including vaginal atrophy and pain during sexual intercourse [91]. They have demonstrated positive impacts on vaginal dryness and other steroid hormone-dependent parameters without any evidence of peripheral changes in serum E2 [92, 93]. In other studies, significant benefit for vaginal health has been demonstrated using estriol gel [94]: these data have collectively provided powerful evidence for intracrine steroid modulation playing a key role in regulation of the vaginal microenvironment which can be harnessed for therapeutic benefit after ovarian secretion of

451 oestrogens stops at menopause [95]. A recent study reported supplementation of culture media
452 with DHEA enhanced expression of receptivity genes by decidualised stromal cells from
453 women with a mean age of 44. Thus there is the potential that administration of DHEA during
454 the secretory phase *alone* may also assist fertility in older women, although this clearly requires
455 further evaluation [38].

456 In other studies the emphasis has been on inhibition of enzymes that appear dysregulated in
457 disease. For example, there are a number of reports that aromatase is expressed in endometriosis
458 lesions with evidence of a positive feed-forward loop involving local biosynthesis of both E2
459 and the pro-inflammatory regulator prostaglandin E2 [67, 96]. These have been complemented
460 by several studies reporting on the positive impact, or lack thereof, of aromatase inhibitors (AI)
461 including letrozole and anastrozole on symptoms of endometriosis in both pre- and post-
462 menopausal women [97, 98]. The current consensus is that AI should be considered as a
463 treatment for endometriosis-associated pain in women who are postmenopausal but still
464 symptomatic as it will target intracrine oestrogen biosynthesis that is thought to play an
465 important role in this age group [97].

466 To target intracrine biosynthesis of steroids and prostaglandins Bayer Pharma have developed
467 an AKR1C3 inhibitor as a therapy for endometriosis [BAY 1128688]. A phase I trial was
468 completed in 2016 and a phase II trial is listed as underway in Spain [EudraCT number 2017-
469 000244-18] with results awaited as to efficacy. Insulin stimulates AKR1C3 expression in
470 adipose tissue of women with PCOS and its inhibition has been suggested as offering a
471 therapeutic target to reduce the hyperandrogenism that is a feature of this disorder [99].

472 A number of 17βHSD inhibitors have been developed to target intracrine biosynthesis of
473 oestrogens in hormone-dependent disorders, including cancer and endometriosis [100].
474 Promising results have been reported using a 17beta HSD1 inhibitor to suppress conversion of
475 E1 to E2 in endometriosis tissue homogenates [101]. Forendo Pharma based in Turku Finland
476 [http://www.finlandhealth.fi/-/forendo-pharma] have developed a specific HSD17B1 Inhibitor
477 (FOR-6219) that is due to enter phase I studies during second quarter of 2018. High expression
478 of 17beta HSD1 is associated with poor prognosis in EC [82]. In a recent study, Konings et al
479 [102] reported detection of 17βHSD1 in EC metastatic lesions and promising results

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demonstrating inhibition of enzyme activity using the FP4643 type 1 inhibitor in both in vitro and ex vivo models.

The evidence that STS is expressed in endometrial cancers [50, 103] and endometriosis [104] has prompted development of specific STS inhibitors as novel therapies. Several potent STS inhibitors have been developed [105] including STX64 which was effective in blocking synthesis in endometrial cancer cells in vitro. STX64 has been renamed as Irosustat (Ipsen) and was tested in a phase 2 open label trial in women with advanced/metastatic or recurrent estrogen receptor-positive endometrial cancer but did not result in better survival rates and has not been developed further.

Another inhibitor, estradiol-3-O-sufamate (E2MATE) used on human endometrial explants and in a mouse model of endometriosis has shown some promising results [106]. Currently under the name PGL2 this compound is listed on the web as being part of a phase II clinical trial for endometriosis [Jenapharm: https://adisinsight.springer.com/drugs/800026648].

Whilst monotherapies are still under test, drugs which have dual actions have also been developed to target both aromatase and STS (DASI) as well as STS and 17βHSD1. A dual STS/17βHSD1 inhibitor has been shown to block proliferation of cancer cells treated with E1S/E1 but not those treated with E2 alone [107] but awaits further testing in vivo. A range of DASI drugs have been developed and tested by Barry Potter and his Colleagues with promising results in cell and animal models (reviewed in [108]) but have yet to be tested as a treatment for women with EC or endometriosis.

8. Conclusions

Intracrine regulation of oestrogens and androgens has emerged as a key regulator of endometrial function both during the normal cycle and in endometrial disorders such as cancer and endometriosis. Further studies are needed to better define their role in the complex cross-talk between different cell types and the interplay between metabolic and inflammatory processes. To date, regulation of normal and abnormal endometrial tissue resulting from intracrine biosynthesis of steroids has focused on regulation of gene expression, however, with new

evidence that a wide range of non-coding RNAs are also likely to play a role in endometrial tissues studies need to be devised to explore whether their availability is also regulated by steroids [109]. Importantly, manipulation of intracrine sex steroid metabolism has emerged as a therapeutic target for treatment of endometriosis and endometrial cancer and the scope of these studies is likely to broaden as we gain a greater understanding of their roles in fertility.

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Figures

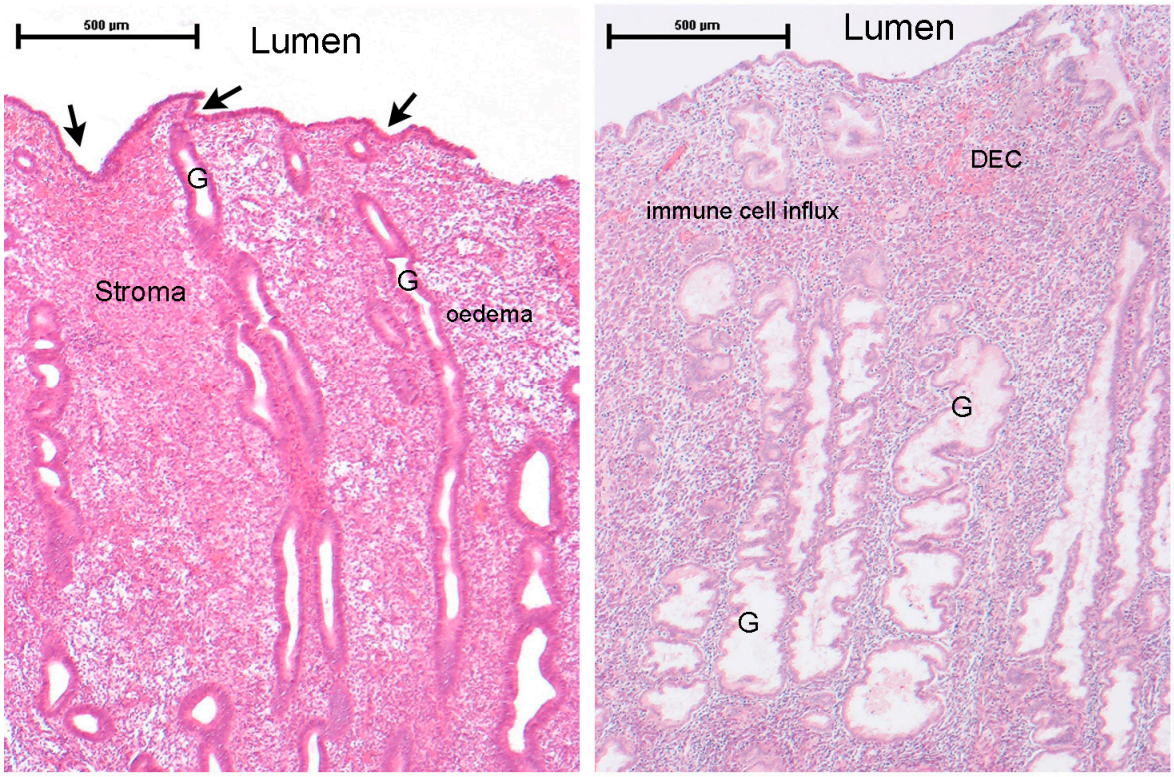
Figure 1. Histology of the human endometrium during the normal cycle.

Full thickness endometrial biopsies from hysterectomy specimens stained with haematoxylin and eosin.

A. Proliferative phase: note the presence of long curving glands (G) and some stromal oedema [110].

B. Secretory phase: note the prominent glands (G) which have a dilated lumen and an irregular outer border stretching down into the basal compartment [111]. In the luminal (functional layer) immune cells are readily detected (most of these are likely to be macrophages and uNK cells [17, 19] as are areas of decidualized fibroblasts (DEC) close to arterioles.

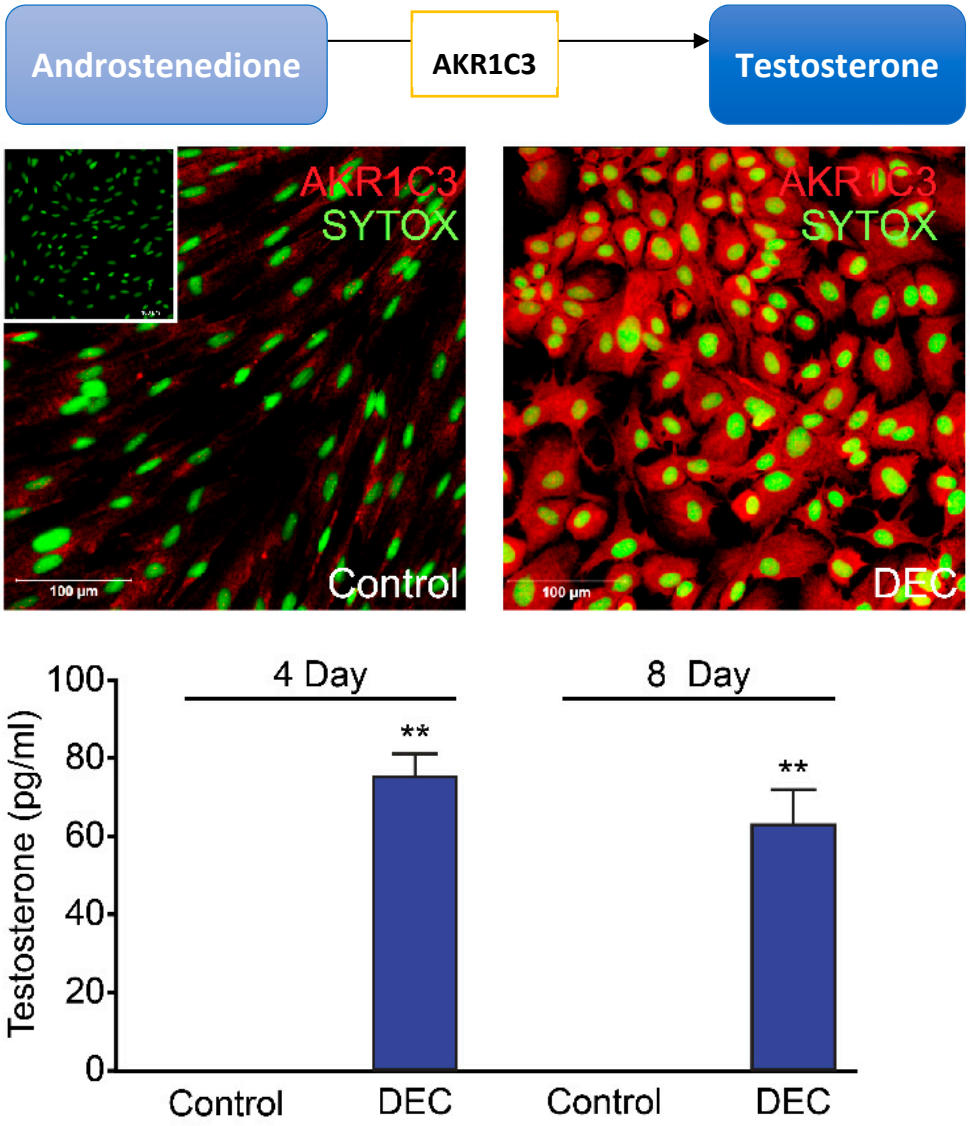
A. B.



797

798 Figure 2: Expression of AKR1C3 in human endometrial stromal fibroblasts is increased in
799 response to a decidualization stimulus resulting in increased biosynthesis and secretion of
800 testosterone. Based on a Figure published in [41] under a CC-BY licence: concentrations of T
801 were determined using an ELISA on days 4 or 8 of the experiment.

802



803 Figure 3: In vitro decidualisation of endometrial stromal fibroblasts results in upregulation of
804 aromatase protein expression (green). Based on data reported in [13]. ESC – primary
805 endometrial stromal fibroblasts; positive control (+) was a protein extract from human placenta,
806 aromatase protein 58kDa.