Estrogen receptor β: the guardian of the endometrium

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**BACKGROUND:** The endometrium is the primary target organ for the 'female' sex steroid hormone estrogen, which exerts effects in the endometrium via two main classical estrogen receptor (ER) isoforms, ERα and ERβ. The main function of the endometrium, embryo implantation, appears unperturbed in ERβ knockout mice, which has led researchers to disregard other potentially important functional roles that ERβ may have in endometrium. This review focuses on ERβ in the human endometrium and its protective role from the undesired effects of ERα.

**METHODS:** We conducted a systematic search using PubMed and Ovid for publications between January 1996 and February 2014. All studies that examined ERβ expression or function in non-pregnant endometrium or cells derived from the endometrium were considered, including human and animal studies.
**RESULTS:** Studies of the basic function of ERβ isoforms in restraining ERα-mediated cell-specific trophic/mitotic responses to estrogen in other tissues has allowed appreciation of the important potential role of ERβ in the regulation of cell fate in the human endometrium. Our current understanding of ERβ expression and function in endometrium is, however, incomplete. ERβ is dynamically expressed in healthy premenstrual endometrium, persists in post-menopausal atrophic endometrium and may play an important role in endometrial disease. All endometrial cell types express ERβ and aberrations in ERβ expression have been reported in almost all benign and malignant endometrial proliferative disease.

**CONCLUSIONS:** The collective evidence suggests that ERβ has an important role in normal endometrial function and also in most, if not all, benign and malignant endometrial diseases. However, the conduct of studies of endometrial ERβ expression needs to be standardized: agreement is needed regarding the most appropriate control tissue for endometrial cancer studies as well as development of standardized methods for the quantification of ERβ immunohistochemical data, similar to those scoring systems employed for other hormonally regulated tissues such as breast cancer, since these data may have direct clinical implications in guiding therapy.

**Key words:** endometrium / estrogen receptor β / hormone receptors / endometrial proliferation / endometrial cancer

**Introduction**

The endometrium is the primary target organ for the ‘female’ sex steroid hormone estrogen (Katzinenllenbogen, 1984; Greaves et al., 2013). The effects of estrogens are exerted in the endometrium via two main classical estrogen receptor (ER) isoforms, ERα and ERβ, and perhaps via the recently described G-protein-coupled estrogen receptor (GPER; formerly GPR30) (Critchley and Saunders, 2009; Holm and Nilsson, 2013). The main function of the endometrium, embryo implantation, appears to be unperturbed in ERβ knockout (KO) mice, which has led researchers to disregard other potentially important functional roles that ERβ may have in endometrium (Burns and Korach, 2012). Studies from other organisms have confirmed the opposing actions of ERβ on ERα function (Gustafsson, 2003; Bottner et al., 2014). Evidence for the involvement of ERβ has been reported in almost all gynaecological pathologies including menorrhagia, endometriosis, infertility and endometrial cancer (EC), as well as in normal and abnormal pregnancy-related conditions (Fernandez et al., 2012; Häring et al., 2012a; Hu et al., 2012). The classical trophic effects of ERα are reviewed elsewhere (Arnal et al., 2013) and this review focuses on the less well-described receptor, ERβ, and its role in protecting the human endometrium from the undesired effects of ERα.

**Human endometrial anatomy**

The endometrium is the mucosal lining of the uterus and is derived from the inner layer of the embryonic paramesonephric ductal mesenchyme (McCuggage, 2011). Endometrial development and function in menstruating upper order primates (including humans) is complex compared with most other mammals (Slayden and Brenner, 2004; Jabbour et al., 2006). The human endometrium is stratified into two functional layers: the transient superficial stratum functionalis and the permanent deeper stratum basalis adjacent to the myometrium (Ferenczy and Bergeron, 1991). The superficial stratum functionalis is lined by luminal epithelium, contains superficial glandular epithelium and stroma and is completely shed and regenerated during the monthly menstrual cycle and after childbirth (Ferenczy and Gurulnicz, 1983; Gargett et al., 2008). It can be divided into the deeper zona spongiosa with a loosely organized stromal zone and a superficial zona compacta with a more compact stroma (Ferenczy, 1980; Wynn, 1989). The stratum basalis contains the terminal part of the endometrial glands and densely organized stroma and is not shed during menses or at parturition; it remains after cessation of ovarian cyclicity as an atrophic, inactive post-menopausal endometrium (Chhieng and Hui, 2011). The germinial layer of the endometrium where the stem cells reside is, therefore, postulated to be the stratum basalis (Padykula et al., 1989; Gargett and Masuda, 2010; Valentin, 2013). The other components of the endometrium, that is the blood vessels and immune cells, exist in both layers (Bulmer et al., 1991a; Spencer et al., 2011).

Access to the full thickness of the endometrium containing both stratum basalis and a stratum functionalis usually requires hysterectomy and, therefore, particularly when studying endometrium either from healthy women or those with benign endometrial disease, researchers have mainly considered the stratum functionalis which is easily obtained with an outpatient endometrial biopsy (Hapangama et al., 2008a). However, the hormonal responsiveness, for example, has been postulated to differ between the stratum functionalis and basalis (Prianishnikov, 1978; Padykula et al., 1989). Many studies of endometrial hormone receptors have not only overlooked the structural and functional differences between the stratum basalis and functionalis, but also have discounted the exceptionally dynamic nature of the stratum functionalis during the normal menstrual cycle (Argenta et al., 2014). Consequently, compared with other estrogen-sensitive organs such as the breast, the exact detailed mechanism of estrogen action in the endometrium remains unclear, with seemingly contradictory results.

**Human endometrium as an estrogen-receptive organ**

Estrogens, progesterone and androgens are the main three classical ovarian steroid hormones that exert their effects on the endometrial cells mainly via their cognate receptors (Hapangama, 2003; Slayden and Brenner, 2004). 17β-estradiol (E2) and estrones are the two main estrogens available for the non-pregnant endometrium, and these exert their cellular functions through nuclear receptors, ERα and ERβ, which are hormone-inducible transcription factors (Vani et al., 2008; Blair, 2010; Crandall and Barrett-Connor, 2013).

Surprisingly, compared with the large number of studies on breast tissue, for example, the number of studies investigating ERβ in endometrium (despite it being a primary target organ for E2) has been modest. There are many reviews of ERβ which discuss most other organs and completely disregard endometrium as an organ in which ERβ has a functional role (Bottner et al., 2014). Furthermore, there are no recent
reviews that specifically discuss the current evidence regarding the potential roles of ERβ in the endometrium.

Although estrogen receptors are responsible for many physiological functions in both females and males (Couse and Korach, 1999; Harris, 2006), the evidence from ERα, ERβ double KO mice confirms that life is possible without E2 action, although ERβ is almost universally expressed in all human organs (Lubahn et al., 1993; Dupont et al., 2000; Weihs et al., 2000). Furthermore, E2 is essential for reproductive function in females, yet only ERα seems to be essential in preserving fertility (Lubahn et al., 1993). The main function of ERβ is therefore thought to be particularly in preventing undesired ERα-mediated actions of E2 (Hall and McDonnell, 2005; Pettersen, 2011). In this review, we focus on the expression and function of ERβ in non-pregnant endometrium. We discuss the available evidence regarding the involvement of ERβ in pre- and post-menopausal healthy endometrium, in benign premenopausal endometrical pathologies and in EC, with reference to animal data where appropriate. ERβ expression and its possible action on the stratum functionalis and the germinal stratum basalis will also be examined in detail. The effects and expression of ERα on the endometrium are reviewed elsewhere (Brosens et al., 2004; Jubbour et al., 2006; Critchley and Saunders, 2009).

Methods

PubMed (Medline) and Ovid searches using the key words ERβ, endometrium, endometrial cancer, endometriosis, polycystic ovarian syndrome, infertility, menorrhagia and uterus were carried out systematically for publications from January 1996 until February 2014. All studies examining ERβ expression or function in non-pregnant endometrium or primary cells or tissue explants in culture derived from the endometrium, including human and animal studies and endometrial cell lines, were considered.

Estrogen receptors

The first known ER subtype, ERα, was identified in the rat uterus in 1966 (Toft and Gorski, 1966), followed by the cloning of human ERα cDNA (gene ESR1) in 1985 (Walter et al., 1985). This was followed by the discovery of a second ER subtype, ERβ, in the prostate and ovaries of rats in 1996 (Kuiper et al., 1996). The human ERβ gene ESR2 was cloned first in 1996 from the testis (Mosselman et al., 1996) and subsequent work has increased understanding of the physiological and pathological action of E2 in human cells and tissues. E2 may also bind to transmembrane GPER, which mediates rapid signalling events traditionally associated with G-protein-coupled receptors; this receptor is reviewed elsewhere (Prossnitz and Barton, 2014), and it is therefore outside the remit of this review. Interestingly, the pivotal accepted role of ERs in reproduction is a late evolutionary development, as there is evidence suggesting that in early order invertebrates, the reproductive role of E2 is not mediated by ER and may take place through ancient, ER-independent pathways (Thornton et al., 2003; Keay et al., 2006). Cloning, genome mapping and phylogenetic analysis studies have indicated that ER isoforms are likely to have been generated by duplication of the Esr gene early in the vertebrate lineage (Thornton 2001; Wu et al., 2003). It is proposed that the functions of an ancestral gene are partitioned among duplicate genes by complementary loss of tissue-specific expression (Thornton, 2001; Wu et al., 2003). The tissue-specific expression of two ER isoforms and their splice variants, therefore, provides the potential for very flexible regulation of target tissues by E2.

ERβ can have opposing actions to ERα on the same gene promoter in response to E2 (Smith et al., 2004; Thomas and Gustafsson, 2011). These inhibitory effects of ERβ on ERα activity may be exerted through a combination of altered recruitment of key transcription factors and increased ERα degradation (Matthews et al., 2006). In contrast, ERβ expression is induced by E2 acting via ERα and may be suppressed by hypermethylation of the ERβ promoter (Rody et al., 2005). The pro-proliferative function of ERα is essential for reproduction, yet is associated with obvious E2-associated health risks in the endometrium (and in other organs) (Koos, 2011; May, 2014). Therefore, the ERα opposing activity of ERβ has been of particular interest with the emergence of new receptor isotype-specific pharmacological modulators.

ERβ structure

ERβ is a member of the Class I nuclear hormone receptor superfamily of ligand-inducible transcription factors and shares the common, evolutionarily conserved structural and functionally distinct domains of other superfamily members (Matthews et al., 2006) (Fig. 1A). This includes a central, highly conserved DNA binding domain (DBD), which binds to the same estrogen responsive element (ERE) as ERα in the target gene promoters; a multifunctional ligand-binding domain (LBD) at the C-terminal; the ligand-dependent activation function 2 (AF2) at the C-terminal; and the constitutively active AF1 at the N-terminal and flexible-hinge D-domain between the LDB and the DBD (Fig. 1A) (Nilsson et al., 2001; Harnish, 2006). Transcriptional activation of ERβ is facilitated by two acidic activation domains, AF1 and AF2, which recruit a range of specific co-regulatory protein complexes to the DNA-bound receptor (Fig. 1B) (Benecke et al., 2001). Although there is close homology in the DBD (97%) and LBD (60%) between the two ER subtypes (Fig. 1A), significant divergence exists between the N-terminal regions, where only 20% of amino acid identity is shared. ER subtype-specific (Mosselman et al., 1996), promoter-specific and cell-specific E2 actions on target genes are therefore thought to be due to this highly variable N-terminal domain and ligand-independent AF1 (Katzenellenbogen et al., 2001; Hawse et al., 2008; Kumar et al., 2012). Despite their close homology, the ERβ (ESR2) gene is located on chromosome 14, whereas ERα protein is coded by a different gene (ESR1) located on chromosome 6 (Menasce et al., 1993; Enmark et al., 1997). The human ERβ (ESR2) gene is highly conserved with that of other higher order primates such as chimpanzee, rhesus monkey and orang-utan (Lewandowski et al., 2002).

Five alternatively spliced transcript variants of the ERβ (ESR2) gene have been described to date as ERβ1–5 (Moore et al., 1998) (Fig. 1A, and published primer sequences for splice variants included in Supplementary data, Table S1), although the characterization of the functional isoform pattern in human endometrium is not complete. The 530-amino acid human ERβ isoform is currently regarded as the wild-type ERβ1 (Leygue et al., 1998). Unlike ERα, in addition to ERβ1 at least two other splice variants, ERβ2 and ERβ5, are transcribed with all three proteins being identical except for the C-terminus; all are expressed as proteins and have been described in the endometrium (Collins et al., 2009). ERβ2 does not bind to the ligand or make homodimers and the C-terminus truncations of both ERβ2 and ERβ5 proteins may affect their ligand binding capacities, although they can form
heterodimers with either ERβ1 or ERα (Ogawa et al., 1998; Fujimura et al., 2001). Very little is known about ERβ5, which has only been described in the context of malignant endometrium (Collins et al., 2009). The isoforms may differentially modulate E2 signalling and, as a consequence, impact target gene regulation (Ramsey et al., 2004). Tissue and species-specific expression of the isoforms of the splice variants of ERβ mRNA and products has been described and may have functional consequences in ERβ-mediated responses (Weiser et al., 2008). Some of the existing contradictory reports of ERβ expression may be explained by the fact that studies may either have employed non-specific primers which did not distinguish the splice variants or examined a single splice variant in isolation, disregarding the potential collective effect of co-existing variants. The lack of commercially available specific antibodies to these alternatively spliced variants of ERβ has been the major obstacle for researchers investigating their specific function in vivo. Reliable and specific antibodies for these splice variants are therefore urgently required to unravel the important information on the functional and clinically relevant involvement of ERβ and its splice variants in human diseases. However, most of the available functional data only mention either ERβ or ERβ1, with little reference to the other variants.

Figure 1 (A) Schematic illustration of the comparative structures of ERβ splice variants and ERα isoform. All receptor isoforms contain the distinct DBD, LBD and activation function 1 and 2 (AF1/AF2) regions. ERβ1 is the wild-type ERβ with 530 amino acids (aa). (B) Co-activators and suppressors of ERβ. A graphical illustration of some known co-activators and repressors of ERB. ER, estrogen receptor; AF1/AF2, activation function 1 and 2; SRA, steroid receptor RNA activator; p68, the DEAD box proteins DDX5; SRC 1, 2 and 3, steroid receptor co-activator 1–3; CBP, CREB-binding protein; p300, E1A-associated protein p300; TRAP220, mediator complex subunit 1; DRIP, vitamin D3 receptor interacting protein; TRAP, thyroid hormone receptor; ASC-1 and 2, activating signal co-integrator 1, 2; RTA, repressor of tamoxifen transcriptional activity; NCoR, nuclear receptor corepressor; SMRT, silencing mediator for retinoic acid and thyroid hormone receptors; RIP140, receptor-interacting protein 140; E6-AP, E6-associated protein; RPF1, ribosome production factor I homologue; PGC1, peroxisome proliferator-activated receptor γ coactivator 1–α; CAPER, coactivator of activating protein-1 and estrogen receptors; CoAA, coactivator activator; CARM1, coactivator-associated arginine methyltransferase 1; PRMT1, protein arginine methyltransferase 1; CoCoA, calcium binding and coiled-coil domain containing protein 1; mSiah2, seven in absentia 2.
This review of the functional role of ERβ therefore mainly focuses on ERβ1, unless otherwise stated.

**ERβ receptor activation**

ERβ binds E2 with similar affinity to that of ERα and upon binding to the ligand, the activated receptor may exert effects involving the classical hormone signalling pathway (Pastore et al., 2012). This involves ERβ dimerizing, translocating to the nucleus, binding to the ERE located in the respective gene promoters to initiate recruitment of co-activators, co-repressors and chromatin-remodelling factors to either activate or repress transcription of target genes (McDonnell and Norris, 2002; Saxon and Turner, 2005; Zhang and Trudeu, 2006; Zhao et al., 2008; Fig. 2). DNA binding of ERβ, and hence its nuclear localization, is reported by some to be rapidly lost at body temperature when the ligand, E2, is absent (Pace et al., 1997; Tan et al., 1999). Although these findings have been described in the context of the classical hormonal pathway, recent literature examining a new class of ERβ-selective compounds has demonstrated activation of multiple endogenous genes through ERβ by selectively recruiting ERβ and co-activators to target genes without binding to ERβ (Vivar et al., 2010). This novel pathway of ligand-independent ERβ activation may play an important role and needs to be explored further in the future to understand the collective and the full impact of both classical and non-classical pathways in ERβ function. ERβ1 can form homodimers or heterodimers with ERα and ERβ splice variants (e.g. ERβ2), and therefore, the expression and availability of each ER subtype in a cell will influence the cell-specific response to E2 (Kuiper and Gustafsson, 1997; Tremblay et al., 1999). At a subcellular level, ERβ is localized in the nucleus, cytoplasm and mitochondria and this is regulated by both the availability of the ligand and by the co-expression of ERα/ERβ2 (Chen et al., 2007; Milanesi et al., 2009). Furthermore, ligand-bound ERβ can interact directly with other transcription factor complexes and influence the transcription of genes that do not possess the ERE in their promoter (Kushner et al., 2000). In the presence of E2, ERβ is able to oppose the effects of tissue-specific ER modulators such as tamoxifen via these indirect pathways (Paech et al., 1997). Therefore, the availability and type of the estrogenic agonist, the cellular expression of steroid receptors (including ERs and their respective isoforms, as well as other hormone receptors) and the expression of co-regulators, all influence cellular expression of ERβ and ERβ-mediated gene expression in response to estrogen in any given tissue type.

**ERβ expression in normal endometrium**

**Evidence from transgenic mice: ERβKO mice**

E2 action is essential for normal development of female sexual characteristics; ERα is the main receptor responsible for these actions, as evidenced by the phenotype of the ERαKO mouse, which demonstrated a non-fertile and infantile phenotype (Lubahn et al., 1993). In contrast the
ERβ KO mouse was fertile; this observation may have led to a relative lack of interest from reproductive biologists to investigate ERβ expression and function in the endometrium (Weihua et al., 2000). Although fertile, ERβ KO mice did display some reproductive deficiencies with a subfertile phenotype and an exaggeration of the endometrial epithelial proliferative response to E2, suggesting a suppressive role of ERβ on the actions of ERα (Wada-Hiraike et al., 2006). However, there is evidence that ERβ could partially compensate for the loss of ERα in the genital tract since the uterine phenotype of ERαβ double KO mice has been described to be similar to that of an aggravated ERαKO uterine phenotype, whereas the ERβ KO genital tract appeared to be normal (Dupont et al., 2000). The decrease in ovarian production of E2 was thought to be responsible for the reduced fertility and smaller litter size seen in ERβ KO animals and therefore, these animals initially were not thought to have a defect in implantation or placentation (Weihua et al., 2000).

Normal endometrial epithelial cell maturation was associated with the attainment of apical—basal polarity, which relied on formation of intercellular adherent junctions that provides a structural foundation for normal epithelial architecture (Valentijn et al., 2013). E-cadherin, localized to the lateral membrane of differentiated epithelia, is essential for the maintenance of functional junctions and was deficient in ERβ KO mouse endometrium (Wada-Hiraike et al., 2006), resulting in deformed glandular genesis and loss of glandular differentiation. Recent work has highlighted the importance of endometrial glands in implantation (Filant and Spencer, 2013), suggesting that, despite earlier beliefs, ERβ may indeed have an important role to play in the reproductive function of the endometrium. Treatment with E2 increased both stromal and epithelial ERα and ERβ expression in wild-type, ovariectomized mice, whilst combined E2 and progesterone treatment decreased expression of ERβ in endometrial epithelium (Wada-Hiraike et al., 2006). Conversely, ERβ KO mice showed up-regulation of progesterone receptor (PR) in response to E2 compared with the wild-type mice, suggesting that ERβ represses epithelial PR expression (Wada-Hiraike et al., 2006). There are no reports of detailed examination of the proliferative effect of endometrial epithelial cells in ERαβ double KO mice in response to E2 and the published studies only describe the uterine phenotype in comparison with wild-type mice (Dupont et al., 2000). ER subtype-specific ligand studies have also indicated that ERβ can modulate ERα activity in a response specific manner (Frasor et al., 2003). When ERα was selectively deleted in the mouse uterine epithelium, although the E2-induced initial epithelial mitogenic response remained intact, prolonged E2 treatment induced an increase in epithelial apoptosis, indicating that the protective effect of E2 against uterine epithelial apoptosis is mediated via ERβ (Winuthayanon et al., 2010). These observations further suggest that the direct action of E2 on endometrial epithelial cells via ERβ is to induce apoptosis.

Ovariectomized ERβ KO mice showed an aberrant hyper-proliferative response in the absence of E2 (Weihua et al., 2000). These mice would have low E2 and absent progesterone, but relatively high androgen levels of adrenal origin. In breast epithelium, androgen up-regulated ERβ via androgen receptor (AR) and inhibited proliferation (Rizza et al., 2014); if a similar mechanism exists in the endometrium, the action of androgens in the absence of ERβ may be to stimulate proliferation.

**Primates studies**

Primates menstruate and have an endometrial cycle and structure similar to that of humans. In primates such as macaques, ERβ expression does not change across the menstrual cycle in either the stratum functionalis or the stratum basalis. Only ERβ has been reported to be expressed in the endometrial endothelial cells throughout the menstrual cycle and ERβ has been proposed to regulate the angiogenic and vascular changes that occur in embryo implantation, early placentation and the maintenance of pregnancy (Slayden and Brenner, 2004). In the marmoset monkey, ERβ was highly expressed in endometrial epithelial cells throughout the menstrual cycle and in pregnancy. Increased stromal ERβ expression was observed in the late proliferative phase with the staining index decreasing by half as the secretory phase progressed and remaining low in pregnancy (Silvestri and Fraser, 2007). Treatment with GnRH agonists or ovariectomy caused significant reductions in PR and ERβ expression, but not in ERα when compared with the late proliferative phase of the normal menstrual cycle. In rhesus macaques, ERβ expression was increased with E2 treatment in simulated cycles and the levels decreased in the epithelial cells in the stratum functionalis with the subsequent combined treatment with E2 and progesterone (Crichtley et al., 2001). These authors also reported static expression of both ER isoforms in the stratum basalis across the menstrual cycle (Crichtley et al., 2001). When compared with other animals, primate endometrium is the closest to that of humans with obvious similarities. However, there are subtle yet striking differences in endocrinology; for example, E2 levels rise in the mid-secretory phase of the cycle in humans, but this is not observed in non-human primates (Narkar et al., 2006). Therefore, caution should be exercised when extrapolating primate data to human endometrial physiology, including ERβ expression and function.

**ERβ in the endometrial vasculature**

Benign angiogenesis is a unique property of endometrium which is essential for normal regeneration of the stratum functionalis after menstrual shedding and is also a fundamental feature of the E2-dominant proliferative endometrium (Nayak and Brenner, 2002). Most described trophic effects of E2 on this benign endometrial angiogenic process are exerted via ERα either directly or indirectly, acting on endometrial epithelial and stromal cells to secrete angiogenic growth factors (Rees and Bicknell, 1998). The evidence that E2 has a direct action on endometrial vessels is suggested by reports describing the presence of ER isoforms in the endometrial vasculature. Nevertheless, there is significant controversy regarding cyclical variation in expression of ER subtypes, including ERβ, in the endometrial vascular cells. Although all studies report expression of ERβ by endometrial vessels (Crichtley et al., 2001; Lecce et al., 2001; Greaves et al., 2013), some have suggested that endometrial endothelial ERβ expression may be dynamically regulated during the menstrual cycle (Lecce et al., 2001), while others reported non-cyclical constitutive expression (Crichtley et al., 2001). Lecce et al. (2001) reported expression of both ERα and ERβ in endometrial endothelial and vascular smooth muscle cells at different phases of the menstrual cycle, although in the menstrual phase, when ovarian hormone levels are at a nadir, both ER isoforms were reported to be absent from the vascular compartment (Lecce et al., 2001). The ERβ expression by the vascular smooth muscle cells was highest in the late secretory phase, whereas those who reported detection of ERα in the endothelium noted the highest endothelial ERα expression to be in E2-dominant mid-cycle endometrium (Lecce et al., 2001). There are also conflicting reports of PR expression by vascular endothelium with some reporting
PR mRNA to be present (Krikun et al., 2005), whilst all existing reports assert that PR protein is not expressed by these cells (Critchley et al., 2001).

In summary, the human data suggest that ERβ is the main ER subtype in endometrial endothelial cells and that expression may be hormonally regulated, although precise receptor expression remains to be fully clarified (Critchley et al., 2001; Lecce et al., 2001; Kayisli et al., 2004; Krikun et al., 2005). Subsequently, in an elegant set of rodent experiments, Masuda et al. (2007) demonstrated that bone marrow-derived endothelial stem/progenitor cells preferentially express ERα, and that physiological post-natal vascular regeneration is E2 regulated via ERα (Masuda et al., 2007). In the endometrium of rhesus macaques (Macaca mulatta), ERβ is the only steroid receptor to be expressed by endothelial cells and perivascular smooth muscle cells, but the perivascular stromal cells expressed all types of steroid receptors (Slayden and Brenner, 2004). The exact ER isoform regulating the vascular remodelling and neovasculogenesis that occurs in regular human endometrial regeneration during the menstrual cycle and after parturition is therefore not yet conclusively established, although there is no doubt that ERβ exists in these cells, with evidence that it is likely to play a pivotal role in that process.

ERβ in endometrial immune cells

Immune cells are a major component (>20% of the stromal cells in the late secretory phase) of the endometrium; the cell numbers change in the stratum functionalis according to the ovarian hormone cycle with higher levels seen in the secretory phase, particularly in late secretory phase endometrium (Bulmer et al., 1991b; Berbic and Fraser, 2013). The majority of endometrial leucocytes in the stratum functionalis consist of three cell types: T cells, macrophages and uterine natural killer (uNK) cells, with very few neutrophils except in menstrual endometrium, and rare B cells (Vassiliadou and Bulmer, 1996), although B lymphocytes are seen in the stratum basalis (Bulmer et al., 1988; Marshall and Jones, 1988). The observation that the stromal leucocytes increase in number during the window of implantation is a compelling reason for implicating endometrial leucocytes with a key role in the implantation process, and the immunological maintenance of pregnancy (Blois et al., 2011; Evans et al., 2011). Because of their frequency in the late secretory phase and early pregnancy, as well as the dramatic increase in numbers around the time of expected implantation in a fertilized cycle, many studies have focused on the uNK cells. Recent work has focused on their potential role in spiral artery remodelling in early pregnancy (Robson et al., 2012), although other roles suggested relate to control of trophoblast invasion, immunosuppression and cytokine secretion, amongst others (reviewed in Lash et al., 2010). The available evidence regarding endometrial stromal immune cell numbers and composition in the endometrial stratum basalis and in post-menopausal endometrium is particularly scarce and, for the reasons previously alluded to, evidence on any aberrations of these cell numbers or their function in endometrial pathologies is generally limited to the stratum functionalis and at best is confusing, due to the lack of established and agreed methods of assessment. In humans, uNK cells increase in number dramatically in the mid-secretory phase of the menstrual cycle, although the explanation for this increase remains uncertain. This has led to investigation of expression of steroid hormone receptors by uNK cells and in particular the expression of PR since uNK cells are also prominent in progesterone-treated endometrium. Mouse uNK cells are devoid of both ER receptor subtypes (Borzychowski et al., 2003), but human uNK cells purified from early pregnancy decidua contained mRNA for both ERβ1 and ERβ2, whilst not expressing ERα or PR transcripts; using immunohistochemistry only ERβ1 protein was expressed in uNK cells of non-pregnant endometrium, with no ERβ2 protein (Henderson et al., 2003). Expression levels for ERβ splice variants in mRNA from purified uNK cells from non-pregnant endometrium are not yet known. This highlights the species-specific differences in regulation and function of the various endometrial cell types. Furthermore, ERβ expression and specific functions have been described in T-cells (Rider et al., 2006) and macrophages (Kramer and Vray, 2002), yet the ERβ expression of endometrial T-cells and macrophages has not been specifically examined. Studies in KO mice and in other murine models have suggested that in T-cells, E2 modulation of the immune response may depend on the origin of the T-cells, and may be tissue-specific (Maret et al., 2003; Wu et al., 2013). Hence, there is a need for further studies to characterize steroid receptor expression by human endometrial immune cells.

ERβ in the human uterus

Expression of ERβ mRNA and proteins has been documented in human endometrium across the menstrual cycle and in post-menopausal endometrium. The method employed in most studies of ERβ expression in endometrium has been polymerase chain reaction (PCR), and the level of mRNA detected for ERβ at any time in the menstrual cycle has been reported by most researchers to be significantly lower than that for ERα (Matsuzaki et al., 2000; O’Neill et al., 2004). However, many studies either using immunohistochemistry to localize ERβ protein at the cellular level with analysis by a variety of semi-quantitative methods or using western blotting reported that the levels of ERβ protein in human endometrium are either comparable with or in excess of those of ERα in the endometrium (Villavicencio et al., 2006; Wu et al., 2012). ERβ is expressed in all endometrial cell types, including glandular epithelium and stromal cells. ERβ is reported by some groups to be the sole ER expressed in many specific cell types within the endometrium, including the endometrial endothelium (Taylor and Al-Azzawi, 2000; Critchley et al., 2001) and uNK cells (Henderson et al., 2003), although as stated before (in the ERβ in endometrial immune cells section), others have reported conflicting results (Lecce et al., 2001).

ERβ expression in the developing uterus

Studies in ERβ KO mice suggest that ERβ may play an important role in maintaining endometrial quiescence, particularly in the immature uterus (Weihua et al., 2000). Similarly in the pre-pubertal human uterus, ERα and ERβ are expressed in both endometrial epithelium and stroma during early development before maturity, whereas menarche (maturation)-associated proliferation coincides with an exclusive increase in ERα expression (Spencer et al., 2011).

ERβ expression in healthy adult human endometrium across the menstrual cycle

The dynamic expression pattern of ovarian steroid receptor proteins and mRNA in the endometrium according to menstrual cycle phase has been well established. Ligand-activated ERα induces expression of both ER subtypes, PR and AR in the endometrium (Brosens et al., 2004). Progesterone, working through PR, counteracts these effects of E2 on steroid receptor expression, and also prevents proliferation of endometrial epithelial cells, promoting epithelial differentiation and stromal
decidualization in preparation for embryo implantation (Critchley and Saunders, 2009).

ERβ mRNA expression across the menstrual cycle is much lower than that of ERα, despite the high levels of ERβ protein expression throughout the cycle (Matsuzaki et al., 1999). ERβ mRNA is present in epithelial, stromal and endothelial cells, with the highest levels seen in the epithelial cells (Matsuzaki et al., 1999; Critchley et al., 2002). In the estrogen-dominant proliferative phase, nuclear ERα protein levels are high in all endometrial cell types with moderate levels of nuclear ERβ protein. Expression of both ER subtypes increases in the late proliferative and early secretory phases and subsequently decreases in the mid-late secretory phase, yet ERβ is the predominant ER subtype in the late secretory phase endometrial stroma (Critchley et al., 2001; Lecce et al., 2001).

Whilst stromal ERβ expression is increased or maintained in the late secretory phase, epithelial expression of ERβ protein decreases in common with ERα (Critchley et al., 2001, 2002; Lecce et al., 2001). Therefore, in the mid-late secretory phase in particular, where endometrial ERα expression diminishes, ERβ remains as the predominant ER isoform in the stratum functionalis (Lecce et al., 2001). Interestingly, ERβ2 is also expressed in both stromal and epithelial cell compartments, similar to ERβ1, but there is a significant decrease in ERβ2 in the glandular epithelium of the stratum functionalis in the mid-secretory phase, whilst ERβ1 persists (Critchley et al., 2002). This may suggest that ERβ1 is able to largely form homo-dimers and become dominant in response to E2 in the mid-secretory phase endometrial stratum functionalis when circulating E2 levels are still high. Intriguingly, the mid-secretory phase of the cycle in humans is also known to be associated with increased E2 levels and a reduction in endometrial glandular proliferative activity (Critchley et al., 2006; Narkar et al., 2006; Cooke et al., 2013). ERβ expression in the epithelial compartment of the stratum functionalis correlates with the time when highest E2 levels are seen in the menstrual cycle (late proliferative, early secretory phases) (Lecce et al., 2001), whereas stromal and vascular ERβ levels peak in the late secretory phase, with the plateaued second peak of circulating E2 (Lecce et al., 2001) (Fig. 3). Since both E2 via ERα and progesterone via PR have been shown to increase ERβ transcripts, we postulate that ERβ is the main safety mechanism whereby the potent mitogenic action of E2 is restricted in the healthy endometrium.

It has been postulated by many authors that the stratum basalis of the endometrium may be less responsive to ovarian hormone regulation than the stratum functionalis (Prianishnikov, 1978; Padykula et al., 1984). Expression of ERβ in the stratum basalis has been reported to remain static across the menstrual cycle (Critchley et al., 2001): this particular study included a modest 32 full-thickness endometrial samples at different time points in the cycle, utilized an immune scoring method that examined only the intensity of the staining and also did not examine co-expression of other hormone receptors such as PR and AR. ERβ2 expression was very low in the stratum basalis compared with ERβ1, suggesting that ERβ1 is the dominant splice variant in the stratum basalis (Critchley et al., 2002).

The reason why the stratum functionalis of the endometrium but not the stratum basalis responds to ovarian hormonal signals, despite both layers expressing all classical ovarian hormone receptors, is not fully understood. ERα and PR are present, but their levels in the stratum basalis and the cyclical changes in their expression levels are controversial with reports ranging from alterations in the secretory phase to no change across the menstrual cycle (Critchley et al., 2001, 2002; Leyendecker et al., 2008; Fig. 3). There are no current studies that examine simultaneously the expression (and therefore interplay) of all ovarian steroid receptors (ERα, ERβ, PR and AR) in these two functionally very distinct endometrial layers of healthy endometrium (Fig. 3). Therefore, data on spatial and temporal differences in the expression of all steroid hormone receptor types in human endometrium remain inconclusive.

**Role of ERβ in endometrial regeneration**

The hypothesis that endometrium regenerates from the hormonally resistant stratum basalis came initially from Prianishnikov (1978). Other studies suggested that although the initial part of post-menstrual endometrial regeneration is independent of estrogen, subsequent post-menstrual repair growth is estrogen-dependent (Ferenczy, 1976). The stratum basalis is widely accepted as the germinal compartment of the endometrium where stem progenitor cells reside (Padykula et al., 1989; Valentijn et al., 2013) and ERβ1 and ERβ2 are expressed by all cell types in the stratum basalis (Critchley et al., 2002). Many investigators of endometrial stem progenitor cells have reported a reduction or lack of ERα and PR in the more primitive cells with progenitor activity (Chan and Gargett, 2006; Valentijn et al., 2013). In mouse endometrium, the endometrial epithelial progenitor cell pool expanded dramatically despite the lack of expression of both ERα and PR (Janzon et al., 2013). However, none of the descriptive papers of endometrial stromal/epithelial stem/progenitor populations in either animal or human studies examined or commented on ERβ expression (Chan and Gargett, 2006; Cervello et al., 2010; Masuda et al., 2010; Janzon et al., 2013), and post-partum vascular regeneration (thought to involve stem cells), although E2-dependent, has been reported to be mediated via ERα rather than ERβ (Masuda et al., 2007). Furthermore, studies on mouse endometrial regeneration provide evidence that the primitive label-retaining cells are estrogen responsive (Gargett et al., 2012). Therefore, further work is needed to examine expression of ERβ and other hormone receptors in the primitive epithelial and stromal stem/progenitor population, since the observed (possible direct) stimulatory effect of E2 on the progenitor population may be mediated via ERβ.

**ERβ expression in the healthy human post-menopausal endometrium**

Normal post-menopausal endometrium is the remaining stratum basalis after the cessation of the ovarian hormonal cycle. Similarities between the premenopausal stratum basalis and post-menopausal endometrium have been described, both in the gene expression profile (Nguyen et al., 2012) and in expression of epithelial markers (Valentijn et al., 2013). However, the hormonal milieu of pre- and post-menopausal endometrium is clearly different (Labrie, 2014). Post-menopausal endometrium is exposed to relatively low E2 levels, absent progesterone and relatively unchanged androgen levels of adrenal origin (Yasui et al., 2012). ERβ expression in post-menopausal endometrium was reported to be weaker than ERα expression in both stromal and epithelial compartments (Zang et al., 2008). Another small immunohistochemical study which included only 11 post-menopausal patients reported down-regulation of both ERs in post-menopausal endometrium compared with the late proliferative phase (Mylonas et al., 2007). Others have reported moderate to strong ERβ immunostaining in all cell types in normal post-menopausal endometrium which did not change in hormone-treated (continuous combined E2 and progesterone hormone
replacement therapy) post-menopausal endometrium (Vani et al., 2008). A further study described expression of ERβ1 and ERβ2 in up to 29 and 21% of healthy post-menopausal endometrium, respectively, and concluded that there is likely to be an antiproliferative role in both endometrium and breast tissue (Cheng et al., 2007). Combined treatment with E2 and testosterone, however, increased ERβ expression in post-menopausal endometrium (Zang et al., 2008), and the antiproliferative effect of androgen treatment on the endometrium is thought to be via increased ERβ expression. However, androgen metabolites generated by the aromatase-independent enzymes activate both ER subtypes and ERβ activation by these metabolites may represses ERα in the post-menopausal endometrium (Hanamura et al., 2014).

**Figure 3** Expression of ERs in normal, healthy human endometrium. Immunohistochemical staining of paraffin-embedded full thickness human endometrial tissue sections demonstrating brown (DAB) positive nuclear ERβ (mouse monoclonal antibody PPG5/10 MCA1974S, Serotec, Kidlington, UK; pretreatment pressure cook in citrate pH6; incubation 1:50 overnight at 4°C) (A–F) and ERα (rabbit polyclonal ab37438, Abcam, Cambridge, UK; pretreatment pressure cook in citrate pH6; incubation 1:50 for 2 h at 20°C) (G–L) in the stratum functionalis (A, B, G and H) and stratum basalis (D, E, J and K) of proliferative (A, D, G and J), secretory (B, E, H and K) and post-menopausal (C, F, I and L) endometrium. Magnification ×200 except C, I ×100.
ERβ in endometrial pathology

Endometriosis and adenomyosis

Endometriosis is a common benign gynaecological condition defined by the presence of endometrium-like tissue outside of the endometrial cavity (Bernardi and Pavone, 2013; Sourial et al., 2014). Adenomyosis is defined as the presence of endometrium within the myometrium (Hapangama and Bulmer, 2014). Aberrant cell proliferation and altered cell fate have been described in eutopic and ectopically grown endometrium and in adenomyosis; estrogen dependence may play a role in the pathophysiology of both conditions (Yang et al., 2007; Hapangama et al., 2009). Although this may immediately suggest a trophic estrogenic effect via ERα, ectopic endometrioid lesions and adenomyosis have been reported to express high levels of ERβ (>100×) compared with the eutopic endometrium (Bulun et al., 2012). The available evidence regarding levels of ERα and ERβ expression in ectopic endometriotic lesions is, however, contradictory (Shao et al., 2008). Factors that are likely to contribute to the variable results include the heterogeneity of the lesions studied, as well as the fact that when surgically excised ectopic endometriotic lesions are examined using techniques such as PCR and western blotting which homogenize the whole tissue the contribution from endometrial-gland and stroma-like tissue to the levels reported can vary widely.

Relative over-expression of ERβ mRNA (therefore decreased ERα:ERβ mRNA ratio) in ovarian endometriomas compared with either peritoneal ectopic lesions (Bukulmez et al., 2007) or eutopic endometrium (Brandenberger et al., 1999; Fujimoto et al., 1999; Bukulmez et al., 2007) has been described suggesting a unique E2-dependent growth of ovarian endometriomas. Deficient methylation of the ERβ promoter resulting in pathological overexpression of ERβ in endometriotic stromal cells has been suggested by some authors who also report relatively low ERα expression in these cells (Bulun et al., 2012). A high level of ERβ has been proposed as the reason for low ERα expression, resulting in low PR levels contributing to progesterone resistance and inflammation. ERβ knockdown has been reported to significantly increase ERα mRNA and protein levels in endometriotic stromal cells (Trukhacheva et al., 2009; Bulun et al., 2012). This theory is reviewed extensively (Bulun et al., 2012). Conversely, ERβ overexpression in endometrial stromal cells was reported to decrease ERα mRNA and protein levels and ERβ knockdown significantly decreased proliferation of endometriotic stromal cells (Trukhacheva et al., 2009). In human endometriotic lesions (Fujimoto et al., 1999; Bukulmez et al., 2007) and in induced lesions in endometriosis models in baboons (Fazleabas et al., 2003), and in rodents (Han et al., 2012), there was decreased ERα, while ERβ was maintained. Compared with healthy women, women with endometriosis and adenomyosis showed a decrease in the ERα/ERβ ratio in proliferative phase eutopic endometrium, suggesting ERβ dominance (Juhasz-Bass et al., 2011; Mehasseb et al., 2011), although there is no conclusive evidence suggesting differential proliferative activity in the proliferative phase endometrium of women with endometriosis. Most cellular aberrations described in the eutopic endometrium have been observed in the stratum functionalis in the secretory phase where a persistence in proliferative activity is detected (Hapangama et al., 2009, 2012) and ERβ expression in women with endometriosis in the secretory phase has been reported to be unchanged (Hudelist et al., 2005) or decreased (Hapangama et al., 2008b). In the window of implantation, endometrial stratum functionalis of women with endometriosis may also have a reduction in ERβ expression compared with healthy controls (Hapangama et al., 2008b). Further studies have shown higher ERα mRNA levels in endometriotic lesions compared with the eutopic endometrium (Matsuzaki et al., 2000, 2001; Fig. 4). Furthermore, ERβ promoter methylation has been proposed as the primary defect resulting in differential ERβ expression between ectopic and eutopic endometrium (Xue et al., 2007).

It is of interest that the pattern of ERβ expression reported in both adenomyosis and ectopic endometriotic lesions was similar to that of the endometrial stratum basalis (Mehasseb et al., 2011), which in turn is proposed to be unresponsive to hormones. There are suggestions from primate (Donnez et al., 2012; Sourial et al., 2014) and human studies (Leyendecker et al., 2002; Valentinj et al., 2013) that endometrial stratum basalis plays an essential role in the pathogenesis of endometriosis. This may suggest that the regulation of cell fate in these pathological lesions is distinct from that of the differentiated stratum functionalis in the eutopic endometrium but rather is similar to the stratum basalis that contains the germinal capacity. Further exploration of their similarities to the germinal stratum basalis may improve our understanding of endometrial cellular growth and regeneration.

Polycystic ovary syndrome

The clinical phenotype of polycystic ovary syndrome (PCOS) includes reproductive and hormonal aberrations. The endometrial consequences are those of a high and unopposed effect of estrogen, and possibly androgens (Hapangama and Bulmer, 2014). The aetiology of PCOS is not fully understood and using genotyping a +1730 G/A polymorphism in the ERβ gene has been proposed to be associated with susceptibility to PCOS (Kim et al., 2010). Homozygous ERβKO mice have defective ovulation reminiscent of PCOS in humans (Imamov et al., 2005). It has been postulated that women with PCOS exhibit a lower pregnancy rate secondary to decreased endometrial expression of both ERα and ERβ during the window of implantation, potentially decreasing endometrial receptivity, reducing conception and hence lowering fertility (Wang et al., 2011). However, investigation of the differential ER subtype expression in women with PCOS compared with healthy women has not revealed a definitive pattern (Maliqueo et al., 2003). Anovulatory PCOS is associated with a 3- to 4-fold increased risk of developing EC compared with unaffected women and the effect is postulated to be due to the progesterone unopposed action of E2 (Pearlney et al., 2010; Hapangama and Bulmer 2014), presumed to be via ERα. A gradual increase in ERβ levels from anovulatory endometrium to endometrial hyperplasia (EH) was seen in women with PCOS, suggesting either direct involvement of ERβ in endometrial proliferation or an indirect effect due to the action of ERα which can cause increased expression of all steroid receptors including ERβ (Villavicencio et al., 2006). Further studies are needed to evaluate the involvement of ER subtypes and the endometrial proliferative aberration that occurs in PCOS.

Endometrial polyps

Endometrial polyps are commonly occurring outgrowths of the endometrium consisting of a monoclonal overgrowth of endometrial stromal cells with inclusion of a non-neoplastic glandular component (Indraccolo et al., 2013). Endometrial polyps are usually benign but are occasionally associated with focal atypical hyperplasia or even
adenocarcinoma, and these findings are more common in post-menopausal women (Costa-Paiva et al., 2011). Endometrial polyps lack the cyclical changes seen in the adjacent endometrium and E2 stimulation is postulated as the main driving force for endometrial polyp formation (Van Bogaert, 1988). This is supported by the observation that the use of tamoxifen, which acts as an ER agonist on the endometrium, increases the risk of endometrial polyps (Erdemoglu et al., 2008; Tokyol et al., 2009). ERβ mRNA expression in benign endometrial polyps has been reported to be similar to the adjacent normal endometrium (Zitao et al., 2010), whilst protein expression in the stromal compartment was increased (Ye et al., 2006). In tamoxifen-treated endometrial polyps, glandular ERβ expression appeared to be lower than that of ERα, suggesting a lack of ERβ mediated opposition of ERα activity (Hachisuga et al., 2003). Further sufficiently powered studies are necessary to evaluate the involvement of ERβ in the pathogenesis of benign endometrial polyps.

Endometrial hyperplasia

EH is histologically defined as the abnormal overgrowth of endometrial glands in relation to the endometrial stroma (Ordi et al., 2013). Several classification schemes have been proposed and reproducibility is variable (Yang et al., 2012; Li and Song, 2013; Ordi et al., 2013). Unopposed estrogen stimulation, usually associated with anovulation or occasional ovulation in peri-menopausal women, is a common cause of EH, which may also be seen in 20% of women with PCOS with oligomenorrhea.
(Prakansamut et al., 2014). When cytological atypia is present, EH is associated with approximately a 30% risk of developing into or co-existing with EC (Lacey et al., 2007; Park et al., 2011). Transcripts of ERβ splice variants have been described in EH (Witek et al., 2001), and ERβ protein expression in EH without cytological atypia in women without PCOS has been reported to remain unchanged compared with normal proliferative endometrium, whilst in pre-cancerous atypical hyperplasia, ERβ expression decreases (Hu et al., 2005). This suggests that the loss of ERβ regulation of ERα-mediated proliferation results in EH.

**Endometrial cancer**

EC is the most common gynaecological malignancy and is usually a disease of the post-menopausal endometrium (Murali et al., 2014). In contrast with atrophic and inactive post-menopausal endometrium (McCluggage, 2011), the hallmark of EC is dysregulated and inexhaustible cellular proliferation (Owings and Quick, 2014). At least Type-I endometrioid EC is thought to be EZ-dependent (although recent results indicate that Types I and II ECs have similar risk factors), and unopposed EZ action is known to increase the risk of endometrial carcinogenesis (Setiawan et al., 2012). Therefore, conditions such as PCOS and obesity, where there is an excessive extra-ovarian conversion of adrenal androgens to estrogens, as well as HRT use, increase the risk of EC (Thanapprapasr and Thanapprapasr, 2013).

Available data on ERβ protein and mRNA expression in EC subtypes are largely confined to endometrioid EC (Table I). The general consensus that the carcinogenesis process in Type II ECs is EZ-independent is likely to be the reason for the lack of data on ERβ expression in Type II ECs in particular, with virtually no studies examining the ERβ expression in serous and clear cell carcinoma. Nevertheless, there are emerging data suggesting involvement of ERβ in carcinogenesis of other tissues, such as the intestine, that are typically not regarded as E2-responsive (Hogan et al., 2009), and hence future studies examining ERβ in type II ECs are warranted.

In addition to the wild-type ERβ1, the expression of splice variant isoforms ERβ2, β3, β4, β5 and exon deletes (βΔ1, βΔ2A5, βΔ4, βΔ6) has been described in endometrioid EC (Saegusa and Okayasu, 2000; Utsunomiya et al., 2000; Sakaguchi et al., 2002; Paul et al., 2004; Skrzypczak et al., 2004; Mylonas et al., 2005; Chakravarty et al., 2007; Collins et al., 2009; Zannoni et al., 2013). Similar expression patterns to ERα have been reported for ERβ variants (ERβ1, ERβ2 and ERβ5) in endometrioid EC samples: ERβ1 and ERβ2 immuno-expression was higher in low-grade ECs, whereas ERβ5 expression was constitutively intense regardless of the grade (Collins et al., 2009). In tamoxifen-associated ECs, ERβ expression was particularly prominent compared with spontaneous ECs (Negoita and Mihalovich, 2011). In a different study that included a semi-quantitative scoring system similar to the quickscore hormone receptor evaluation in breast cancer, ERβ1 and ERβ2 expression did not correlate with tumour grade, FIGO (International Federation of Gynecology and Obstetrics) stage or myometrial invasion, but the ERα/ERβ2 ratio was reported to be an independent prognostic factor of overall survival (Zannoni et al., 2013). The ERα/ERβ ratio has been used to evaluate the imbalance in the relative expression of ER isoforms in endometrial (Fujimoto et al., 2000; Jazaeri et al., 2001; Takama et al., 2001; Jongen et al., 2009; Šmuc and Ržner, 2009) and other hormonal-regulated malignancies, such as breast cancer, and has been suggested as a potential predictor of disease outcome (Sastre-Serra et al., 2013). Nevertheless, the existing data on the correlation between clinical outcome and the ERα/ERβ ratio are conflicting (Jongaen et al., 2009; Zannoni et al., 2013). Methodological differences (sample size and scoring methods) are likely to explain the observed discrepancies in the available literature. There are over 160 publications describing ERβ involvement in EC, yet the data on ERβ expression, correlation with disease stage and grade of histological differentiation remain controversial (Table I). Another reason for this is that not all studies included healthy control tissue comparators. Some studies have only described ERβ expression in neoplastic cells and the surrounding niche (Šmuc and Ržner, 2009). When healthy controls were used, they included either pre- or post-menopausal women, although in some studies, the menopausal status of the healthy control women was not even mentioned (Jarzabek et al., 2013; Knapp et al., 2013). When premenopausal control tissue was used, attention was not given to the known differences between menstrual cycle phase (Jazaeri et al., 2001), or in full thickness endometrial samples from hysterectomies, the differences between stratum basalis and functionalis, with no information provided regarding which layer was included in the analysis (Chakravarty et al., 2007; Knapp et al., 2013). Early studies also suffered from deficiencies in antibody and primer specificities, particularly when splice variants were not appreciated.

Breast and prostatic carcinogenesis has been reported to be associated with a decrease or loss of ERβ expression, and this is the basis for the hypothesis that ERβ plays a tumour suppressor role (Bottner et al., 2014). In breast and ovarian cancer, ERβ exerted anti-proliferative effects via prevention of ERα transcriptional complexes from activating c-myc, cyclin-1 and cyclin-A genes and induction of cyclin-dependent kinase inhibitors leading to arrest of the cell cycle in G2 phase (Paruthiyil et al., 2004). Similarly, in the endometrium (as described above), some authors have reported a decrease in ERβ expression in EC suggesting an analogous function. A decrease in both ERβ mRNA levels and protein (immunohistochemistry) has been reported in endometrioid EC compared with either adjacent normal endometrium or normal proliferative endometrium from healthy premenopausal control women (Paul et al., 2004; Hong-bing et al., 2008; Šmuc and Ržner, 2009). However, a potential oncogenic role has also been proposed by other authors showing an up-regulation of ERβ5 transcript in high-grade endometrioid EC, which was also associated with HER2 and MYBL2 oncogene expression (Skrzypczak et al., 2004; Häring et al., 2012b).

Since ovarian steroid hormone receptor expression is a feature of differentiated endometrial cells, with primitive or undifferentiated cells not expressing ER or PR, the diminishing steroid receptor expression levels in EC, including ERβ, may merely be a sign of loss of cellular differentiation and/or cellular transformation (Fig. 4). Alternatively, dysregulation of steroid receptor homeostasis owing to loss of PR and ERα with possible persistant expression and up-regulation of ERβ isoforms (ERβ3) and splice variants, particularly in high-grade and advanced stage endometrioid EC, may reflect a tumour-promoting role for ERβ in high-grade ECs. Data from ERαβKO mice may suggest ERβ could partially compensate for the loss of ERα (Dupont et al., 2000) and therefore the reported increase in ERβ in high-grade ECs may have a tumour-promoting effect similar to ERα. However, conclusive data on the functional role of ERβ or the splice variants on regulating EC growth or metastasis does not exist to date. The reason for the apparent contradictory data for ERβ to be a tumour suppressor and a promotor may be due to a tissue-specific dual role of ERβ; a tumour suppressor role in healthy
<table>
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<td>Utsunomiya et al. (2000)</td>
<td>ERβ</td>
<td>45 endometrioid G1 – 3</td>
<td>RT–PCR</td>
<td>No control</td>
<td>% positive</td>
<td>Polyclonal; Immunotech; ERβ Protein +, mRNA +, ERβ m RNA + in the cytoplasm of malignant cells</td>
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<td>Jazaeri et al. (2001)</td>
<td>ERβ</td>
<td>7 endometrioid G1</td>
<td>RT–PCR</td>
<td>7 pre M</td>
<td>ERβ mRNA + in PM and cancer groups</td>
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<td>Takama et al. (2001)</td>
<td>ERβI</td>
<td>33 endometrioid</td>
<td>Multiplex</td>
<td>I S H</td>
<td>ERβ/ERα ratio ↑ in cancer</td>
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<td>Fujimoto et al. (2000)</td>
<td>ERβI</td>
<td>20 stage I</td>
<td>RT–PCR</td>
<td>20 normal</td>
<td>H-score</td>
<td>Goat polyclonal L-20, Santa Cruz ≠ ERβ (protein and mRNA) were significantly lower than ERα in normal and cancer tissue</td>
</tr>
<tr>
<td>Paul et al. (2004)</td>
<td>ERβI, ERβΔ6</td>
<td>14 endometrioid G1, G2</td>
<td>RT–PCR</td>
<td>11 PP</td>
<td>ERβ1 ↓ in EC compared with PP</td>
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<tr>
<td>Skrzypczak et al. (2004)</td>
<td>ERβI – 5</td>
<td>19 stage I (A,B)</td>
<td>RT–PCR</td>
<td>6 PM</td>
<td>ERβ1, β2 and β5 + in EC</td>
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<td>Chakravarty et al. (2007)</td>
<td>ERβI, ERβ2/βcx</td>
<td>26 endometrioid cancer</td>
<td>RT–PCR</td>
<td>22 PP</td>
<td>ERβ1 + ↔ in benign and EC</td>
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<td>ERβI</td>
<td>315 endometrioid</td>
<td>TMA IHC</td>
<td>No control</td>
<td>ERβ/ERα ratio &gt; 1 associated with disease free and overall survival</td>
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<tr>
<td>Hong-Bing et al. (2008)</td>
<td>ERβI</td>
<td>68 endometrioid</td>
<td>IHC photo analysis</td>
<td>a</td>
<td>ERβ ↓ in atypical hyperplasia and EC (ERβ inversely correlate with ki67)</td>
<td></td>
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<tr>
<td>Collins et al. (2009)</td>
<td>ERβI, ERβ2, ERβ5</td>
<td>30 endometrioid G1 – 3</td>
<td>RT–PCR</td>
<td>No control</td>
<td>ERβ1, ERβ2 and ERβ5 (mRNA and protein) + in EC ↔ between the grades</td>
<td></td>
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<tr>
<td>Šmuc and Ržner (2009)</td>
<td>ERβ</td>
<td>24 endometrioid G1 – 3</td>
<td>RT–PCR</td>
<td>Normal adjacent</td>
<td>ERβ (and ERα) m RNA ↓, ERβ/ERα ratio ↑ (not significant)</td>
<td></td>
</tr>
<tr>
<td>Häring et al. (2012b)</td>
<td>ERβI – 5, ERβΔ1, ERβΔ2, ERβΔ3</td>
<td>46 endometrioid G1 – 3</td>
<td>RT–PCR</td>
<td>15 PP</td>
<td>ERβ1, ERβ2 ↔ ERβΔ1 ↑ in G1, 2 EC; ERβΔ4 ↓ in G2, 3 EC (ERβ1, ERβ2, ERβ5 correlate with HER2, 6 exon skip isoforms of ERβ correlate with HER2, MYBL, cyclin B1,D1,A1)</td>
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endometrium which expresses the full complement of other ovarian hormone receptor types and particularly ERα, and a potential tumour promoter role in high-grade EC cells that have lost other receptor subtypes. This is an interesting possibility that needs to be explored in future studies.

In summary, the exact role of ERβ in endometrial carcinogenesis and progression of EC is not yet fully known. Therefore, comprehensive characterization of ERβ expression in EC subtypes and further functional studies are required to reveal the involvement and contribution of ERβ in endometrial carcinogenesis. Since at least some ECs are likely to respond to hormonal chemotherapeutic agents, a detailed examination of all steroid receptor types and standardization of the methodology for quantification of hormone receptors in EC in a similar approach to that used for other hormone responsive cancers, such as in breast cancer, is urgently needed.

### Pharmacological regulation of endometrial ERβ expression

#### Evidence for regulation of endometrial ERβ expression by exogenous hormone treatment

There is evidence from both in vivo and in vitro studies that ERβ plays an important role in determining cellular responses to estrogens and anti-estrogens (Hall and McDonnell, 1999). Progesterone induced ERβ transcription whilst down-regulating the PR gene in telomerase immortalized endometrial epithelial cells (Hombach-Klonisch et al., 2005). In contrast, treatment of women with an intrauterine system containing the androgenic progestagen, Levenorgestrel, decreased epithelial and stromal ERβ (and also ERα and PR) expression after 3 months (Engemise et al., 2008). Therefore, the expected uterine/ovarian selective agonists in ovariectomized animals (Wang et al., 2002). The available evidence therefore suggests that the endometrial ERβ expression in response to exogenous hormones depends on the duration and dose as well as the presence of other steroid hormones and their receptors.

#### Evidence for SERMs (ERβ selective pharmacological agents) in endometrium

Studies in rodents have shown that Puerarin, a selective estrogen receptor modulator (SERM) with ERβ preferential agonist activity, may affect endometrial expression of implantation-specific proteins and may have a contraceptive effect (Saha et al., 2012). In agreement with these data, a Phase 2 trial investigating the use of a selective ERβ agonist for climacteric symptoms in 164 post-menopausal women reported an increased incidence of endometrial thickening (18 versus 6% in the placebo group) but with benign histology (Grady et al., 2009). In contrast, others have reported non-uterotrophic activity by ERβ selective agonists in ovariectomized rats (Hertrampf et al., 2008). Therefore, the expected uterine/endometrial neutral effect of novel ERβ selective SERMs has not been conclusively confirmed in long-term studies and should be explored prior to administering these agents for their other potential health benefits. However, studies of these agents in other tissues have highlighted the complexities of interplay between ERβ and other hormonal nuclear receptor functions. Differential actions of these agents in the endometrial epithelial and stromal compartments also have to be fully
elucidated in health and in endometrial pathologies prior to considering the therapeutic potential of the many available selective pharmacological modulators of ERβ for patient benefit.

Summary/conclusion

Despite early predictions to the contrary, the collective evidence suggests that ERβ has an important role in normal endometrial tissue homeostasis, cell turnover and regeneration and also in most if not all benign and malignant endometrial diseases. Information gained from studies of other tissues on the basic function of ERβ isoforms in restraining the ERα-mediated cell-specific trophic/mitotic responses to E2 has allowed appreciation of the important role of ERβ in regulation of cell fate in human endometrium. Our current understanding of ERβ expression and function in endometrium is, however, far from complete. ERβ is dynamically expressed in healthy premenstrual endometrium and persists in post-menopausal atrophic endometrium. The possible dysregulation of ERβ expression that has been reported in benign endometrial proliferative diseases, such as endometriosis and PCOS, as well as in EC highlights the importance of exploring the potential use of this ER isoform for targeted therapy with estrogenSERMs. However, there are several voids in our current knowledge; for example, the existence of ERβ splice variants and their influence on ERβ1 function and E2 responsiveness in health and in endometrial disease. Research clarifying these areas and functional studies may explain conflicting data that exist in the current literature. Future studies should specifically examine the inter-regulatory effect of the ERβ splice variants, whereby each of the splice variants regulates the effect of the other variants, as well as the co-regulatory effect of ERβ with other steroid hormone receptors and their combined effects on endometrial cellular responses to steroid hormone signals, as these are all pivotal for predicting the effects of pharmacological hormone receptor modulators in endometrial function. Our current understanding of the role of ERβ in the endometrium from many animal studies remains to be confirmed in vitro and in vivo) on human endometrium, or human endometrial epithelial cells in particular. However, there is also an urgent need to standardize the way that immunohistochemical studies of steroid receptor expression in benign and malignant endometrium are conducted and assessed, especially in EC; agreement is needed regarding the most appropriate control tissue (post- or premenopausal endometrium), especially in the light of increased levels of obesity and the associated estrogenic effects on the endometrium, as well as the development of a robust method for quantification/assessment of immunohistochemical data on ERβ expression, similar to the scoring systems that are employed in other hormonally regulated tissues, such as breast cancer, since these data may have obvious direct clinical implications in guiding therapy.

Supplementary data

Supplementary data are available at http://humupd.oxfordjournals.org/ online.

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Authors’ roles

D.K.H. conceived the manuscript, D.K.H. and A.M.K. prepared the first draft and J.N.B. revised the manuscript critically for important intellectual content. A.M.K. drafted the figures and tables. All authors revised and read the manuscript and approved the submitted final version.

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Conflict of interest

None declared.

References


Bukulmez O, Hardy DB, Carr BR, Word RA, Mendelson CR. Inflammatory status scoring systems that are employed in other hormonally regulated tissues, such as breast cancer, since these data may have obvious direct clinical implications in guiding therapy.

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Menasce LP, White GR, Harrison CJ, Boyle JM. Localization of the estrogen receptor
Mosselman S, Polman J, Dijkema R. ER beta: identification and characterization of a


Zhao C, Dahlman-Wright K, Gustafsson JA. Estrogen receptor beta: an overview and update. *Nucl Receptor Signal* 2008; **6**:e003.