REVIEW

The importance of ER β signalling in the ovary

Ann E Drummond and Peter J Fuller

Prince Henry's Institute of Medical Research, PO Box 5152, Clayton, Victoria 3168, Australia

(Correspondence should be addressed to A E Drummond; Email: ann.drummond@princehenrys.org)

Abstract

This review examines the evidence for a central role of oestrogen receptor β (ER β or ESR2 as listed in the MGI Database) in folliculogenesis and hence reproductive biology. Knockout mouse models have been a valuable resource in this respect. The ER β -null mouse exhibits a granulosa cell phenotype associated with the partial arrest of folliculogenesis and ovulatory dysfunction. Phyto-oestrogens such as genistein, which preferentially activate ER β , have been shown to alleviate the ovarian phenotype of the oestrogen-depleted aromatase knockout mouse. In normal adult mice, genistein has been shown to cause reproductive defects following neonatal administration. Studies of ovarian cancer have also informed the literature. A decline in ER β levels in epithelial ovarian cancers has been hypothesised to be

associated with severity of disease and prognosis. Whereas the abundant expression of ER β in granulosa cell tumours (GCT) of the ovary and evidence that ER β signalling is transrepressed by the nuclear factor- κ B pathway in GCT cell lines suggest a pathogenetic role for ER β in GCT. In recent years, studies into the impact of environmental oestrogens (either in the form of pesticides or plastics) on reproductive function have shown that ER β -selective toxins cause reproductive dysfunction and impair fertility. It remains to be established as to what genes are regulated by ER β in the ovary. Finally, ER β has been shown to be regulated by gonadotrophins, the pituitary hormones mediating ovarian function.

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Introduction

The identification of the second oestrogen receptor (ER β or ESR2 as listed in the MGI Database) in 1996 (Kuiper *et al.* 1996, Mosselman *et al.* 1996) reignited research into the action of oestrogen throughout the body. Knockout mouse models were developed that either eliminated one or both of the receptors (ER α (ESR1) and ER β ; Lubahn *et al.* 1993, Couse *et al.* 1997, 1999, 2000, Krege *et al.* 1998, Dupont *et al.* 2000) or prevented oestrogen production (aromatase knockout mouse, ArKO: Fisher *et al.* 1998, Britt *et al.* 2000). ER α and ER β exhibit species-/tissue-/cell-specific localisation and levels of expression (Fig. 1). ER β has been detected primarily in the ovary (granulosa cells, theca cells, corpora lutea (CL), and oocyte), colon, brain, Fallopian tube, lung, adipose, kidney, bone, heart, bladder, adrenal, testis, and prostate (Enmark *et al.* 1997, Grohe *et al.* 1997, Kuiper *et al.* 1997, Saunders *et al.* 1997, 2002, Taylor & Al-Azzawi 2000, Anwar *et al.* 2001, Bord *et al.* 2001, Bocca *et al.* 2008, van den Driesche *et al.* 2008, Morani *et al.* 2008, Weiser *et al.* 2008), while ER α has been found predominantly in testis, epididymis, ovary (granulosa cells and theca cells), mammary gland, brain (pituitary gland), adipose, bone, heart, and uterus (Grohe *et al.* 1997, Kuiper *et al.* 1997, Drummond *et al.* 1999,

Pelletier *et al.* 2000, Taylor & Al-Azzawi 2000, Anwar *et al.* 2001, Bord *et al.* 2001, Morani *et al.* 2008, Weiser *et al.* 2008). Although there has been considerable interest, driven by the pharmaceutical industry, in the role of ER β in bone, the cardiovascular system, and in inflammation (for a review of ER β 's roles in non-reproductive tissues, see Harris (2007)), its role in the ovary remains to be determined.

Oestrogen plays a pivotal role as an intrafollicular modulator, stimulating granulosa cell proliferation and facilitating the differentiative actions of FSH and LH on these cells (Richards 1980). Granulosa cells produce oestrogens, principally oestradiol (E₂) from androgens via aromatisation, in response to FSH. The contribution of each ER to mediating these effects is becoming clearer: ER β appears to be more directly implicated in ovarian development than ER α (Lubahn *et al.* 1993, Krege *et al.* 1998). This review will address the actions of ER β in the ovary and its importance for normal ovarian function.

Oestrogen receptors

Steroid receptors are composed of five domains (Fig. 2) denoted A–F (Mangelsdorf *et al.* 1995). The DNA-binding domain (domain C) is highly conserved between ER α and

Figure 1 Schematic representation of oestrogen receptor localisation in human tissues. The level of expression of each receptor is not indicated. Structures shown are not to scale.

ER β with an amino acid identity of 95%, whereas the homology in the ligand-binding domain (domain E) is only 55% (Kuiper *et al.* 1996). By way of perspective, this level of identity is also seen between the ligand-binding domains of the androgen, glucocorticoid, mineralocorticoid, and progesterone receptors, and is associated with both unique and shared ligand binding. The N-terminal (domains A/B), hinge (domain D), and C-terminal regions (domain F) have the greatest sequence diversity (Kuiper *et al.* 1996).

Multiple isoforms of the ER β subtype have now been described (Chu & Fuller 1997, Petersen *et al.* 1998, Matthews & Gustafsson 2003, Poola *et al.* 2005, Zhao *et al.* 2005, 2008), although it is not clear whether these forms are all biologically active. Chu & Fuller (1997) reported the existence of a 54 nucleotide insert (Fig. 3A) in the ligand-binding domain of rat ER β . Termed ER β 2, this isoform, present only in rodents (Chu & Fuller 1997, Lu *et al.* 1998), acts as a dominant negative regulator of ER β - and ER α -mediated transcription (Maruyama *et al.* 1998). While this isoform has not been detected in humans, shortened transcripts and alternatively spliced forms of ER β have been reported in normal ovary and ovarian tumours (Maruyama *et al.* 1998, Moore *et al.* 1998, Ogawa *et al.* 1998). These forms designated ER β 1, ER β 2 (also known as ER β cx), ER β 3, ER β 4, and ER β 5 (Ogawa *et al.* 1998, Inoue *et al.* 2000, Poola *et al.* 2005) each produce a full-length transcript despite truncations at the 3'-end (Fig. 3B). Initially, it was thought that ER β 4 and ER β 5 existed only as truncated transcripts but this has proven not to be the case (Poola *et al.* 2002, 2005).

The affinity of ligands for the respective receptor subtypes and isoforms present in the rat differs (Petersen *et al.* 1998). Of the human isoforms, ER β 1 is the only one that has been shown to have full function (Leung *et al.* 2006). Homodimeric forms of ER β 2, ER β 4, and ER β 5 have no activity but monomers of these forms can heterodimerise with ER β 1 to enhance its activation (Leung *et al.* 2006), raising the possibility of tissue-specific dimerisation. The response to oestrogen in a given tissue is defined by the ER expressed and the matrix of ER-interacting proteins present within the cells. These coregulatory molecules may influence the response in both a ligand- and promoter-dependent context, which, in turn, may be influenced by other signalling pathways. Nuclear hormone receptors interact with coregulatory proteins, either coactivators, which enhance transcription, or corepressors, which repress transcription. ER contains two 'activation functions' (AF) (see Fig. 2) that interact with coactivators:

Figure 2 Schematic representation of human ER α and ER β . The amino acid sequence is numbered according to published data by Katzenellenbogen *et al.* (2000). The steroid receptor domains are indicated.

Figure 3 (A) Schematic representation of the rat ER β isoforms. ER β 2 and ER β 2- δ 3 contain a 54 nucleotide (18 amino acid) insert in the ligand-binding domain. ER β 1- δ 3 and ER β 2- δ 3 have a 117 nucleotide (39 amino acid) deletion in the DNA-binding domain (Pettersson *et al.* 1997). (B) Schematic representation of the human ER β isoforms (ER β 1–5). Each isoform produces a full-length transcript with identical sequences to ER β 1, between exons 1 and 7. In place of exon 8 are unique sequences (Zhao *et al.* 2008). The steroid receptor domains are indicated.

AF-1 that is ligand-independent lies within the N-terminal domain, whereas for AF-2 that lies in the ligand binding domain (LBD), its activity is dependent on ligand-induced conformational changes. The relative contribution of each AF is cell and promoter dependent. Transcription of the human ER β genes occurs from at least two promoters, 0N and 0K (Zhao *et al.* 2008), with the same transcript produced.

Localisation and regulation of ER β in the ovary

ER β is present in the ovaries of a wide number of species including mouse, rat, rabbit, sheep, cow, baboon, marmoset, hamster, pig, and human (Byers *et al.* 1997, Couse *et al.* 1997, Tremblay *et al.* 1997, Lu *et al.* 1998, Drummond *et al.* 1999, Rosenfeld *et al.* 1999, Pelletier & El-Alfy 2000, Saunders *et al.* 2000, Monje & Boland 2001, Slomczynska *et al.* 2001, Pepe *et al.* 2002, Yang *et al.* 2002, Juengel *et al.* 2006). While ER β is predominantly expressed by the granulosa cells, theca cells, surface epithelium, and CL, oocytes have also been reported to express the receptor (Hild-Petito *et al.* 1988, Revelli *et al.* 1996, Byers *et al.* 1997, Brandenberger *et al.* 1998, Hillier *et al.* 1998, Tetsuka *et al.* 1998, Hosokawa *et al.* 2001, Juengel *et al.* 2006). Species differences are evident in the localisation profiles.

Definitive information on the expression of the respective ER mRNAs and proteins in granulosa cells of different follicle sizes is limited. *In situ* hybridisation and RT-PCR studies in the rat indicate that there is more ER β than ER α mRNA in the ovary, and further analysis revealed more ER β 2 than ER β 1 in ovarian RNA collected from post-natal rats (Drummond *et al.* 1999). mRNAs for ER α and ER β 1 and ER β 2 are present in granulosa cells of follicles with at most two to three layers of granulosa cells (Drummond *et al.* 1996, 1999), and ER β 1 and ER β 2 proteins are present in rat granulosa cells (Drummond *et al.* 1996, Byers *et al.* 1997, Sar & Welsch 1999). In the human and marmoset, ER β protein is expressed by granulosa cells of all follicle types, whereas ER α is not expressed by follicles containing one to two layers of granulosa cells (Saunders *et al.* 2000).

A convergence between gonadotrophin signalling and ER β -mediated transcription in the ovary has been noted, unlike ER α . Gonadotrophins are important regulators of ovarian function and it makes sense for them to regulate ER β expression if indeed ER β is important for ovarian function. The LH surge has been found to downregulate ER β mRNA in the ovaries of rats and hamsters (Byers *et al.* 1997, Yang *et al.* 2002), and gonadotrophin-induced cofactor-4 (GIOT-4 or ZFP709 as listed in the MGI Database), which is induced by FSH, coactivated ER β in granulosa cells (Kouzu-Fujita *et al.* 2009).

Transducing ER β signals

ER β s mediate transcription as dimers. Both homodimers and heterodimers of the ER activate transcription of reporter gene constructs containing oestrogen response elements (EREs), although heterodimers form preferentially in the presence of E₂ (Pettersson *et al.* 1997, Petersen *et al.* 1998). It has been suggested that ER β activity is compromised in the absence of ER α (Couse *et al.* 1997), further supporting the heterodimer as the functional form of ER. Studies in other tissues suggest that ER β may antagonise/oppose the effects of ER α , thereby serving to limit cellular proliferation, promote differentiation (luteinisation), and modulate apoptosis (atresia).

Although a biological role for ER β 2 has not yet been elucidated, the studies of Maruyama *et al.* (1998) suggest that ER β 2 may be a negative regulator of oestrogen action, given that it dose dependently suppressed ER α - and ER β 1-mediated transcriptional activation. Thus, the formation of dimers containing ER β 2 may well induce very different effects on gene expression relative to those induced by receptor dimers, which do not contain ER β 2. In human cell lines, ER α and ER β have been shown to signal in different ways depending on the ligand and response element (ERE or AP1) involved (Paech *et al.* 1997). It has also been demonstrated that the coexpression of ER α and ER β in HeLa cells led to the suppression of the ER α -mediated gene cyclin D1 (Liu *et al.* 2002). There are other instances where functional antagonism of ER α by ER β has been demonstrated, notably in models of fat reduction and cellular proliferation (Weihua *et al.* 2001). Microarray analyses

of bone and liver tissue from ER-null mice support the inhibitory action of ER β on ER α -mediated transcriptional activity (Lindberg *et al.* 2003).

ER β plays a direct role in follicle development being required for antrum formation and preovulatory follicle maturation (Hegele-Hartung *et al.* 2004, Emmen *et al.* 2005). Ovulatory defects have been linked with polymorphisms of human ER β (Sundarajan *et al.* 2001). Haemorrhagic and cystic follicles of ER α and LH β CTP (these mice express elevated levels of LH in the absence of ER β) transgenic mice require ER β for development (Couse *et al.* 2004). Polyovular follicles were induced by both ER α and ER β agonists in neonatal mice (Nakamura *et al.* 2008). However, mice lacking ER β do not produce polyovular follicles when challenged with genistein or diethylstilboestrol (DES) (Jefferson *et al.* 2002, Kim *et al.* 2009a), whereas ER α deplete mice do, suggesting that ER β is directly involved in polyovular follicle formation. In human CL, oestrogenic activity is mediated by ER β with both protein and mRNA localised to luteal cells, perivascular cells, and fibroblasts within the CL (Hosokawa *et al.* 2001). ER β 1 and ER β 2 mRNAs were differentially expressed across the luteal phase with ER β 1 maximally expressed in the mid-luteal phase and ER β 2 maximally expressed in the early luteal phase (van den Driesche *et al.* 2008). Colocalisation of the two forms was noted but not obligatory.

ER β -regulated genes

Studies to identify genes regulated by ER β in normal tissues have yet to be published; the few undertaken to date have utilised cancer lines (see Zhao *et al.* (2008) for a summary). Chang *et al.* (2006) investigated the effect of ER β on gene regulation by MCF-7 cells expressing ER α . Microarray analyses revealed that genes regulating signal transduction pathways, cell cycle progression, and apoptosis were modulated by ER β . These included members of the transforming growth factor- β superfamily (which are normally associated with suppression of breast cancer cell growth), class 3 and 4 semaphorin pathways, member of the forkhead box transcription factor family, only expressed in proliferating cells (*FOXM1*), cell division cycle 25 homologue A (*CDC25A*), transcription factor (*E2F1*), *SURVIVIN* (member of the inhibitor of apoptosis protein family that acts as a suppressor of apoptosis and plays a central role in cell division), and cyclin-dependent kinase inhibitor (*p21WAF1*; Chang *et al.* 2006). Proliferation of MCF-7 cells declined when ER β was present, consistent with the repression of *FOXM1*, *CDC25A*, *E2F1*, and *SURVIVIN* mRNAs and the upregulation of *p21WAF1*, an inhibitor of cell proliferation and *SEMA3B*, a tumour suppressor (Chang *et al.* 2006).

In the presence of E₂, ER β enhanced the repression of *THROMBOSPONDIN 1* (THBS1), reduced the repression of *INTEGRIN-6* and *BMP-7*, and downregulated stromal cell-derived factor-1 (*SDF-1* or *CXCL12*; Chang *et al.* 2006), which has previously been shown to act as an autocrine

growth factor for breast cancer cells (Hall & Korach 2003, Chang *et al.* 2006). Interestingly, *SDF-1* has also been shown to interfere with semaphorin signalling. What is clear from these recent studies is that it is the relative levels of ER β and ER α in a cell line/tissue which will determine the response to oestrogen.

ER β knockout mice

Despite normal levels of gonadotrophins and ovaries that contain follicles of all stages of development and CL, ER β knockout mice (β ERKO) are subfertile producing fewer pups and litters and the ovaries yield fewer oocytes following superovulation (Krege *et al.* 1998, Couse *et al.* 2000, 2003, 2005, Dupont *et al.* 2000). A granulosa cell-specific phenotype of the β ERKO mouse is evident (Couse *et al.* 2000). Ovaries of β ERKO contain fewer large antral follicles and CL, and apoptosis in large follicles is increased (Emmen *et al.* 2005). It is clear that ER β is important for follicle maturation from the antral stage of development and follicle rupture but not luteinisation (Emmen *et al.* 2005). The expression of genes important for ovarian differentiation is altered with reduced expression of aromatase, (*Cyp19a1*) LH receptor, (*Lhgr*) and prostaglandin-synthase 2 (*Ptgs2*) mRNAs (Emmen *et al.* 2005) and increased expression of the androgen receptor in antral follicles (Cheng *et al.* 2002). Follicles from these mice produce significantly less E₂ compared to wild type *in vitro* indicating an attenuated response to FSH (Couse *et al.* 2005).

It is apparent from the ER α knockout (ERKO) and β ERKO ovarian phenotypes that ER α and ER β have different roles to play in folliculogenesis. It has been hypothesised that the proliferative action of oestrogen is transmitted preferentially via ER α , whereas the differentiative effects of oestrogen are mediated principally by ER β (Britt & Findlay 2002). This hypothesis is supported by the differentiation of granulosa cells into male-type Sertoli cells in the oestrogen-deficient, aromatase knockout (ArKO) (Britt *et al.* 2002). These Sertoli cells disappear from the ovaries of mice treated with E₂ or phyto-oestrogens, principally genistein (Britt *et al.* 2002), an ER β -selective ligand (Pettersson *et al.* 1997). However, interpreting the consequences of ER α and ER β deletion in these models is complicated by the inability of these receptors to form heterodimers of ER α and ER β . Homodimers of these transcription factors may induce very different effects on gene expression compared with ER heterodimers.

Ovarian cancer

The majority of ovarian cancers are epithelial in origin. Preliminary studies suggest that ER β levels (protein and mRNA) in epithelial ovarian cancer decline relative to levels in normal ovary (Li *et al.* 2003, Bardin *et al.* 2004, Lindgren *et al.* 2004, Lazennec 2006). Overexpressing ER β in an ovarian adenocarcinoma cancer cell line PEO14 led to a 50%

reduction in proliferative capacity (Lindgren *et al.* 2004). The prognostic significance of ER expression by ovarian cancers has received little attention although one study reported a correlation between levels of ER β expression and cancer disease stage, with levels declining with increased severity of disease (Chan *et al.* 2008). In addition, breast cancer studies indicate that tumours positive for ER β respond better to endocrine therapy (Fox *et al.* 2008). Thus, loss of ER β expression may be a feature of malignant transformation.

An antitumoural role of ER β in SK-OV-3 ovarian cancer cells that do not express functional ER α has been reported (Treeck *et al.* 2007). Reduced proliferation, inhibited motility, and increased apoptosis of SK-OV-3 cells overexpressing ER β 1 were noted. Exon-deleted ER β 1 splice variants ER β - δ 125 and ER β - δ 1256, which lack the AF-1 domain and have deletions in their DNA and ligand-binding domains, had no effect on proliferation or apoptosis but partly inhibited motility of these cells (Treeck *et al.* 2007). Genes associated with these physiological changes include an increase in p21 (WAF1), a cell cycle inhibitor, downregulation of cyclin A2, an oestrogen-responsive cell cycle regulator (Vendrell *et al.* 2004), and an increase in fibullin-1c, an extracellular matrix protein overexpressed in epithelial ovarian cancers and involved in motility (Hayashido *et al.* 1998). ER β expression may be reduced as a result of DNA methylation (Zhao *et al.* 2008). Studies investigating epithelial ovarian carcinoma revealed that human promoter 0N was significantly methylated in ovarian cancer cell lines and tissues, and that this methylation correlated with decreases in the expression of exon 0N, ER β 1, ER β 2, and ER β 4 (Suzuki *et al.* 2008).

Granulosa cell tumours (GCT) account for about 5% of all ovarian cancers. GCT and GCT-derived cell lines abundantly express ER β (Chu *et al.* 2000, Fuller *et al.* 2002, Fuller & Chu 2004), and their molecular phenotype is similar to preovulatory granulosa cells. As in other endocrine tumours, ER β may be of pathogenetic significance. The steroid receptor coactivators SRC-1, -2, and -3 and the corepressors NcoR and SMRT are also expressed by GCT (Fuller *et al.* 2002). Despite ER β expression and E₂ binding, when GCT cell lines were transfected with oestrogen-responsive reporter genes and treated with E₂, there was no response (Chu *et al.* 2004). The activation state of several signalling pathways in these lines was examined with both nuclear factor- κ B (NF κ B or NFkB) and AP-1 signalling found to be constitutively active. When the NF κ B activity is inhibited by BAY 11-7082, ligand-dependent steroid receptor-mediated transactivation occurs for both exogenous and endogenous ER β (Chu *et al.* 2004). Thus, ER β signalling in GCT cell lines is transrepressed via the NF κ B pathway.

Few studies have examined NF κ B signalling in normal granulosa cells. We have localised p65 (RelA), a member of the NF κ B family to granulosa cells, theca cells, oocytes, and luteal cells of adult rat ovary with both cytoplasmic and nuclear staining evident. Wang *et al.* (2002) reported that the NF κ B pathway mediates the FSH-induced expression of X-linked inhibitor of apoptosis by granulosa cells. These data

are consistent with a role for NF κ B signalling in granulosa cells and indicate that ER β signalling may be modulated by NF κ B, perhaps through mutual transrepression. In malignant granulosa cells, inhibition of ER β signalling by NF κ B may be enhanced by cyclin D2 (Chu *et al.* 2004). Together, these data suggest that in both normal granulosa cell proliferation and in malignancy (GCT), the action of ER β is inhibited by pro-proliferative signalling pathways, arguing that its role may be primarily to inhibit proliferation and/or promote differentiation. In GCT, this may contribute to the pathogenesis by interrupting part of an autocrine loop that contributes to limiting the FSH-like growth stimulation (Schumer & Cannistra 2003).

Environmental oestrogens

Ovarian-derived oestrogens are not the only compounds that can activate ER. Phyto-oestrogens are plant compounds with intrinsic oestrogen-like biological activity mainly due to the presence of a phenolic A ring, which is crucial for receptor binding (Brzozowski *et al.* 1997, Kuiper *et al.* 1998). There are two major classes of phyto-oestrogens: lignans and isoflavones. Soya protein contains the isoflavones, genistein and daidzein (Price & Fenwick 1985). Phyto-oestrogens are believed to signal predominantly via ER β (Kuiper *et al.* 1997, Whitten & Naftolin 1998), and genistein, in particular, has a 20-fold higher binding affinity for ER β compared to ER α (Kuiper *et al.* 1997, Whitten & Patisaul 2001, Martin *et al.* 2004). Feeding oestrogen-depleted arKO mice diets containing either soy or genistein in part ameliorated the reproductive phenotype of female mice (Britt *et al.* 2005). Ovarian and uterine weights increased, although not to wild-type levels and haemorrhagic cysts disappeared with the addition of genistein. These effects of genistein are thought to be mediated via ER β and are supported by the identification of ER β in the uterus (Saunders *et al.* 1997, Matsuzaki *et al.* 1999, Sasano *et al.* 1999, Wang *et al.* 1999) and evidence that oestrogen directly (and not elevated LH) is responsible for the development of haemorrhagic cysts (Couse *et al.* 2004, Britt *et al.* 2005). Adverse effects of genistein on rodent reproductive function have also been reported (Jefferson *et al.* 2002, 2007b), notably reduced fertility, the formation of polyovular follicles, and altered oestrous cycles. The doses of genistein given neonatally to mice in these studies while high were environmentally relevant and led to the manifestation of reproductive abnormalities in adult life (Jefferson *et al.* 2007a).

Exposure of adult females to oestrogen either via the environment or clinically can have consequences for reproductive function. Adult rats treated with E₂ valerate had abnormal oestrus cycles, while the ovaries contained reduced numbers of CL, developed follicular cysts, theca cell hyperplasia, and had an increase in apoptosis of granulosa cells from primary and secondary follicles (Shirwalkar *et al.* 2007). ER β and PR proteins expressed by granulosa cells declined in

follicles larger than secondary follicles suggesting abnormal differentiation of the granulosa cells.

Women exposed to endocrine-disrupting chemicals have impaired fertility and irregular menstrual cycles, and experience pregnancy loss (Fuortes *et al.* 1997, Younglai *et al.* 2005). Methoxychlor (MXC), an organochloride pesticide with oestrogenic activity (Kuiper *et al.* 1997) mediated primarily via ER β (Zama & Uzumcu 2009), caused ovarian dysfunction in the adult rodent following exposure to rats during the foetal or neonatal period. Follicle composition was altered with more preantral and early antral follicles present and fewer CL (Gray *et al.* 1989). ER β expression declined (no effect on ER α), and there were reduced expression of *LHR* and *P450SCC* mRNAs. Accelerated entry into puberty and to first oestrus, irregular cyclicity and reduced litter sizes have also been reported (Armenti *et al.* 2008). The bisphenol demethylated form HPTE (2,2-bis-(p-hydroxyphenyl)-1,1,1-trichloroethane) is believed to be responsible for the oestrogenic activity of MXC (Waters *et al.* 2001). HPTE analogues act as ER α agonists and ER β antagonists in a range of cell lines (Gaido *et al.* 2000, Safe *et al.* 2001). ER β was found to be hypermethylated, i.e. inactivated, whereas ER α was not (Zama & Uzumcu 2009).

Bisphenol A (BPA) exposure results from interactions with polycarbonate plastics or epoxy resins in food packaging (Kang *et al.* 2006). BPA acts as an agonist of oestrogen via ER β , whereas it acts as both an agonist and antagonist in some cell types via ER α . The effect of BPA is likely to be determined on a tissue-specific basis (Safe *et al.* 2001, Kurosawa *et al.* 2002). Neonatal exposure to DES or BPA induces anovulation and persistent oestrus in female rodents (Iguchi 1992, Kato *et al.* 2003, Nakamura *et al.* 2008) and induces polyovular follicles (Kim *et al.* 2009b). The observed anovulation and induced oestrus are thought to be mediated via ER α , given that diarylpropionitrile, an ER β -selective agonist, had no effect on these parameters (Nakamura *et al.* 2008).

Resveratrol (RES), a phyto-oestrogen found in grapes, binds equally to ER α and ER β (Henry & Witt 2002). RES decreased body weight and induced ovarian hypertrophy potentially via ER β in gonadally intact rats. RES-liganded ER β induced significantly higher levels of transcriptional activity than E₂-liganded ER β , suggesting that tissues expressing ER β will be more transcriptionally active in response to RES than those expressing ER α (Bowers *et al.* 2000).

Conclusion

ER β still remains the most poorly understood of all steroid receptors despite more than 10 years elapsing since its identification. The studies reviewed here provide evidence for a role of ER β in ovarian function, although the story is far from complete. Further studies are required to elaborate on the role of coactivators and corepressors in ER β signalling, to elucidate the structure of the physiologically active dimer, and to identify genes specifically regulated by ER β in the ovary.

Declaration of interest

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

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References

- Anwar A, McTernan PG, Anderson LA, Askaa J, Moody CG, Barnett AH, Eggo MC & Kumar S 2001 Site-specific regulation of oestrogen receptor- α and - β by oestradiol in human adipose tissue. *Diabetes, Obesity and Metabolism* **3** 338–349.
- Armenti AE, Zama AM, Passantino L & Uzumcu M 2008 Developmental methoxychlor exposure affects multiple reproductive parameters and ovarian folliculogenesis and gene expression in adult rats. *Toxicology and Applied Pharmacology* **233** 286–296.
- Bardin A, Boule N, Lazennec G, Vignon F & Pujol P 2004 Loss of ER β expression as a common step in estrogen-dependent tumor progression. *Endocrine-Related Cancer* **11** 537–551.
- Bocca SM, Billiar RB, Albrecht ED & Pepe GJ 2008 Oocytes of baboon fetal primordial ovarian follicles express estrogen receptor beta mRNA. *Endocrine* **33** 254–260.
- Bord S, Horner A, Beavan S & Compston J 2001 Estrogen receptors alpha and beta are differentially expressed in developing human bone. *Journal of Clinical Endocrinology and Metabolism* **86** 2309–2314.
- Bowers JL, Tyulmenkov VV, Jernigan SC & Klinge CM 2000 Resveratrol acts as a mixed agonist/antagonist for estrogen receptors alpha and beta. *Endocrinology* **141** 3657–3667.
- Brandenberger AW, Tee MK & Jaffe RB 1998 Estrogen receptor alpha (ER- α) and beta (ER- β) mRNAs in normal ovary, ovarian serous cystadenocarcinoma and ovarian cancer cell lines: down-regulation of ER- β in neoplastic tissues. *Journal of Clinical Endocrinology and Metabolism* **83** 1025–1028.
- Britt KL & Findlay JK 2002 Estrogen actions in the ovary revisited. *Journal of Endocrinology* **175** 269–276.
- Britt KL, Drummond AE, Cox VA, Dyson M, Wreford NG, Jones ME, Simpson ER & Findlay JK 2000 An age-related ovarian phenotype in mice with targeted disruption of the Cyp 19 (aromatase) gene. *Endocrinology* **141** 2614–2623.
- Britt KL, Kerr J, O'Donnell L, Jones ME, Drummond AE, Davis SR, Simpson ER & Findlay JK 2002 Estrogen regulates development of the somatic cell phenotype in the eutherian ovary. *FASEB Journal* **16** 1389–1397.
- Britt KL, Simpson ER & Findlay JK 2005 Effects of phytoestrogens on the ovarian and pituitary phenotypes of estrogen-deficient female aromatase knockout mice. *Menopause* **12** 174–185.
- Brzozowski AM, Pike AC, Dauter Z, Hubbard RE, Bonn T, Engstrom O, Ohman L, Greene GL, Gustafsson JA & Carlquist M 1997 Molecular basis of agonism and antagonism in the oestrogen receptor. *Nature* **389** 753–758.
- Byers M, Kuiper GG, Gustafsson JA & Park-Sarge OK 1997 Estrogen receptor-beta mRNA expression in rat ovary: down-regulation by gonadotropins. *Molecular Endocrinology* **11** 172–182.
- Chan KK, Wei N, Liu SS, Xiao-Yun L, Cheung AN & Ngan HY 2008 Estrogen receptor subtypes in ovarian cancer: a clinical correlation. *Obstetrics and Gynecology* **111** 144–151.

- Chang EC, Frasor J, Komm B & Katzenellenbogen BS 2006 Impact of estrogen receptor beta on gene networks regulated by estrogen receptor alpha in breast cancer cells. *Endocrinology* **147** 4831–4842.
- Cheng G, Weihua Z, Makinen S, Makela S, Saji S, Warner M, Gustafsson JA & Hovatta O 2002 A model for the androgen receptor in follicular atresia of estrogen receptor beta knockout mouse ovary. *Biology of Reproduction* **66** 77–84.
- Chu S & Fuller PJ 1997 Identification of a splice variant of the rat estrogen receptor beta gene. *Molecular and Cellular Endocrinology* **132** 195–199.
- Chu S, Marners P, Burger HG & Fuller PJ 2000 Estrogen receptor isoform gene expression in ovarian stromal and epithelial tumors. *Journal of Clinical Endocrinology and Metabolism* **85** 1200–1205.
- Chu S, Nishi Y, Yanase T, Nawata H & Fuller PJ 2004 Transrepression of estrogen receptor beta signaling by nuclear factor-kappaB in ovarian granulosa cells. *Molecular Endocrinology* **18** 1919–1928.
- Couse JF, Lindzey J, Grandien K, Gustafsson JA & Korach KS 1997 Tissue distribution and quantitative analysis of estrogen receptor-alpha (ERalpha) and estrogen receptor-beta (ERbeta) messenger ribonucleic acid in the wild-type and ERalpha-knockout mouse. *Endocrinology* **138** 4613–4621.
- Couse JF, Hewitt SC, Bunch DO, Sar M, Walker VR, Davis BJ & Korach KS 1999 Postnatal sex reversal of the ovaries in mice lacking estrogen receptors alpha and beta. *Science* **286** 2328–2331.
- Couse JF, Curtis Hewitt S & Korach KS 2000 Receptor null mice reveal contrasting roles for estrogen receptor alpha and beta in reproductive tissues. *Journal of Steroid Biochemistry and Molecular Biology* **74** 287–296.
- Couse JF, Yates MM, Walker VR & Korach KS 2003 Characterization of the hypothalamic–pituitary–gonadal axis in estrogen receptor (ER) null mice reveals hypergonadism and endocrine sex reversal in females lacking ERalpha but not ERbeta. *Molecular Endocrinology* **17** 1039–1053.
- Couse JF, Yates MM, Sanford R, Nyska A, Nilson JH & Korach KS 2004 Formation of cystic ovarian follicles associated with elevated luteinizing hormone requires estrogen receptor-beta. *Endocrinology* **145** 4693–4702.
- Couse JF, Yates MM, Deroo BJ & Korach KS 2005 Estrogen receptor-beta is critical to granulosa cell differentiation and the ovulatory response to gonadotropins. *Endocrinology* **146** 3247–3262.
- van den Driesche S, Smith VM, Myers M & Duncan WC 2008 Expression and regulation of oestrogen receptors in the human corpus luteum. *Reproduction* **135** 509–517.
- Drummond AE, Dyson M, Mercer JE & Findlay JK 1996 Differential responses of post-natal rat ovarian cells to FSH and activin. *Molecular and Cellular Endocrinology* **122** 21–32.
- Drummond AE, Baillie AJ & Findlay JK 1999 Ovarian estrogen receptor alpha and beta mRNA expression: impact of development and estrogen. *Molecular and Cellular Endocrinology* **149** 153–161.
- Dupont S, Krust A, Gansmuller A, Dierich A, Chambon P & Mark M 2000 Effect of single and compound knockouts of estrogen receptors alpha (ERalpha) and beta (ERbeta) on mouse reproductive phenotypes. *Development* **127** 4277–4291.
- Emmen JM, Couse JF, Elmore SA, Yates MM, Kissling GE & Korach KS 2005 *In vitro* growth and ovulation of follicles from ovaries of estrogen receptor (ER){alpha} and ER{beta} null mice indicate a role for ER{beta} in follicular maturation. *Endocrinology* **146** 2817–2826.
- Enmark E, Peltö-Huikko M, Grandien K, Lagercrantz S, Lagercrantz J, Fried G, Nordenskjöld M & Gustafsson JA 1997 Human estrogen receptor beta-gene structure, chromosomal localization, and expression pattern. *Journal of Clinical Endocrinology and Metabolism* **82** 4258–4265.
- Fisher CR, Graves KH, Parlow AF & Simpson ER 1998 Characterization of mice deficient in aromatase (ArKO) because of targeted disruption of the cyp19 gene. *PNAS* **95** 6965–6970.
- Fox EM, Davis RJ & Shupnik MA 2008 ERbeta in breast cancer – onlooker, passive player, or active protector? *Steroids* **73** 1039–1051.
- Fuller PJ & Chu S 2004 Signalling pathways in the molecular pathogenesis of ovarian granulosa cell tumours. *Trends in Endocrinology and Metabolism* **15** 122–128.
- Fuller PJ, Chu S, Fikret S & Burger HG 2002 Molecular pathogenesis of granulosa cell tumours. *Molecular and Cellular Endocrinology* **191** 89–96.
- Fuortes L, Clark MK, Kirchner HL & Smith EM 1997 Association between female infertility and agricultural work history. *American Journal of Industrial Medicine* **31** 445–451.
- Gaido KW, Maness SC, McDonnell DP, Dehal SS, Kupfer D & Safe S 2000 Interaction of methoxychlor and related compounds with estrogen receptor alpha and beta, and androgen receptor: structure–activity studies. *Molecular Pharmacology* **58** 852–858.
- Gray LE Jr, Ostby J, Ferrell J, Rehnberg G, Linder R, Cooper R, Goldman J, Slott V & Laskey J 1989 A dose–response analysis of methoxychlor-induced alterations of reproductive development and function in the rat. *Fundamental and Applied Toxicology* **12** 92–108.
- Grohe C, Kahlert S, Lobbek K, Stimpel M, Karas RH, Vetter H & Neyses L 1997 Cardiac myocytes and fibroblasts contain functional estrogen receptors. *FEBS Letters* **416** 107–112.
- Hall JM & Korach KS 2003 Stromal cell-derived factor 1, a novel target of estrogen receptor action, mediates the mitogenic effects of estradiol in ovarian and breast cancer cells. *Molecular Endocrinology* **17** 792–803.
- Harris HA 2007 Estrogen receptor-beta: recent lessons from *in vivo* studies. *Molecular Endocrinology* **21** 1–13.
- Hayashido Y, Lucas A, Rougeot C, Godyna S, Argraves WS & Rochefort H 1998 Estradiol and fibulin-1 inhibit motility of human ovarian- and breast-cancer cells induced by fibronectin. *International Journal Cancer* **75** 654–658.
- Hegele-Hartung C, Siebel P, Peters O, Kosemund D, Müller G, Hillisch A, Walter A, Kraetzschmar J & Fritzscheimer KH 2004 Impact of isotype-selective estrogen receptor agonists on ovarian function. *PNAS* **101** 5129–5134.
- Henry LA & Witt DM 2002 Resveratrol: phytoestrogen effects on reproductive physiology and behavior in female rats. *Hormones and Behavior* **41** 220–228.
- Hild-Petito S, Stouffer RL & Brenner RM 1988 Immunocytochemical localization of estradiol and progesterone receptors in the monkey ovary throughout the menstrual cycle. *Endocrinology* **123** 2896–2905.
- Hillier SG, Anderson RA, Williams AR & Tetsuka M 1998 Expression of oestrogen receptor alpha and beta in cultured human ovarian surface epithelial cells. *Molecular Human Reproduction* **4** 811–815.
- Hosokawa K, Ottander U, Wahlberg P, Ny T, Cajander S & Olofsson IJ 2001 Dominant expression and distribution of oestrogen receptor beta over oestrogen receptor alpha in the human corpus luteum. *Molecular Human Reproduction* **7** 137–145.
- Iguchi T 1992 Cellular effects of early exposure to sex hormones and antihormones. *International Review of Cytology* **139** 1–57.
- Inoue S, Ogawa S, Horie K, Hoshino S, Goto W, Hosoi T, Tsutsumi O, Muramatsu M & Ouchi Y 2000 An estrogen receptor beta isoform that lacks exon 5 has dominant negative activity on both ERalpha and ERbeta. *Biochemical and Biophysical Research Communications* **279** 814–819.
- Jefferson WN, Couse JF, Padilla-Banks E, Korach KS & Newbold RR 2002 Neonatal exposure to genistein induces estrogen receptor (ER){alpha} expression and multioocyte follicles in the maturing mouse ovary: evidence for ERbeta-mediated and nonestrogenic actions. *Biology of Reproduction* **67** 1285–1296.
- Jefferson WN, Padilla-Banks E & Newbold RR 2007a Disruption of the developing female reproductive system by phytoestrogens: genistein as an example. *Molecular Nutrition & Food Research* **51** 832–844.
- Jefferson WN, Padilla-Banks E & Newbold RR 2007b Disruption of the female reproductive system by the phytoestrogen genistein. *Reproductive Toxicology* **23** 308–316.
- Juengel JL, Heath DA, Quirke LD & McNatty KP 2006 Oestrogen receptor alpha and beta, androgen receptor and progesterone receptor mRNA and protein localisation within the developing ovary and in small growing follicles of sheep. *Reproduction* **131** 81–92.
- Kang JH, Kondo F & Katayama Y 2006 Human exposure to bisphenol A. *Toxicology* **226** 79–89.
- Kato H, Ota T, Furuhashi T, Ohta Y & Iguchi T 2003 Changes in reproductive organs of female rats treated with bisphenol A during the neonatal period. *Reproductive Toxicology* **17** 283–288.

- Katzenellenbogen BS, Choi I, Delage-Mourroux R, Ediger TR, Martini PG, Montano M, Sun J, Weis K & Katzenellenbogen JA 2000 Molecular mechanisms of estrogen action: selective ligands and receptor pharmacology. *Journal of Steroid Biochemistry and Molecular Biology* **74** 279–285.
- Kim H, Hayashi S, Chambon P, Watanabe H, Iguchi T & Sato T 2009a Effects of diethylstilbestrol on ovarian follicle development in neonatal mice. *Reproductive Toxicology* **27** 55–62.
- Kim H, Nakajima T, Hayashi S, Chambon P, Watanabe H, Iguchi T & Sato T 2009b Effects of diethylstilbestrol on programmed oocyte death and induction of polyovular follicles in neonatal mouse ovaries. *Biology of Reproduction* **81** 1002–1009.
- Kouzu-Fujita M, Mezaki Y, Sawatsubashi S, Matsumoto T, Yamaoka I, Yano T, Taketani Y, Kitagawa H & Kato S 2009 Coactivation of estrogen receptor beta by gonadotropin-induced cofactor GIOT-4. *Molecular and Cellular Biology* **29** 83–92.
- Krege JH, Hodgins JB, Couse JF, Enmark E, Warner M, Mahler JF, Sar M, Korach KS, Gustafsson JA & Smithies O 1998 Generation and reproductive phenotypes of mice lacking estrogen receptor beta. *PNAS* **95** 15677–15682.
- Kuiper GG, Enmark E, Peltö-Huikko M, Nilsson S & Gustafsson JA 1996 Cloning of a novel receptor expressed in rat prostate and ovary. *PNAS* **93** 5925–5930.
- Kuiper GG, Carlsson B, Grandien K, Enmark E, Haggblad J, Nilsson S & Gustafsson JA 1997 Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. *Endocrinology* **138** 863–870.
- Kuiper GJM, Lemmen JG, Carlsson B, Corton JC, Safe SH, van der Sagg PT, van der Berg B & Gustafsson J-A 1998 Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor b. *Endocrinology* **139** 4252–4263.
- Kurosawa T, Hiroi H, Tsutsumi O, Ishikawa T, Osuga Y, Fujiwara T, Inoue S, Muramatsu M, Momoeda M & Taketani Y 2002 The activity of bisphenol A depends on both the estrogen receptor subtype and the cell type. *Endocrine Journal* **49** 465–471.
- Lazennec G 2006 Estrogen receptor beta, a possible tumor suppressor involved in ovarian carcinogenesis. *Cancer Letters* **231** 151–157.
- Leung YK, Mak P, Hassan S & Ho SM 2006 Estrogen receptor (ER)-beta isoforms: a key to understanding ER-beta signaling. *PNAS* **103** 13162–13167.
- Li AJ, Baldwin RL & Karlan BY 2003 Estrogen and progesterone receptor subtype expression in normal and malignant ovarian epithelial cell cultures. *American Journal of Obstetrics and Gynecology* **189** 22–27.
- Lindberg MK, Moverare S, Skrtic S, Gao H, Dahlman-Wright K, Gustafsson JA & Ohlsson C 2003 Estrogen receptor (ER)-beta reduces ERalpha-regulated gene transcription, supporting a “ying yang” relationship between ERalpha and ERbeta in mice. *Molecular Endocrinology* **17** 203–208.
- Lindgren PR, Cajander S, Backstrom T, Gustafsson JA, Makela S & Olofsson JI 2004 Estrogen and progesterone receptors in ovarian epithelial tumors. *Molecular and Cellular Endocrinology* **221** 97–104.
- Liu MM, Albanese C, Anderson CM, Hilty K, Webb P, Uht RM, Price RH Jr, Pestell RG & Kushner PJ 2002 Opposing action of estrogen receptors alpha and beta on cyclin D1 gene expression. *Journal of Biological Chemistry* **277** 24353–24360.
- Lu B, Leygue E, Dotzlaw H, Murphy LJ, Murphy LC & Watson PH 1998 Estrogen receptor-beta mRNA variants in human and murine tissues. *Molecular and Cellular Endocrinology* **138** 199–203.
- Lubahn DB, Moyer JS, Golding TS, Couse JF, Korach KS & Smithies O 1993 Alteration of reproductive function but not prenatal sexual development after insertional disruption of the mouse estrogen receptor gene. *PNAS* **90** 11162–11166.
- Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schutz G, Umesono K, Blumberg B, Kastner P, Mark M, Chambon P *et al.* 1995 The nuclear receptor superfamily: the second decade. *Cell* **83** 835–839.
- Martin C, Ross M, Chapman KE, Andrew R, Bollina P, Seckl JR & Habib FK 2004 CYP7B generates a selective estrogen receptor beta agonist in human prostate. *Journal of Clinical Endocrinology and Metabolism* **89** 2928–2935.
- Maruyama K, Endoh H, Sasaki-Iwaoka H, Kanou H, Shimaya E, Hashimoto S, Kato S & Kawashima H 1998 A novel isoform of rat estrogen receptor beta with 18 amino acid insertion in the ligand binding domain as a putative dominant negative regulator of estrogen action. *Biochemical and Biophysical Research Communications* **246** 142–147.
- Matsuzaki S, Fukaya T, Suzuki T, Murakami T, Sasano H & Yajima A 1999 Oestrogen receptor alpha and beta mRNA expression in human endometrium throughout the menstrual cycle. *Molecular Human Reproduction* **5** 559–564.
- Matthews J & Gustafsson JA 2003 Estrogen signaling: a subtle balance between ER alpha and ER beta. *Molecular Interventions* **3** 281–292.
- Monje P & Boland R 2001 Subcellular distribution of native estrogen receptor alpha and beta isoforms in rabbit uterus and ovary. *Journal of Cellular Biochemistry* **82** 467–479.
- Moore JT, McKee DD, Slentz-Kesler K, Moore LB, Jones SA, Horne EL, Su JL, Klier SA, Lehmann JM & Willson TM 1998 Cloning and characterization of human estrogen receptor beta isoforms. *Biochemical and Biophysical Research Communications* **247** 75–78.
- Morani A, Warner M & Gustafsson JA 2008 Biological functions and clinical implications of oestrogen receptors alpha and beta in epithelial tissues. *Journal of Internal Medicine* **264** 128–142.
- Mosselman S, Polman J & Dijkema R 1996 ER beta: identification and characterization of a novel human estrogen receptor. *FEBS Letters* **392** 49–53.
- Nakamura T, Katsu Y, Watanabe H & Iguchi T 2008 Estrogen receptor subtypes selectively mediate female mouse reproductive abnormalities induced by neonatal exposure to estrogenic chemicals. *Toxicology* **253** 117–124.
- Ogawa S, Inoue S, Watanabe T, Orimo A, Hosoi T, Ouchi Y & Muramatsu M 1998 Molecular cloning and characterization of human estrogen receptor betacx: a potential inhibitor of estrogen action in human. *Nucleic Acids Research* **26** 3505–3512.
- Paech K, Webb P, Kuiper GG, Nilsson S, Gustafsson J, Kushner PJ & Scanlan TS 1997 Differential ligand activation of estrogen receptors ERalpha and ERbeta at AP1 sites. *Science* **277** 1508–1510.
- Pelletier G & El-Alfy M 2000 Immunocytochemical localization of estrogen receptors alpha and beta in the human reproductive organs. *Journal of Clinical Endocrinology and Metabolism* **85** 4835–4840.
- Pelletier G, Labrie C & Labrie F 2000 Localization of oestrogen receptor alpha, oestrogen receptor beta and androgen receptors in the rat reproductive organs. *Journal of Endocrinology* **165** 359–370.
- Pepe GJ, Billiar RB, Leavitt MG, Zachos NC, Gustafsson JA & Albrecht ED 2002 Expression of estrogen receptors alpha and beta in the baboon fetal ovary. *Biology of Reproduction* **66** 1054–1060.
- Petersen DN, Tkalecic GT, Koza-Taylor PH, Turi TG & Brown TA 1998 Identification of estrogen receptor beta2, a functional variant of estrogen receptor beta expressed in normal rat tissues. *Endocrinology* **139** 1082–1092.
- Pettersson K, Grandien K, Kuiper GG & Gustafsson JA 1997 Mouse estrogen receptor beta forms estrogen response element-binding heterodimers with estrogen receptor alpha. *Molecular Endocrinology* **11** 1486–1496.
- Poola I, Abraham J & Baldwin K 2002 Identification of ten exon deleted ERbeta mRNAs in human ovary, breast, uterus and bone tissues: alternate splicing pattern of estrogen receptor beta mRNA is distinct from that of estrogen receptor alpha. *FEBS Letters* **516** 133–138.
- Poola I, Abraham J, Baldwin K, Saunders A & Bhatnagar R 2005 Estrogen receptors beta4 and beta5 are full length functionally distinct ERbeta isoforms: cloning from human ovary and functional characterization. *Endocrine* **27** 227–238.
- Price KR & Fenwick GW 1985 Naturally occurring oestrogens in food – a review. *Food Additives and Contaminants* **2** 73–106.
- Revelli A, Pacchioni D, Cassoni P, Bussolati G & Massobrio M 1996 *In situ* hybridization study of messenger RNA for estrogen receptor and immunohistochemical detection of estrogen and progesterone receptors in the human ovary. *Gynecological Endocrinology* **10** 177–186.
- Richards JS 1980 Maturation of ovarian follicles: actions and interactions of pituitary and ovarian hormones on follicular cell differentiation. *Physiological Reviews* **60** 51–89.

- Rosenfeld CS, Yuan X, Manikkam M, Calder MD, Garverick HA & Lubahn DB 1999 Cloning, sequencing, and localization of bovine estrogen receptor-beta within the ovarian follicle. *Biology of Reproduction* **60** 691–697.
- Safe SH, Pallaroni L, Yoon K, Gaido K, Ross S, Saville B & McDonnell D 2001 Toxicology of environmental estrogens. *Reproduction, Fertility, and Development* **13** 307–315.
- Sar M & Welsch F 1999 Differential expression of estrogen receptor-beta and estrogen receptor-alpha in the rat ovary. *Endocrinology* **140** 963–971.
- Sasano H, Suzuki T, Matsuzaki Y, Fukaya T, Endoh M, Nagura H & Kimura M 1999 Messenger ribonucleic acid *in situ* hybridization analysis of estrogen receptors alpha and beta in human breast carcinoma. *Journal of Clinical Endocrinology and Metabolism* **84** 781–785.
- Saunders PT, Maguire SM, Gaughan J & Millar MR 1997 Expression of oestrogen receptor beta (ER beta) in multiple rat tissues visualised by immunohistochemistry. *Journal of Endocrinology* **154** R13–R16.
- Saunders PT, Millar MR, Williams K, Macpherson S, Harkiss D, Anderson RA, Orr B, Groome NP, Scobie G & Fraser HM 2000 Differential expression of estrogen receptor-alpha and -beta and androgen receptor in the ovaries of marmosets and humans. *Biology of Reproduction* **63** 1098–1105.
- Saunders PT, Millar MR, Macpherson S, Irvine DS, Groome NP, Evans LR & Sharpe RM 2002 ER beta1 and the ERbeta2 splice variant (ERbetacx/beta2) are expressed in distinct cell populations in the adult human testis. *Journal of Clinical Endocrinology and Metabolism* **87** 2706–2715.
- Schumer ST & Cannistra SA 2003 Granulosa cell tumor of the ovary. *Journal of Clinical Oncology* **21** 1180–1189.
- Shirwalkar H, Modi DN & Maitra A 2007 Exposure of adult rats to estradiol valerate induces ovarian cyst with early senescence of follicles. *Molecular and Cellular Endocrinology* **272** 22–37.
- Slomczynska M, Duda M & Galas J 2001 Estrogen receptor alpha and beta expression in the porcine ovary. *Folia Histochemica et Cytobiologica* **39** 137–138.
- Sundararajan C, Liao WX, Roy AC & Ng SC 2001 Association between estrogen receptor-beta gene polymorphisms and ovulatory dysfunctions in patients with menstrual disorders. *Journal of Clinical Endocrinology and Metabolism* **86** 135–139.
- Suzuki F, Akahira J, Miura I, Suzuki T, Ito K, Hayashi S, Sasano H & Yaegashi N 2008 Loss of estrogen receptor beta isoform expression and its correlation with aberrant DNA methylation of the 5'-untranslated region in human epithelial ovarian carcinoma. *Cancer Science* **99** 2365–2372.
- Taylor AH & Al-Azzawi F 2000 Immunolocalisation of oestrogen receptor beta in human tissues. *Journal of Molecular Endocrinology* **24** 145–155.
- Tetsuka M, Milne M & Hillier SG 1998 Expression of oestrogen receptor isoforms in relation to enzymes of oestrogen synthesis in rat ovary. *Molecular and Cellular Endocrinology* **141** 29–35.
- Treec O, Pfeiler G, Mitter D, Latrich C, Piendl G & Ortmann O 2007 Estrogen receptor {beta}1 exerts antitumoral effects on SK-OV-3 ovarian cancer cells. *Journal of Endocrinology* **193** 421–433.
- Tremblay GB, Tremblay A, Copeland NG, Gilbert DJ, Jenkins NA, Labrie F & Giguere V 1997 Cloning, chromosomal localization, and functional analysis of the murine estrogen receptor beta. *Molecular Endocrinology* **11** 353–365.
- Vendrell JA, Magnino F, Danis E, Duchesne MJ, Pinloche S, Pons M, Birnbaum D, Nguyen C, Theillet C & Cohen PA 2004 Estrogen regulation in human breast cancer cells of new downstream gene targets involved in estrogen metabolism, cell proliferation and cell transformation. *Journal of Molecular Endocrinology* **32** 397–414.
- Wang H, Masironi B, Eriksson H & Sahlin L 1999 A comparative study of estrogen receptors alpha and beta in the rat uterus. *Biology of Reproduction* **61** 955–964.
- Wang Y, Chan S & Tsang BK 2002 Involvement of inhibitory nuclear factor-kappaB (Nf-kappaB)-independent Nf-kappaB activation in the gonadotropic regulation of X-linked inhibitor of apoptosis expression during ovarian follicular development *in vitro*. *Endocrinology* **143** 2732–2740.
- Waters KM, Safe S & Gaido KW 2001 Differential gene expression in response to methoxychlor and estradiol through ERalpha, ERbeta, and AR in reproductive tissues of female mice. *Toxicology Science* **63** 47–56.
- Weihua Z, Makela S, Andersson LC, Salmi S, Saji S, Webster JL, Jensen EV, Nilsson S, Warner M & Gustafsson JA 2001 A role for estrogen receptor beta in the regulation of growth of the ventral prostate. *PNAS* **98** 6330–6335.
- Weiser MJ, Foradori CD & Handa RJ 2008 Estrogen receptor beta in the brain: from form to function. *Brain Research Reviews* **57** 309–320.
- Whitten PL & Naftolin F 1998 Reproductive actions of phytoestrogens. *Bailliere's Clinical Endocrinology and Metabolism* **12** 667–689.
- Whitten PL & Patisaul HB 2001 Cross-species and interassay comparisons of phytoestrogen action. *Environmental Health Perspectives* **109** (Suppl 1) 5–20.
- Yang P, Kriatchko A & Roy SK 2002 Expression of ER-alpha and ER-beta in the hamster ovary: differential regulation by gonadotropins and ovarian steroid hormones. *Endocrinology* **143** 2385–2398.
- Younglai EV, Holloway AC & Foster WG 2005 Environmental and occupational factors affecting fertility and IVF success. *Human Reproduction Update* **11** 43–57.
- Zama AM & Uzumcu M 2009 Fetal and neonatal exposure to the endocrine disruptor methoxychlor causes epigenetic alterations in adult ovarian genes. *Endocrinology* **150** 4681–4691.
- Zhao C, Toresson G, Xu L, Koehler KF, Gustafsson JA & Dahlman-Wright K 2005 Mouse estrogen receptor beta isoforms exhibit differences in ligand selectivity and coactivator recruitment. *Biochemistry* **44** 7936–7944.
- Zhao C, Dahlman-Wright K & Gustafsson JA 2008 Estrogen receptor beta: an overview and update. *Nuclear Receptor Signaling* **6** e003.

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