

## Review

# Estrogen Receptor Knockout Mice and Their Effects on Fertility

Ivan Nalvarte  and Per Antonson \* 

Department of Biosciences and Nutrition, Karolinska Institutet, SE 14157 Huddinge, Sweden

\* Correspondence: per.antonson@ki.se; Tel.: +46-8-52481049

**Abstract:** Estrogens play a crucial role in sexual development and fertility as well as many other physiological processes, and it is estrogen receptors that mediate the physiological responses. To study the role of the estrogen receptors in these processes, several genetic mouse models have been developed using different strategies, which also in some cases yield different results. Here, we summarize the models that have been made and their impact on fertility in relation to known cases of human estrogen receptor mutations.

**Keywords:** *Esr1*; *Esr2*; estrogen receptor alpha; estrogen receptor beta; knockout mice; estrogen; fertility

## 1. Introduction

In this review, we focus on genetic animal models that have been created to study estrogen signaling and how fertility is affected in these models. The first generation of animal models targeting estrogen receptors were made by insertion of a neomycin cassette into a coding exon to interrupt the coding region; however, this strategy is not efficient, since there is a risk that the neomycin cassette can be spliced out, leaving partial receptor activity. Later mouse models developed have used the Cre/loxP system [1] to delete a specific exon to cause premature stop codons or delete specific domains from the receptor. This system can be used either to make germline deletions or conditional deletions. Several knock-in mouse lines with specific point mutations have also been made to determine the function of specific amino acids in the estrogen receptors. Recently, the CRISPR/Cas9 system [2] has been used to make large or specific deletions in the estrogen receptor genes. We will discuss how these different strategies to genetically target estrogen receptors yield different effects on male and female fertility in rodents in relation to known cases of human estrogen receptor mutations.

## 2. Estrogen Receptors

### 2.1. Overview

Physiological responses to estrogen are mediated by the two classic estrogen receptors, estrogen receptor alpha (ER $\alpha$ , NR3A1) and estrogen receptor beta (ER $\beta$ , NR3A2), and the G protein-coupled receptor 1 (GPER1) [3]. GPER1 mediates rapid non-genomic effects of estradiol (E2) but does not affect fertility in mice [4]. The human ER $\alpha$  gene, *ESR1*, was cloned in 1985 [5], and the ER $\beta$  gene, *ESR2*, was discovered in the laboratory of Jan-Åke Gustafsson and cloned from rat prostate in 1996 [6].

ER $\alpha$  and ER $\beta$  are nuclear receptors (NR) and belong to the nuclear hormone receptor superfamily of ligand-regulated transcription factors. Humans have 48 NRs and mice 49, and the ligands for NRs are generally small hydrophobic molecules, such as steroid hormones, retinoids, or metabolic intermediates, and regulate all aspects of life [7]. Ligand binding results in conformational changes that allow DNA binding and interaction with coregulator proteins, which affects gene transcription either positively or negatively, as reviewed in [8].



**Citation:** Nalvarte, I.; Antonson, P. Estrogen Receptor Knockout Mice and Their Effects on Fertility.

*Receptors* **2023**, *2*, 116–126. <https://doi.org/10.3390/receptors2010007>

Academic Editor: Anders Strom

Received: 22 December 2022

Revised: 10 February 2023

Accepted: 28 February 2023

Published: 7 March 2023

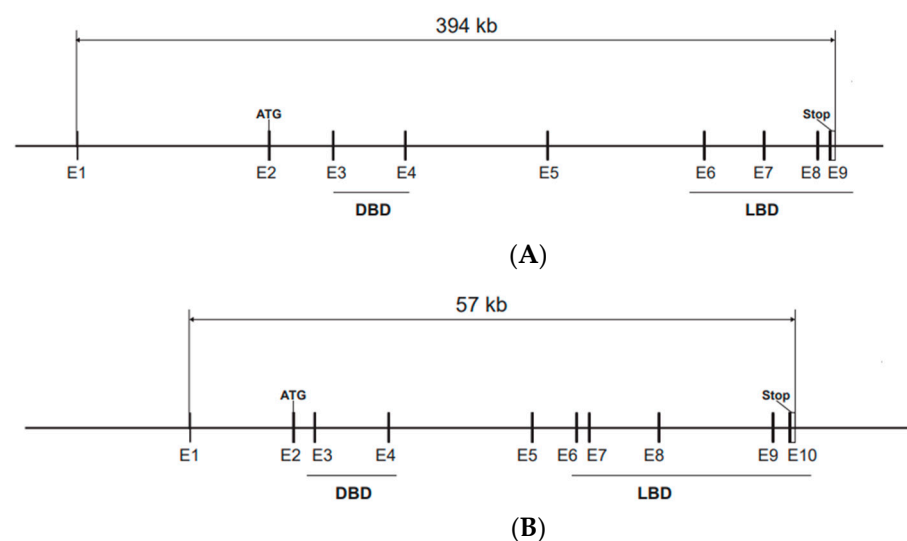


**Copyright:** © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

NRs have a conserved domain structure with a variable N-terminal domain that contains a constitutively active transcriptional activation function (AF-1) region, a DNA-binding domain (DBD), a flexible hinge region, and a ligand-binding domain (LBD), with a ligand-dependent AF-2 region in the C-terminal domain, as reviewed in [9]. The DBD consists of two zinc finger motifs that facilitate binding to specific regions in the genome, called hormone response elements (HRE). Steroid hormone receptors bind as homodimers, while most other NRs bind as heterodimers with the retinoid X receptor (RXR). Sequences responsible for dimerization are localized both in the DBD and the LBD. The LBD has a structurally conserved fold that upon agonist binding stabilizes a transcriptionally active conformation of the receptor, exposing a docking site for coactivators, that can modify the chromatin structure or interact directly with the transcription pre-initiation complex to facilitate transcription. In the absence of ligands or bound to antagonists, the LBD recruits corepressors that repress transcription [10]. The LBD is about 56% similar between ER $\alpha$  and ER $\beta$ , and both receptors bind E2 with high affinity. ERs can also bind to other estrogen metabolites and plant estrogens, called phytoestrogens, as well as synthetic ligands, although the affinity is lower than for E2 [11]. The DBD is 97% identical between ER $\alpha$  and ER $\beta$  and it directs binding to estrogen response elements (EREs) located in promoter and enhancer regions. The mouse ER $\alpha$  protein is 599 amino acids long with the DBD located at positions 184–265 and the LBD/AF2 at 315–599. Mouse ER $\beta$  contains 530 amino acids, with the DBD and LBD/AF2 at positions 149–214 and 264–530, respectively.

## 2.2. Genes

In mice, ER $\alpha$  and ER $\beta$ , encoded by the *Esr1* and *Esr2* genes, respectively, share a common genomic organization with the start codon in exon 2, according to the most used nomenclature, although alternative exons are present in both genes (Figure 1A,B). The sequences encoding the DBD are in exon 3 and exon 4, with one zinc finger in each of these exons [12]. Both *Esr1* and *Esr2* produce alternatively spliced mRNA transcripts [13,14]. Mouse *Esr1* produce several mRNA variants with different 5'-untranslated regions by alternative promoter usage and alternative splicing [15]. The most common alternatively spliced *Esr2* variant in mice and rats is ER $\beta$ -2, also called ER $\beta$ -ins, that has an extra exon in the region encoding the LBD, resulting in a lower affinity for ligands, and functions as a dominant negative receptor that represses estrogen signaling [16–18]. In humans, the splice variant ER $\beta$ -2, also called ER $\beta$ -cx, has a similar role [19]. The mouse *Esr1* gene is localized on chromosome 10 and human *ESR1* on chromosome 6, while the mouse *Esr2* gene is localized on chromosome 12 and the human ortholog is on chromosome 14.



**Figure 1.** Genomic structure of the *Esr1* and *Esr2* genes. Start and stop codons are indicated and the exons encoding the DBD and LBD are underlined. (A) The *Esr1* gene is localized on chromosome 10

and spans 394 kb and 9 exons (E1–E9) according to reference sequence NM\_007956.5, *Esr1* transcript variant 1. The start codon (ATG) is located in exon 2 and the stop codon in exon 9. (B) The *Esr2* gene is localized on chromosome 12 and spans 57 kb and contains 10 exons (E1–E10) according to reference sequence NM\_207707.1, *Esr2* transcript variant 1. This variant produces a protein with an insertion of 18 amino acids that results in decreased ligand-binding affinity. *Esr2* transcript variant 2, that lacks exon 7, is the variant that has the highest transcriptional activation activity.

### 2.3. Expression Patterns of *Esr1* and *Esr2*

*Esr1* is expressed in reproductive organs, including the uterus, ovary, and testis, as well as several non-reproductive organs, including the pituitary, liver, mammary gland, bone, and hypothalamus [20,21]. The expression of mouse *Esr2* is more restricted, with the highest protein levels in granulosa cells in the ovary and parts of the brain [22–27]. Rat and human *ESR2* is expressed in more tissues, including prostate [6,23], and in humans the testis is the organ that expresses the highest levels of ER $\beta$  [22], suggesting species-specific functions of this receptor.

## 3. Animal Models

Several mouse models with disrupted *Esr1* and *Esr2* genes, resulting in compromised *Esr* expression, have been generated, and the mice are viable and grossly normal, but fertility is affected in both *Esr1* and *Esr2* null mutated mice. In addition, many *Esr1* knock-in mouse lines have been developed. In these, mutations have been introduced in different parts of the *Esr1* gene, resulting in different reproductive phenotypes. A summary of genetic mouse models with information about the roles of *Esr1* and *Esr2* on fertility is provided in Table 1.

### 3.1. *Esr1* Mouse Models

The first *Esr1* knockout mice were generated by insertion of a neomycin cassette into exon 3 and showed that female mice were infertile and male mice had reduced fertility [28]. More recent mouse models have used the Cre/LoxP system to excise *Esr1* exon 3, that produces a frame-shift mRNA transcript with a premature stop codon short after the deleted exon, and the putative translated truncated protein lacks both the DBD and the LBD [29–32]. This mutation results in infertility in both male and female mice. The infertility in male knockout mice has been determined to be due to reduced expression of the Na<sup>+</sup>/H<sup>+</sup> exchanger and water channel genes in the efferent ducts that connect the rete testis to the proximal portion of the duct of the epididymis, resulting in disrupted fluid reabsorption, causing a harmful environment, with increased pH and decreased luminal osmolality, that kills sperm [33–35]. Female knockout mice have increased E2 levels, no estrous cycle, a hypoplastic uterus, and hemorrhagic cysts in the ovaries. *Esr1* knockout mice do not produce oocytes in response to superovulation [29]. The ovarian phenotype has been proposed to be due to elevated luteinizing hormone (LH) levels and hyperstimulation of the ovary as a result of aberrant estradiol feedback inhibition in the pituitary [36].

**Table 1.** Selected mouse lines with *Esr* mutations that affect fertility.

Mouse Line	Mutation	Cell Type/Effect	Fertility	References
ERKO	Neo insertion into <i>Esr1</i> exon 3	Global KO	F: infertile M: reduced	[28]
ER $\alpha$ KO, Ex3 $\alpha$ ERKO	Deletion of <i>Esr1</i> exon 3	Global KO	F: infertile M: infertile	[29–32]
ER $\alpha$ <sup>fl/fl</sup>	Deletion of <i>Esr1</i> exon 3	Neurons	F: infertile	[37]
ER $\alpha$ <sup>fl/fl</sup> ;CamKII $\alpha$ -Cre	Deletion of <i>Esr1</i> exon 3	Adipocytes and hypothalamus	F: infertile M: fertile	[38]

Table 1. Cont.

Mouse Line	Mutation	Cell Type/Effect	Fertility	References
ER $\alpha^{\text{flox/flox}}$ $\alpha\text{GSU}^{\text{cre}}$	Deletion of <i>Esr1</i> exon 3	pituitary gonadotroph	F: infertile	[39]
UtEpi $\alpha\text{ERKO}$	Deletion of <i>Esr1</i> exon 3	uterine epithelial cells	F: infertile	[40]
WE $^{\text{d/d}}$ (Wnt7a-Cre)	Deletion of <i>Esr1</i> exon 3	uterine cells	F: infertile	[41]
Amhr2 $^{\text{Cre/+}}$ ; <i>Esr1</i> $^{\text{f/-}}$	Deletion of <i>Esr1</i> exon 3	uterine stromal cells	F: reduced	[42]
NERKI+/–	E207A/G208A in ER $\alpha$	no DNA binding, DN	F: infertile M: fertile	[43]
ER $\alpha_{(\text{EAAE})}$	Y201E/K210A/K214A/R215E in ER $\alpha$	no DNA binding	F: infertile M: infertile	[44]
ENERKI	G525L in ER $\alpha$	no E2 binding	F: infertile M: sub-fertile	[45]
AF2ERKI	L543A/L544A in ER $\alpha$	no E2 binding	F: infertile M: fertile	[46]
ER $\alpha$ C451A, NOER	C451A in ER $\alpha$	no membrane ER $\alpha$	F: infertile M: sub-fertile	[47–49]
ESR1 $^{\text{Y541S}}$ / $\beta\text{Actin-Cre}$	Y541S, inducible	constitutively active	F: infertile M: infertile	[50]
ER $\beta$ –/–	Neo insertion into <i>Esr2</i> exon 3	Global	F: reduced M: fertile	[51]
Er $\beta\text{KO}$	Replacement of <i>Esr2</i> exon 3 with Neo	Global	F: reduced M: fertile	[29]
ER $\beta_{\text{ST}}^{\text{L-/-}}$ , Ex3 $\beta\text{ERKO}$	Deletion of <i>Esr1</i> exon 3	Global	F: reduced * M: fertile *	[52–55]
ER $\beta$ - $\Delta\text{ex3}$ , CER $\beta\text{KO}$	Deletion of <i>Esr2</i> exon 1–10	Global	F: sub-fertile	[24]
<i>Esr2</i> $^{\Delta\text{E1-10}}$			M: fertile	
<i>Esr2</i> $^{\text{Y55F/Y55F}}$	Y55F in ER $\beta$	reduced activity	F: reduced	[56]
$\alpha\beta\text{ERKO}$	<i>Esr1</i> / <i>Esr2</i> double knockout	Global	F: infertile	[57]
ER $\alpha\beta\text{KO}$	ERKO $\times$ ER $\beta$ –/–	Global	M: infertile	[29]
	<i>Esr1</i> / <i>Esr2</i> double knockout		F: infertile	
	ER $\alpha\text{KO}$ $\times$ ER $\beta\text{KO}$		M: infertile	

F, Female; M, Male; DN, dominant-negative function; \* infertility has been reported.

For neuron-specific *Esr1* deletion, ER $\alpha^{\text{fl/fl}}$  CamKII $\alpha$ -Cre mice have demonstrated a critical role for ER $\alpha$  in the estrogen positive feedback on gonadotropin-releasing hormone (GnRH) neurons, where the lack of ER $\alpha$  results in infertility due to the absence of the pre-ovulatory gonadotropin surge [37]. In addition, an increased serum E2 level and infertility have been reported as a consequence of deletion of *Esr1* in the hypothalamus using *aP2*-Cre or *RIP*-Cre transgenic mice, which directs Cre expression to adipocytes or the pancreas and hypothalamus [38,58]. Uterine-specific knockout mice that lack ER $\alpha$  in epithelial cells are infertile due to a lack of implantation [40,41], and mice with *Esr1* deleted from uterine stromal cells have reduced fertility [42]. Finally, a critical role for ER $\alpha$  in the pituitary was further demonstrated since female pituitary-specific *Esr1* knockout mice, ER $\alpha^{\text{flox/flox}}$   $\alpha\text{GSU}^{\text{cre}}$ , lack estrus cyclicity and the LH surge and are infertile [39]. In conclusion, studies indicate that it is ER $\alpha$ -mediated functions in the brain on several levels, in the uterus, and in the pituitary that are responsible for the infertility, rather than ER $\alpha$ 's effects on the ovary.

Mouse lines with entire ER $\alpha$  domains have been developed and showed that removal of the exons coding for the AF-1 domain or DBD or LBD results in infertility in both male and female mutated mice, as reviewed in [59]. Several mouse lines with *Esr1* germline point mutations have been generated which display severe fertility deficits. A knock-in mouse line termed NERKI with mutations in *Esr1*, ER $\alpha$  E207A/G208A, that make ER $\alpha$  unable to bind to DNA, displayed infertility in female heterozygous mice. This mouse model showed the importance of the classical pathway, where ERs binds to DNA, but also nonclassical pathways, since the phenotype differed from the global ER $\alpha$  most strikingly in the uterus which displayed hyperplasia [43]. Another ER $\alpha$  DBD mutant knock-in mouse line termed EAAE, with the mutations Y201E, K210A, K214A, and R215E, displayed a

phenotype similar to the global ER $\alpha$  knockout mice with infertility in both sexes when homozygous for the mutation [44]. Furthermore, ER $\alpha$  LBD is also needed for fertility, exemplified by an estrogen-non-responsive ER $\alpha$  knock-in mice (ENERKI) model with a point mutation in the LBD coding region, G525L [45]. This mutation resulted in a reduced response to endogenous estrogens and showed an anovulatory and hemorrhagic cystic follicle phenotype in female homozygous mice that in turn resulted in infertility [45]. Interestingly, the ENERKI mice do respond to the ER $\alpha$ -selective agonist propyl pyrazole triol (PPT), and treatment prevented the formation of hemorrhagic cysts.

A different ER $\alpha$  LBD knock-in mouse model, AF2ERKI, with the ER $\alpha$  mutations L543A and L544A, displayed infertility in both male and female mice with similar phenotypes as in global ER $\alpha$  knockout mice [46,60]. Interestingly, this mutation changes antagonists to agonists and the male infertility can be restored by Tamoxifen treatment [46]. A mouse line with a Cre-inducible germline point mutation in the *Esr1* LBD coding region, Y541S, corresponding to the human metastatic breast cancer mutation ESR1<sup>Y537S</sup>, resulted in constitutive activation of the receptor, which led to severe developmental defects in the reproductive organs that in turn resulted in infertility in both male and female mice and cysts detected in the uterus [50]. ER $\alpha$  non-genomic functions are mediated by a cell membrane-bound form of ER $\alpha$  that is directed to the cell membrane by the post-translational modification palmitoylation of a cysteine at position 451 in ER $\alpha$ , and position 447 in human ER $\alpha$ . The membrane-bound fraction of ER $\alpha$  interacts with the membrane protein caveolin-1 and acts together with membrane-associated kinases to mediate rapid effects of E2 [61]. Knock-in mouse models with a mutation of ER $\alpha$  cysteine 451 to alanine have been made independently by two research groups, and these two mouse lines, termed ER $\alpha$  C451A [47] and NOER [48], display severe effects on fertility in both female and male mice [47–49]. The female mice are infertile with ovaries lacking corpora lutea and have an excess of hemorrhagic follicles and increased luteinizing hormone levels. The phenotype of uteri was reported to be normal in ER $\alpha$  C451A mice but hypoplastic in NOER mice. The male phenotype is milder than the global ER $\alpha$  knockout and young males produce litters [49]. Finally, a targeted mutation, using the CRISPR/Cas9 technique, that specifically reduces the expression of an ovary-, uterus-, and pituitary-specific long variant of ER $\alpha$  (ER $\alpha$ -66), resulted in subfertility, an irregular estrus cycle, and defects in maintaining litters, although no obvious morphological or pathological changes were observed in the reproductive organs [62].

### 3.2. *Esr2* Mouse Models

The first *Esr2* knockout mouse line was made in the laboratories of Jan-Åke Gustafsson and Oliver Smithies by insertion of a neomycin cassette and stop codons into exon 3, encoding the DBD. These mice lack expression of full-length ER $\beta$  mRNA and displayed reduced female fertility, while male mice remained fertile [51]. Subsequent mouse models have used the Cre/loxP system to target exon 3, similar to what was done to generate *Esr1* knockout mice [29,52–55]. Since targeting exon 3 may still produce low amounts of ER $\beta$  mRNA splice variants, later strategies have used the CRISPR/Cas9 system to delete the entire gene [24,63]. The overall conclusion from these studies is that male *Esr2* knockout mice are fertile and female mice are sub-fertile, with fewer litters and reduced litter sizes. Interestingly, the complete deletion of the *Esr2* gene results in not only female subfertility but also premature female infertility at around 6 months of age [24]. Transplantation experiments in which ovaries were transplanted from WT into *Esr2* knockout mice restored the fertility in knockout females [64], which strongly supports that ER $\beta$  in the ovary is responsible for the effects on fertility and is in line with high levels of ER $\beta$  in the granulosa cells of the mouse ovary. Serum levels of E2 are normal in *Esr2* knockout mice, but at the diestrus/proestrus stage of the estrous cycle, the levels have been reported to be lower than in WT mice [24,55,64] and it is likely that this affects the LH surge that is blunted in *Esr2* knockout mice [55,64]. Furthermore, the ovaries of the *Esr2* knockout mice display a reduced number of corpora lutea, a sign of reduced ovulation, as the most



striking phenotype [24,29,51,55]. Analysis of gene expression in follicles and granulosa cells from *Esr2* knockout mice have identified several genes with reduced expression that might explain the fertility defects. These include *Cyp19a1*, encoding the aromatase enzyme responsible for final stages of E2 biosynthesis, and after gonadotropin stimulation, the *Lhcgr*, *Cyp11a1*, and *Ptgs2* genes were expressed at lower levels [53,65].

In superovulation experiments, *Esr2* knockout mice produce less ovulating follicles in response to gonadotropin injections compared to their WT littermates [29,51,53,66]. Surprisingly, mice with a complete deletion of *Esr2* do not show such superovulation deficits [24], which may suggest that the ovarian response to gonadotropins is not compromised upon total deletion of *Esr2*, and that the previous *Esr2* knockout models targeting the exon 3 produce ovarian artefacts. ER $\beta$  has also been reported as expressed in GnRH-releasing neurons in the hypothalamus [67] that receive input from ER $\alpha$ - and Kiss1-positive afferent neurons. However, a clear role of ER $\beta$  in GnRH neurons has so far not been established, and likely the effect of ER $\beta$  on fertility is not mediated via its function in the adult brain.

In contrast to the many *Esr1* knock-in mouse models, only one *Esr2* knock-in model has been generated to our knowledge (Table 1). This mouse line has a point mutation in a tyrosine phosphorylation site in the N-terminal AF1 domain, *Esr2*<sup>Y55F/Y55F</sup>, and displays reduced female fertility and ovarian size [56]. The mutation was shown to reduce the ER $\beta$  chromatin binding to promoter regions. The involvement of *Esr2* LBD or DBD in rodent fertility is, however, still not well-established, since there are no mice with point mutations in these regions. However, data from human patients with point mutations in the *ESR2* gene suggest that both the LBD and DBD participate in male and female gonadal development and fertility (discussed in Section 4).

### 3.3. *Esr1/Esr2* Double Knockouts

Mice that lack both *Esr1* and *Esr2*, *Esr1/Esr2* double knockouts, are viable, but both male and female mice are infertile with an ovarian phenotype different from the single knockouts, with structures similar to seminiferous tubules of the testis [29,57]. Interestingly, the adult, but not prepuberal, ovary exhibits follicles that lack oocytes but contain what appears to be seminiferous tube-like structures and Sertoli cells, normally found in testis, suggesting trans-differentiated follicles and a potential sex reversal [57]. Thus, a combined role of *Esr1* and *Esr2* may be needed to maintain oocyte integrity and prevent follicular degeneration.

### 3.4. Rat *Esr1* and *Esr2* Knockout Models

Knockout models of *Esr1* and *Esr2* have been made in rats using zinc finger nuclease genome targeting [68,69]. Both these models targeted and deleted exon 3, which encodes the DBD, similar to the mouse exon 3, and creates premature stop codons. The *Esr1* rat knockout displays many phenotypes similar to the mouse *Esr1* knockout, including infertility in both sexes, increased serum E2 levels, a hypoplastic uterus, and hemorrhagic cysts in the ovaries [68]. The phenotype in the null mutated rat *Esr2* line is more severe than in mice, since females are infertile and do not produce oocytes in response to gonadotropin stimulation, while the males are fertile [69]. In addition, an exon 4-deleted rat model was made that mimics an endogenous splice variant encoding a transcript that lacks DNA-binding capacity. This rat model displays a similar phenotype as the null mutated rat, indicating that the regulation of female fertility depends on ER $\beta$  acting through binding to ERE motifs [69].

## 4. Human Mutations in *ESR1* and *ESR2*

Data on human loss-of-function *ESR1* or *ESR2* mutations are sparse, but identified cases confirm a role for these receptors in human reproduction. A male patient with a homozygous point mutation causing a premature stop codon before the sequences encoding the DBD in *ESR1* has been identified [70]. The mutation resulted in abnormal bone maturation and mineralization, but seemingly normal testosterone levels and sperm

count, although a lower sperm viability [70]. A female patient with a homozygous loss-of-function *ESR1* variant, Q375H, affecting the LBD, was identified in 2013, with a lack of breast development, pelvic pain, increased serum E2 levels, and hemorrhagic ovarian cysts [71]. This phenotype resembles that of female *Esr1* knockout mice. Similar phenotypes were reported from a family with another homozygous loss-of-function *ESR1* mutation, R394H, affecting the LBD [72]. Recently, two sisters were identified with a homozygous loss-of-function *ESR1* variant in the LBD, E385V, but displaying different severities of their ovarian and hormonal phenotypes, suggesting that different compensating mechanisms exist [73]. Three case studies with *ESR2* mutations have been reported. The first study identifies a heterozygous *ESR2* missense variant, A432D, in an exon encoding the LBD in a girl with primary amenorrhea [74]. The second describes a female patient with a heterozygous missense *ESR2* mutation in the LBD, K314R, that was shown to produce a dominant negative form of ER $\beta$  that also affects the activity of ER $\alpha$  [75]. This patient displayed complete ovarian failure and a lack of puberty, as well as juvenile uterus, small ovaries, and severe osteoporosis due to the lack of E2-producing ovary function. Interestingly, E2 supplementation resulted in menarche and regular menses, as well as in an increased uterus volume and ovarian growth, however with a lack of follicles [75], which contrasts with E2 supplementation in *ESR1* mutant females. This confirms animal model data showing that ER $\beta$  is important for fertility via its ovarian function rather than via any direct effect on the hypothalamus or pituitary. The third case study describes three patients with disorders or differences of sex development (46,XY DSD) [76]. One of these patients had a germline homozygous 3 bp deletion in the DBD encoding region of *ESR2*, N181del, and displayed an absence of gonads. The other two patients displayed different heterozygous missense mutations in the N-terminal region, G84V, and the LBD, L426R, coding sequence. However, the association of *ESR2* variants with 46,XY DSD remains to be established since the clinical outcomes are different and the functions of the different ER $\beta$  variants are not fully understood [77]. Although these human studies confirm a role for ERs in human reproduction, they also suggest important species differences, where *ESR2* mutations in humans appear to yield a more severe gonadal phenotype than in mouse models.

## 5. Conclusions and Future Perspectives

Estrogen signaling via ER $\alpha$  and ER $\beta$  plays a key role for fertility in mice, rats, and humans. Null mutations of *Esr1* lead to infertility in both mice and rats and in both males and females, and the phenotypes are comparable to what has been described in patients with homozygous loss-of-function mutations. *Esr2* null mutations lead to reduced fertility in female mice and infertility in female rats. *ESR2* mutations identified in patients suggest a more critical function of *ESR2* on fertility in humans than in rodents. Thus, it appears that important species differences exist regarding ER $\beta$ 's role on fertility. However, this needs to be verified since the number of human cases is low and the function of most of these mutated variants is not fully understood when it comes to interactions with ER $\alpha$  or their transcriptional activity. The results from the 46,XY DSD patients suggest a role for ER $\beta$  in male gonadal development that is not seen in mouse models, and one explanation for this difference could be that humans express high levels of ER $\beta$  in testis that is not seen in rodents [22]. Identifying ER $\beta$  target genes in the ovary that can explain the reduced ovulation in ER $\beta$ -KO mice is an important future step to clarify the role of ER $\beta$  in fertility, and this may identify new targets for fertility treatment. Techniques such as Chip-Seq or single-cell sequencing may hold great promise to advance knowledge in this field. The roles of ER $\beta$  splice variants in fertility are not known, but the presence of two variants, that can either activate or repress E2-regulated transcriptional responses, suggests an important role for these in regulating fertility since the results from mouse models have shown that both enhanced and reduced estrogenic signaling affect fertility.

**Author Contributions:** I.N., writing the manuscript, review and editing, visualization; P.A., writing the manuscript, review and editing, visualization. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by Karolinska Institute and I.N. is in part funded by the National Institutes of Health (R01AG065209).

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Not applicable.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Sauer, B.; Henderson, N. Site-Specific DNA Recombination in Mammalian Cells by the Cre Recombinase of Bacteriophage P1. *Proc. Natl. Acad. Sci. USA* **1988**, *85*, 5166–5170. [[CrossRef](#)] [[PubMed](#)]
2. Ran, F.A.; Hsu, P.D.; Wright, J.; Agarwala, V.; Scott, D.A.; Zhang, F. Genome Engineering Using the Crispr-Cas9 System. *Nat. Protoc.* **2013**, *8*, 2281–2308. [[CrossRef](#)] [[PubMed](#)]
3. Eyster, K.M. The Estrogen Receptors: An Overview from Different Perspectives. *Methods Mol. Biol.* **2016**, *1366*, 1–10. [[CrossRef](#)]
4. Prossnitz, E.R.; Hathaway, H.J. What Have We Learned About Gper Function in Physiology and Disease from Knockout Mice? *J. Steroid Biochem. Mol. Biol.* **2015**, *153*, 114–126. [[CrossRef](#)] [[PubMed](#)]
5. Walter, P.; Green, S.; Greene, G.; Krust, A.; Bornert, J.M.; Jeltsch, J.M.; Staub, A.; Jensen, E.; Scrace, G.; Waterfield, M. Cloning of the Human Estrogen Receptor Cdna. *Proc. Natl. Acad. Sci. USA* **1985**, *82*, 7889–7893. [[CrossRef](#)]
6. Kuiper, G.G.; Enmark, E.; Peltö-Huikko, M.; Nilsson, S.; Gustafsson, J.A. Cloning of a Novel Receptor Expressed in Rat Prostate and Ovary. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 5925–5930. [[CrossRef](#)]
7. Mangelsdorf, D.J.; Thummel, C.; Beato, M.; Herrlich, P.; Schütz, G.; Umesono, K.; Blumberg, B.; Kastner, P.; Mark, M.; Chambon, P.; et al. The Nuclear Receptor Superfamily: The Second Decade. *Cell* **1995**, *83*, 835–839. [[CrossRef](#)] [[PubMed](#)]
8. Rosenfeld, M.G.; Lunyak, V.V.; Glass, C.K. Sensors and Signals: A Coactivator/Corepressor/Epigenetic Code for Integrating Signal-Dependent Programs of Transcriptional Response. *Genes Dev.* **2006**, *20*, 1405–1428. [[CrossRef](#)]
9. Gronemeyer, H.; Gustafsson, J.; Laudet, V. Principles for Modulation of the Nuclear Receptor Superfamily. *Nat. Rev. Drug Discov.* **2004**, *3*, 950–964. [[CrossRef](#)]
10. Heldring, N.; Pike, A.; Andersson, S.; Matthews, J.; Cheng, G.; Hartman, J.; Tujague, M.; Strom, A.; Treuter, E.; Warner, M.; et al. Estrogen Receptors: How Do They Signal and What Are Their Targets. *Physiol. Rev.* **2007**, *87*, 905–931. [[CrossRef](#)] [[PubMed](#)]
11. Kuiper, G.G.; Carlsson, B.; Grandien, K.; Enmark, E.; Haggblad, J.; Nilsson, S.; Gustafsson, J.A. Comparison of the Ligand Binding Specificity and Transcript Tissue Distribution of Estrogen Receptors Alpha and Beta. *Endocrinology* **1997**, *138*, 863–870. [[CrossRef](#)] [[PubMed](#)]
12. Zhang, Z.; Burch, P.E.; Cooney, A.J.; Lanz, R.B.; Pereira, F.A.; Wu, J.; Gibbs, R.A.; Weinstock, G.; Wheeler, D.A. Genomic Analysis of the Nuclear Receptor Family: New Insights into Structure, Regulation, and Evolution from the Rat Genome. *Genome Res.* **2004**, *14*, 580–590. [[CrossRef](#)] [[PubMed](#)]
13. Kos, M.; O'Brien, S.; Flouriot, G.; Gannon, F. Tissue-Specific Expression of Multiple Mrna Variants of the Mouse Estrogen Receptor Alpha Gene. *FEBS Lett.* **2000**, *477*, 15–20. [[CrossRef](#)] [[PubMed](#)]
14. Lewandowski, S.; Kalita, K.; Kaczmarek, L. Estrogen Receptor Beta. Potential Functional Significance of a Variety of Mrna Isoforms. *FEBS Lett.* **2002**, *524*, 1–5. [[CrossRef](#)]
15. Ishii, H.; Sakuma, Y. Complex Organization of the 5'-Untranslated Region of the Mouse Estrogen Receptor Alpha Gene: Identification of Numerous Mrna Transcripts with Distinct 5'-Ends. *J. Steroid Biochem. Mol. Biol.* **2011**, *125*, 211–218. [[CrossRef](#)]
16. Lu, B.; Leygue, E.; Dotzlaw, H.; Murphy, L.C.; Watson, P. Estrogen Receptor-Beta Mrna Variants in Human and Murine Tissues. *Mol. Cell. Endocrinol.* **1998**, *138*, 199–203. [[CrossRef](#)]
17. Maruyama, K.; Endoh, H.; Sasaki-Iwaoka, H.; Kanou, H.; Shimaya, E.; Hashimoto, S.; Kato, S.; Kawashima, H. A Novel Isoform of Rat Estrogen Receptor Beta with 18 Amino Acid Insertion in the Ligand Binding Domain as a Putative Dominant Negative Regular of Estrogen Action. *Biochem. Biophys. Res. Commun.* **1998**, *246*, 142–147. [[CrossRef](#)]
18. Zhao, C.; Toresson, G.; Xu, L.; Koehler, K.F.; Gustafsson, J.A.; Dahlman-Wright, K. Mouse Estrogen Receptor Beta Isoforms Exhibit Differences in Ligand Selectivity and Coactivator Recruitment. *Biochemistry* **2005**, *44*, 7936–7944. [[CrossRef](#)]
19. Ogawa, S.; Inoue, S.; Watanabe, T.; Orimo, A.; Hosoi, T.; Ouchi, Y.; Muramatsu, M. Molecular Cloning and Characterization of Human Estrogen Receptor Betacx: A Potential Inhibitor of Estrogen Action in Human. *Nucleic Acids Res.* **1998**, *26*, 3505–3512. [[CrossRef](#)] [[PubMed](#)]
20. Couse, J.F.; Lindzey, J.; Grandien, K.; Gustafsson, J.A.; Korach, K.S. 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* **1997**, *138*, 4613–4621. [[CrossRef](#)]
21. Irsik, D.L.; Carmines, P.K.; Lane, P.H. Classical Estrogen Receptors and Eralpha Splice Variants in the Mouse. *PLoS ONE* **2013**, *8*, e70926. [[CrossRef](#)]
22. Andersson, S.; Sundberg, M.; Pristovsek, N.; Ibrahim, A.; Jonsson, P.; Katona, B.; Clausson, C.M.; Zieba, A.; Ramstrom, M.; Soderberg, O.; et al. Insufficient Antibody Validation Challenges Oestrogen Receptor Beta Research. *Nat. Commun.* **2017**, *8*, 15840. [[CrossRef](#)] [[PubMed](#)]



23. Ishii, H.; Otsuka, M.; Kanaya, M.; Higo, S.; Hattori, Y.; Ozawa, H. Applicability of Anti-Human Estrogen Receptor Beta Antibody Ppz0506 for the Immunodetection of Rodent Estrogen Receptor Beta Proteins. *Int. J. Mol. Sci.* **2019**, *20*, 6312. [[CrossRef](#)]
24. Antonson, P.; Apolinario, L.M.; Shamekh, M.M.; Humire, P.; Poutanen, M.; Ohlsson, C.; Nalvarte, I.; Gustafsson, J.A. Generation of an All-Exon ESR2 Deleted Mouse Line: Effects on Fertility. *Biochem. Biophys. Res. Commun.* **2020**, *529*, 231–237. [[CrossRef](#)]
25. Ozawa, M.; Hattori, Y.; Higo, S.; Otsuka, M.; Matsumoto, K.; Ozawa, H.; Ishii, H. Optimized Mouse-on-Mouse Immunohistochemical Detection of Mouse ESR2 Proteins with Ppz0506 Monoclonal Antibody. *Acta Histochem. Cytochem.* **2022**, *55*, 159–168. [[CrossRef](#)] [[PubMed](#)]
26. Schröder, S.K.; Tag, C.G.; Kessel, J.C.; Antonson, P.; Weiskirchen, R. Immunohistochemical Detection of Estrogen Receptor-Beta (ER $\beta$ ) with Ppz0506 Antibody in Murine Tissue: From Pitfalls to Optimization. *Biomedicines* **2022**, *10*, 3100. [[PubMed](#)]
27. Maioli, S.; Leander, K.; Nilsson, P.; Nalvarte, I. Estrogen Receptors and the Aging Brain. *Essays Biochem.* **2021**, *65*, 913–925. [[CrossRef](#)]
28. Lubahn, D.B.; Moyer, J.S.; Golding, T.S.; Couse, J.F.; Korach, K.S.; Smithies, O. Alteration of Reproductive Function but Not Prenatal Sexual Development after Insertional Disruption of the Mouse Estrogen Receptor Gene. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 11162–11166. [[CrossRef](#)]
29. Dupont, S.; Krust, A.; Gansmuller, A.; Dierich, A.; Chambon, P.; Mark, M. Effect of Single and Compound Knockouts of Estrogen Receptors Alpha (ER $\alpha$ ) and Beta (ER $\beta$ ) on Mouse Reproductive Phenotypes. *Development* **2000**, *127*, 4277–4291. [[CrossRef](#)] [[PubMed](#)]
30. Chen, M.; Wolfe, A.; Wang, X.; Chang, C.; Yeh, S.; Radovick, S. Generation and Characterization of a Complete Null Estrogen Receptor Alpha Mouse Using Cre/LoxP Technology. *Mol. Cell Biochem.* **2009**, *321*, 145–153. [[CrossRef](#)]
31. Hewitt, S.C.; Kissling, G.E.; Fieselman, K.E.; Jayes, F.L.; Gerrish, K.E.; Korach, K.S. Biological and Biochemical Consequences of Global Deletion of Exon 3 from the ER Alpha Gene. *FASEB J.* **2010**, *24*, 4660–4667. [[CrossRef](#)]
32. Antonson, P.; Omoto, Y.; Humire, P.; Gustafsson, J.A. Generation of ER $\alpha$ -Floxed and Knockout Mice Using the Cre/LoxP System. *Biochem. Biophys. Res. Commun.* **2012**, *424*, 710–716. [[CrossRef](#)] [[PubMed](#)]
33. Eddy, E.M.; Washburn, T.F.; Bunch, D.O.; Goulding, E.H.; Gladen, B.C.; Lubahn, D.B.; Korach, K.S. Targeted Disruption of the Estrogen Receptor Gene in Male Mice Causes Alteration of Spermatogenesis and Infertility. *Endocrinology* **1996**, *137*, 4796–4805. [[CrossRef](#)]
34. Hess, R.A.; Bunick, D.; Lee, K.H.; Bahr, J.; Taylor, J.A.; Korach, K.S.; Lubahn, D.B. A Role for Oestrogens in the Male Reproductive System. *Nature* **1997**, *390*, 509–512. [[CrossRef](#)]
35. Hess, R.A.; Cooke, P.S. Estrogen in the Male: A Historical Perspective. *Biol. Reprod.* **2018**, *99*, 27–44. [[CrossRef](#)] [[PubMed](#)]
36. Curtis Hewitt, S.; Couse, J.F.; Korach, K.S. Estrogen Receptor Transcription and Transactivation: Estrogen Receptor Knockout Mice: What Their Phenotypes Reveal About Mechanisms of Estrogen Action. *Breast Cancer Res.* **2000**, *2*, 345–352. [[CrossRef](#)]
37. Wintermantel, T.M.; Campbell, R.E.; Porteous, R.; Bock, D.; Grone, H.J.; Todman, M.G.; Korach, K.S.; Greiner, E.; Perez, C.A.; Schutz, G.; et al. Definition of Estrogen Receptor Pathway Critical for Estrogen Positive Feedback to Gonadotropin-Releasing Hormone Neurons and Fertility. *Neuron* **2006**, *52*, 271–280. [[CrossRef](#)]
38. Antonson, P.; Matic, M.; Portwood, N.; Kuiper, R.V.; Bryzgalova, G.; Gao, H.; Windahl, S.H.; Humire, P.; Ohlsson, C.; Berggren, P.-O.; et al. Ap2-Cre-Mediated Inactivation of Estrogen Receptor Alpha Causes Hydrometra. *PLoS ONE* **2014**, *9*, e85581. [[CrossRef](#)]
39. Gieske, M.C.; Kim, H.J.; Legan, S.J.; Koo, Y.; Krust, A.; Chambon, P.; Ko, C. Pituitary Gonadotroph Estrogen Receptor-Alpha Is Necessary for Fertility in Females. *Endocrinology* **2008**, *149*, 20–27. [[CrossRef](#)] [[PubMed](#)]
40. Winuthayanon, W.; Hewitt, S.C.; Orvis, G.D.; Behringer, R.R.; Korach, K.S. Uterine Epithelial Estrogen Receptor Alpha Is Dispensable for Proliferation but Essential for Complete Biological and Biochemical Responses. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 19272–19277. [[CrossRef](#)]
41. Pawar, S.; Laws, M.J.; Bagchi, I.C.; Bagchi, M.K. Uterine Epithelial Estrogen Receptor-Alpha Controls Decidualization Via a Paracrine Mechanism. *Mol. Endocrinol.* **2015**, *29*, 1362–1374. [[CrossRef](#)]
42. Winuthayanon, W.; Lierz, S.L.; Delarosa, K.C.; Sampels, S.R.; Donoghue, L.J.; Hewitt, S.C.; Korach, K.S. Juxtacrine Activity of Estrogen Receptor Alpha in Uterine Stromal Cells Is Necessary for Estrogen-Induced Epithelial Cell Proliferation. *Sci. Rep.* **2017**, *7*, 8377. [[CrossRef](#)] [[PubMed](#)]
43. Jakacka, M.; Ito, M.; Martinson, F.; Ishikawa, T.; Lee, E.J.; Jameson, J.L. An Estrogen Receptor (ER) Alpha Deoxyribonucleic Acid-Binding Domain Knock-in Mutation Provides Evidence for Nonclassical ER Pathway Signaling in Vivo. *Mol. Endocrinol.* **2002**, *16*, 2188–2201. [[CrossRef](#)] [[PubMed](#)]
44. Ahlbory-Dieker, D.L.; Stride, B.D.; Leder, G.; Schkoldow, J.; Trolenberg, S.; Seidel, H.; Otto, C.; Sommer, A.; Parker, M.G.; Schutz, G.; et al. DNA Binding by Estrogen Receptor-Alpha Is Essential for the Transcriptional Response to Estrogen in the Liver and the Uterus. *Mol. Endocrinol.* **2009**, *23*, 1544–1555. [[CrossRef](#)]
45. Sinkevicius, K.W.; Burdette, J.E.; Woloszyn, K.; Hewitt, S.C.; Hamilton, K.; Sugg, S.L.; Temple, K.A.; Wondisford, F.E.; Korach, K.S.; Woodruff, T.K.; et al. An Estrogen Receptor-Alpha Knock-in Mutation Provides Evidence of Ligand-Independent Signaling and Allows Modulation of Ligand-Induced Pathways in Vivo. *Endocrinology* **2008**, *149*, 2970–2979. [[CrossRef](#)]
46. Arao, Y.; Hamilton, K.J.; Ray, M.K.; Scott, G.; Mishina, Y.; Korach, K.S. Estrogen Receptor Alpha Af-2 Mutation Results in Antagonist Reversal and Reveals Tissue Selective Function of Estrogen Receptor Modulators. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 14986–14991. [[CrossRef](#)]

47. Adlanmerini, M.; Solinhac, R.; Abot, A.; Fabre, A.; Raymond-Letron, I.; Guihot, A.-L.; Boudou, F.; Sautier, L.; Vessi res, E.; Kim, S.H.; et al. Mutation of the Palmitoylation Site of Estrogen Receptor Alpha in Vivo Reveals Tissue-Specific Roles for Membrane Versus Nuclear Actions. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, E283–E290. [[CrossRef](#)] [[PubMed](#)]
48. Pedram, A.; Razandi, M.; Lewis, M.; Hammes, S.; Levin, E.R. Membrane-Localized Estrogen Receptor Alpha Is Required for Normal Organ Development and Function. *Dev. Cell* **2014**, *29*, 482–490. [[CrossRef](#)]
49. Nanjappa, M.K.; Hess, R.A.; Medrano, T.I.; Locker, S.H.; Levin, E.R.; Cooke, P.S. Membrane-Localized Estrogen Receptor 1 Is Required for Normal Male Reproductive Development and Function in Mice. *Endocrinology* **2016**, *157*, 2909–2919. [[CrossRef](#)]
50. Simond, A.M.; Ling, C.; Moore, M.J.; Condotta, S.A.; Richer, M.J.; Muller, W.J. Point-Activated Esr1(Y541s) Has a Dramatic Effect on the Development of Sexually Dimorphic Organs. *Genes Dev.* **2020**, *34*, 1304–1309. [[CrossRef](#)]
51. Krege, J.H.; Hodgins, J.B.; Couse, J.F.; Enmark, E.; Warner, M.; Mahler, J.F.; Sar, M.; Korach, K.S.; Gustafsson, J.A.; Smithies, O. Generation and Reproductive Phenotypes of Mice Lacking Estrogen Receptor Beta. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 15677–15682. [[CrossRef](#)] [[PubMed](#)]
52. Antal, M.C.; Krust, A.; Chambon, P.; Mark, M. Sterility and Absence of Histopathological Defects in Nonreproductive Organs of a Mouse Erbeta-Null Mutant. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 2433–2438. [[CrossRef](#)]
53. Binder, A.K.; Rodriguez, K.F.; Hamilton, K.J.; Stockton, P.S.; Reed, C.E.; Korach, K.S. The Absence of Er-Beta Results in Altered Gene Expression in Ovarian Granulosa Cells Isolated from in Vivo Preovulatory Follicles. *Endocrinology* **2013**, *154*, 2174–2187. [[CrossRef](#)] [[PubMed](#)]
54. Maneix, L.; Antonson, P.; Humire, P.; Rochel-Maia, S.; Castaneda, J.; Omoto, Y.; Kim, H.J.; Warner, M.; Gustafsson, J.A. Estrogen Receptor Beta Exon 3-Deleted Mouse: The Importance of Non-Ere Pathways in Erbeta Signaling. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 5135–5140. [[CrossRef](#)]
55. Novaira, H.J.; Negron, A.L.; Graceli, J.B.; Capellino, S.; Schoeffield, A.; Hoffman, G.E.; Levine, J.E.; Wolfe, A.; Wondisford, F.E.; Radovick, S. Impairments in the Reproductive Axis of Female Mice Lacking Estrogen Receptor Beta in GnRH Neurons. *Am. J. Physiol. Endocrinol. Metab.* **2018**, *315*, E1019–E1033. [[CrossRef](#)] [[PubMed](#)]
56. Yuan, B.; Yang, J.; Dubeau, L.; Hu, Y.; Li, R. A Phosphotyrosine Switch in Estrogen Receptor Beta Is Required for Mouse Ovarian Function. *Front. Cell Dev. Biol.* **2021**, *9*, 649087. [[CrossRef](#)]
57. Couse, J.F.; Hewitt, S.C.; Bunch, D.O.; Sar, M.; Walker, V.R.; Davis, B.J.; Korach, K.S. Postnatal Sex Reversal of the Ovaries in Mice Lacking Estrogen Receptors Alpha and Beta. *Science* **1999**, *286*, 2328–2331. [[CrossRef](#)]
58. Antonson, P.; Nalvarte, I.; Varshney, M.; Xu, L.; Windahl, S.H.; Humire, P.; Ohlsson, C.; Gustafsson, J.A.; Dahlman-Wright, K. Identification of Proteins Highly Expressed in Uterine Fluid from Mice with Hydrometra. *Biochem. Biophys. Res. Commun.* **2015**, *466*, 650–655. [[CrossRef](#)]
59. Hewitt, S.C.; Korach, K.S. Estrogen Receptors: New Directions in the New Millennium. *Endocr. Rev.* **2018**, *39*, 664–675. [[CrossRef](#)] [[PubMed](#)]
60. Arao, Y.; Hamilton, K.J.; Goulding, E.H.; Janardhan, K.S.; Eddy, E.M.; Korach, K.S. Transactivating Function (Af) 2-Mediated Af-1 Activity of Estrogen Receptor Alpha Is Crucial to Maintain Male Reproductive Tract Function. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 21140–21145. [[CrossRef](#)] [[PubMed](#)]
61. Acconcia, F.; Ascenzi, P.; Bocedi, A.; Spisni, E.; Tomasi, V.; Trentalance, A.; Visca, P.; Marino, M. Palmitoylation-Dependent Estrogen Receptor Alpha Membrane Localization: Regulation by 17beta-Estradiol. *Mol. Biol. Cell* **2005**, *16*, 231–237. [[CrossRef](#)] [[PubMed](#)]
62. Saito, K.; Dickey, J.E.; Rodeghiero, S.R.; Toth, B.A.; Kelly, M.J.; Deng, Y.; Singh, U.; Deng, G.; Jiang, J.; Cui, H. Hypomorphism of a Novel Long Eralpha Isoform Causes Severe Reproductive Dysfunctions in Female Mice. *Endocrinology* **2022**, *163*, bqac160. [[CrossRef](#)] [[PubMed](#)]
63. Warner, M.; Wu, W.F.; Montanholi, L.; Nalvarte, I.; Antonson, P.; Gustafsson, J.A. Ventral Prostate and Mammary Gland Phenotype in Mice with Complete Deletion of the Erbeta Gene. *Proc. Natl. Acad. Sci. USA* **2020**, *117*, 4902–4909. [[CrossRef](#)]
64. Jayes, F.L.; Burns, K.A.; Rodriguez, K.F.; Kissling, G.E.; Korach, K.S. The Naturally Occurring Luteinizing Hormone Surge Is Diminished in Mice Lacking Estrogen Receptor Beta in the Ovary. *Biol. Reprod.* **2014**, *90*, 24. [[CrossRef](#)]
65. Emmen, J.M.; Couse, J.F.; Elmore, S.A.; Yates, M.M.; Kissling, G.E.; Korach, K.S. In Vitro Growth and Ovulation of Follicles from Ovaries of Estrogen Receptor (Er)Alpha and Erbeta Null Mice Indicate a Role for Erbeta in Follicular Maturation. *Endocrinology* **2005**, *146*, 2817–2826. [[CrossRef](#)] [[PubMed](#)]
66. Couse, J.F.; Yates, M.M.; Deroo, B.J.; Korach, K.S. Estrogen Receptor-Beta Is Critical to Granulosa Cell Differentiation and the Ovulatory Response to Gonadotropins. *Endocrinology* **2005**, *146*, 3247–3262. [[CrossRef](#)] [[PubMed](#)]
67. Hrabovszky, E.; Shughrue, P.J.; Merchenthaler, I.; Hajszan, T.; Carpenter, C.D.; Liposits, Z.; Petersen, S.L. Detection of Estrogen Receptor-Beta Messenger Ribonucleic Acid and 125i-Estrogen Binding Sites in Luteinizing Hormone-Releasing Hormone Neurons of the Rat Brain. *Endocrinology* **2000**, *141*, 3506–3509. [[CrossRef](#)]
68. Rumi, M.A.; Dhakal, P.; Kubota, K.; Chakraborty, D.; Lei, T.; Larson, M.A.; Wolfe, M.W.; Roby, K.F.; Vivian, J.L.; Soares, M.J. Generation of Esr1-Knockout Rats Using Zinc Finger Nuclease-Mediated Genome Editing. *Endocrinology* **2014**, *155*, 1991–1999. [[CrossRef](#)]
69. Rumi, M.A.K.; Singh, P.; Roby, K.F.; Zhao, X.; Iqbal, K.; Ratri, A.; Lei, T.; Cui, W.; Borosha, S.; Dhakal, P.; et al. Defining the Role of Estrogen Receptor Beta in the Regulation of Female Fertility. *Endocrinology* **2017**, *158*, 2330–2343. [[CrossRef](#)]

70. Smith, E.P.; Boyd, J.; Frank, G.R.; Takahashi, H.; Cohen, R.M.; Specker, B.; Williams, T.C.; Lubahn, D.B.; Korach, K.S. Estrogen Resistance Caused by a Mutation in the Estrogen-Receptor Gene in a Man. *N. Engl. J. Med.* **1994**, *331*, 1056–1061. [[CrossRef](#)]
71. Quaynor, S.D.; Stradtman, E.W., Jr.; Kim, H.-G.; Shen, Y.; Chorch, L.P.; Schreihof, D.A.; Layman, L.C. Delayed Puberty and Estrogen Resistance in a Woman with Estrogen Receptor Alpha Variant. *N. Engl. J. Med.* **2013**, *369*, 164–171. [[CrossRef](#)] [[PubMed](#)]
72. Bernard, V.; Kherra, S.; Francou, B.; Fagart, J.; Viengchareun, S.; Guéchet, J.; Ladjouze, A.; Guiochon-Mantel, A.; Korach, K.S.; Binart, N.; et al. Familial Multiplicity of Estrogen Insensitivity Associated with a Loss-of-Function Esr1 Mutation. *J. Clin. Endocrinol. Metab.* **2017**, *102*, 93–99. [[CrossRef](#)]
73. Delcour, C.; Khawaja, N.; Gonzalez-Duque, S.; Lebon, S.; Talbi, A.; Drira, L.; Chevenne, D.; Ajlouni, K.; de Roux, N. Estrogen Receptor Alpha Inactivation in 2 Sisters: Different Phenotypic Severities for the Same Pathogenic Variant. *J. Clin. Endocrinol. Metab.* **2022**, *107*, e2553–e2562. [[CrossRef](#)] [[PubMed](#)]
74. Asadi, M.; Ghafouri-Fard, S.; Zare-Abdollahi, D.; Ebrahim-Habibi, A.; Matin, N. Estrogen Receptor Mutation in a Girl with Primary Amenorrhea. *Clin. Genet.* **2013**, *83*, 497–498. [[CrossRef](#)]
75. Lang-Muritano, M.; Sproll, P.; Wyss, S.; Kolly, A.; Hurlimann, R.; Konrad, D.; Bason-Laubert, A. Early-Onset Complete Ovarian Failure and Lack of Puberty in a Woman with Mutated Estrogen Receptor Beta (Esr2). *J. Clin. Endocrinol. Metab.* **2018**, *103*, 3748–3756. [[CrossRef](#)] [[PubMed](#)]
76. Baetens, D.; Güran, T.; Mendonça, B.B.; Gomes, N.L.; De Cauwer, L.; Peelman, F.; Verdin, H.; Vuylsteke, M.; Van der Linden, M.; Stoop, H.; et al. Biallelic and Monoallelic Esr2 Variants Associated with 46,Xy Disorders of Sex Development. *Genet. Med.* **2018**, *20*, 717–727. [[CrossRef](#)] [[PubMed](#)]
77. McElreavey, K.; Bashamboo, A. Monogenic Forms of Dsd: An Update. *Horm. Res. Paediatr.* **2021**, 1–25. [[CrossRef](#)]

**Disclaimer/Publisher’s Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.