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Estrogen receptors in mitochondrial metabolism: age-related changes and implications for pregnancy complications

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Abstract

Estrogen hormones are primarily associated with their role as female sex hormones responsible for primary and secondary sexual development. Estrogen receptors are known to undergo age-dependent decreases due to age-related changes in hormone production. In the mitochondria, estrogen functions by reducing the production of reactive oxygen species in the electron transport chain, inhibiting apoptosis, and regulating mitochondrial DNA content. Moreover, estrogen receptors may be the key components in maintaining mitochondrial membrane potential and structure. Although estrogen plays a crucial role in the development of pregnancy, our understanding of how estrogen receptors change with aging during pregnancy remains limited. During pregnancy, estrogen levels are significantly elevated, with a corresponding upregulation of estrogen receptors, which play various roles in pregnancy. However, the exact role of estrogen receptors in pregnancy complications remains to be further investigated. The paper reviews the role of estrogen receptors in the regulation of mitochondrial metabolism and in pregnancy complications, with a special focus on the effect of age-related changes on estrogen levels and estrogen receptors function. We also address how estrogen maintains mitochondrial function, including reducing the production of reactive oxygen species in the electron transport chain, inhibiting apoptosis, regulating mitochondrial DNA content, and maintaining mitochondrial membrane potential and structure. However, the effects of estrogen on mitochondria-endoplasmic reticulum contacts have not been well studied. Based on these emergent roles in mitochondria, the differential roles of estrogen receptors in pregnancy complications are of great relevance. The paper emphasizes the association between maternal health and estrogen receptors and indicates the need for future research to elucidate the interdependence of estrogen receptor-regulated maternal

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Author contributions

AHJ generated ideas for article. All authors performed the literature search and data analysis, and drafted and/or critically revised the work, and approved the final manuscript.

Conflicts of interest

The authors declare that there is no conflict of interest.

health with mitochondrial function and their relationship with the gut microbiome. Overall, we summarize the important role of estrogen receptors during pregnancy and highlight the need for further research to better understand the role of estrogen receptors in aging and pregnancy complications. This not only helps to reveal the mechanism underlying the role of estrogen in maternal health but also has potential clinical implications for the development of new therapies targeting age-related diseases and pregnancy complications.

Keywords

age-related changes; aging; estrogen; estrogen receptors; mitochondria; pregnancy complications

Introduction

Estrogens are recognized to be important molecules in sexual development, which have been studied intensely (Figure 1).[1–3] Estrogens are a class of sex hormones defined by their ability to promote female sexual development.[4] Despite this characteristic, 17β-estradiol (E2), a major product of estrogen synthesis, is produced in both men and women.[5] In reproductive-aged women, E2 is primarily synthesized in the ovaries.[5] In men, the testes are capable of producing estradiol. Estradiol production also occurs in non-gonadal tissue, such as the brain, liver, muscle, and bone.[6] The conversion of androgens to estrogens is recognized as a critical process in the body, with important implications for reproductive health, bone health, and the pathogenesis of certain diseases such as breast cancer.[6, 7] Increasingly, the literature shows that mitochondria must be considered in the roles of estrogen and its receptors. Beyond simply considering estrogen as a steroid, the roles of its receptors have similarly arisen as important effectors, especially when it comes to maternal health. In this narrative review, we highlight the important role of mitochondria in estrogen receptors during pregnancy and aging.

Search Strategy

For this review, literature published from 2000 to 2024 was searched using PubMed. The following keywords were used: “pregnancy” OR “estrogen” OR “estradiol” AND “mitochondria.” The articles included in this review were selected based on their relevance to the topic, along with additional historical articles or related articles. The results were further screened according to the title and abstract, and whether they included animal experiments, *in vitro* studies, clinical trials, and database or software applications.

Estrogen Receptors in Cellular Signaling

Estrogen receptor α (ERα) and estrogen receptor β (ERβ) are estradiol receptors that are expressed in a variety of cell types (as extensively reviewed in the reference 8). Beyond only different amino acid sequences, ERα and ERβ have differential affinities, with alpha having a higher affinity for E2 than estradiol 17α (E1), while ERß favors estriol.[9] Notably, different isoforms of ERα have different affinity for E2.[10] ERα and ERβ can act through genomic signaling by activating transcription cascade(s).[9] ERβ can notably localize to the mitochondria to restore bioenergetics and improve resilience to oxidative stress in disease

states.[11] Specifically, when cytosolic ERα and ERβ bind to estradiol, they become activated and dimerize.[5] Dimerized receptors will then translocate to the nucleus where they bind to specific DNA sequences called estrogen response elements (EREs), which function similarly to transcription factors.[12] The ERs also interact with transcription factors and stabilize them. This process ultimately facilitates transcription complex assembly, resulting in gene expression.[12] Notably, the linkage between ERs and mitochondria is evident through the modulation of estradiols by mitochondria.[5, 13] Exposure to E2 in cells leads to an increase in mRNA that originates from mitochondrial DNA (mtDNA).[11] E2 also serves as an inhibitor of apoptosis through signaling pathways that involve membrane ERs and mitochondrial ERs.[5]

Additionally, E2 was shown to act through non-genomic signaling. E2 is involved in signaling cascades through protein-kinase activation by binding to membrane-bound ERα, ERβ, and G-Protein Coupled Estrogen Receptor (GPER, also known as GPR30, as extensive reviewed in the reference 14).[15] Structurally, ERα and ERβ differ in the N-terminal A/B domain and to a lesser degree in their ligand-binding domain.[16] GPER is located predominantly in the plasma membrane of the endoplasmic reticulum.[17] Despite limited research on interactions with mitochondria, GPER activation has been suggested to increase mitochondria biogenesis in myoblast cell line C2C12.[18] GPER is activated by estradiol and is responsible for the rapid, non-genomic effects of estradiol.[19] Structurally, GPER consists of seven transmembrane domain receptors.[15] When estradiol activates GPER, downstream pathways are activated that are responsible for regulating factors including cell growth, cell migration, and apoptosis.[15] The four main signaling cascades that E2 has been found to be involved in are the phospholipase C/protein kinase C pathway, the Ras/Raf/MAPK cascade, the phosphatidylinositol 3 kinase (PI_3K)/Akt cascade, and the cyclic adenosine monophosphate (cAMP)/protein kinase A cascade.[20] The phospholipase C/protein kinase C pathway is involved in intracellular calcium mobilization, protein phosphorylation, and activation of transcription factors.[21, 22] The Ras/Raf/MAPK cascade is involved in cell proliferation; the PI_3K/Akt cascade is involved in cell growth and metabolism; and the cAMP/protein kinase A cascade is involved in gene transcription and cell differentiation.[20, 22] Together, these pathways serve crucial roles in cellular survival. ERα has also been found to be involved in increasing ERK, PI_3K, cAMP, and calcium signaling in the breast cancer cell line MCF7.[23] By activating these pathways, E2 can directly affect protein activity as well as indirectly affect gene transcription. However, how E2 interacts with other proteins involved in mTOR and PI_3K/Akt pathways, such as SWELL1 and PTEN,[24, 25] remains unclear.

Estrogen Receptors are Key Regulators of Glucose and Insulin Metabolism

Both ERα and ERβ receptors are found in skeletal muscle, but the effects of estradiol binding to these receptors are different. In E2-depleted mice, the binding of an ERβ agonist led to a decrease in the expression of the insulin-regulated glucose transporter type 4 (GLUT4) in both the cytosol and cell membrane of skeletal muscle.[26] Furthermore, the binding of an ER antagonist in ERα silenced cells led to increased GLUT4 expression, which did not occur in ERβ silenced cells. This demonstrates that the blocking of ERβ leads to increased GLUT4 expression,[27] and suggest that that ERβ binding is necessary for

GLUT4 downregulation in skeletal muscle. In contrast, ERα silenced cells demonstrated a lower level of GLUT4 mRNA compared to wild-type and ERβ silenced cells, which implies that ERα binding increases GLUT4 expression.[26] As skeletal muscle is active in the uptake of glucose by insulin signaling, decreased GLUT4 expression would be predicted to lead to insulin insensitivity and hyperglycemia, which has been demonstrated in ERα silenced mice.[28] When insulin binds to its receptors, it initiates a phosphorylation cascade that ultimately results in the translocation of GLUT4 from cytoplasmic vesicles to the cell membrane, whereby it allows the cellular intake of glucose. E2 plays a critical role in the expression and translocation of GLUT4 as well as in the modulation of glucose through the insulin signaling pathway.[29]

Adipocytes, which function in lipid mobilization as well as leptin secretion, also contain estrogen receptors, primarily in the nucleus.[30] In female mice with removed ovaries, E2 treatment has been found to reverse increased adipocyte size as well as lipogenic gene expression resulting in restored protection to weight gain than their male and female counterparts.[30] It has also been demonstrated that mice fed on a high-fat diet had a decreased level of lipogenic expression when given E2 treatment.[28] These mice also had higher levels of insulin sensitivity, which was implied to be a result of decreased adipose weight.[28] Beyond this, when the aromatase enzyme, critical for E2 synthesis, was deactivated in transgenic mice, the mice developed increased adiposity and insulin levels, which underscores E2's role in preventing metabolic syndrome (MS).[29] Even within male mice, aromatase activity within adipose tissue is protective against inflammation and bolsters insulin sensitivity.[31] However, in some endocrine therapies, such as breast cancer endocrine therapy, hyperinsulinemia occurs frequently in obese women due to the hormones disrupting adipocyte progenitors.[32] In combination, this suggests that E2 is a potential target for obesity protection.

Notably, the role of ERα, Erβ, and GPER in regulating metabolism is beyond only E2 action. Broadly, ERα expression is associated with obesity,[33] with its decrease or mutations in it paralleling metabolic syndrome through inflammation and glucose intolerance.[34, 35] A proper balance of the ERα to ERβ ratio is critical for maintaining carbohydrate and lipid homeostasis in the brain, skeletal muscle, adipose tissue, liver, pancreas, and cardiovascular system.[36] An imbalance of ERα an ERβ leads to greater risk for issues such as MS, which is characterized by obesity, hypertension, and insulin resistance.[37] Notably, while few studies have directly compared them, ERα may be particularly important in metabolic syndrome. A large case-control study in China found polymorphisms in ERα, but not ERβ, were associated with MS in postmenopausal women.[38] Recently, this has led to therapies targeting ERα and administering derived peptides for ERα, thus suppressing ubiquitination-induced IRS1 degradation, which improves insulin sensitivity, glucose homeostasis, and serum lipid profiles.[39]

Given these plentiful roles of E2 in general metabolism, the distinct roles of ERs in facets of mitochondrial function are of great relevance. Roles of estrogen and its receptors in mitochondrial function are plentiful (as extensively reviewed in the references 7, 40, and 41), yet the multifaceted nature of mitochondria (as described in the references 42 and 43) underscores that many characteristics have not yet been elucidated in the context

of ERs. Thus, the sections “Effects of Estrogen Receptors on Mitochondrial Structure,” “Effects of Estrogen Receptors on Mitochondrial DNA,” “Effects of Estrogen Receptor on Mitochondria-Endoplasmic Reticulum Contacts and Calcium are Not Well Characterized,” and “Estrogen Receptor-Dependent Remodeling of Mitochondrial Membrane” focus on promising and nascent areas of mitochondrial research of ERs.

Effects of Estrogen Receptors on Mitochondrial Structure

Recognizing that structure influences function, alterations in mitochondrial morphology can significantly affect calcium signaling, ROS production, and biosynthetic pathways.[44–46] However, improved methods to measure both mitochondrial dynamics and visualize ERs are necessary for advanced morphology analysis. Recently, ZsGreen was used in mice to image ERα,[47] which could be used in collaboration with existing 2D and 3D methods of quantifying mitochondrial structure,[48–50] including serial block-facing-scanning electron microscopy[51, 52] and focus ion beam-scanning electron microscopy.[53] Using techniques such as these and correlative light and electron microscopy[54] may allow for additional findings of associations in E2 regulation and mitochondria structure to be understood.

Mitochondria are double membrane-bound organelles, whose membranes are composed of lipid bilayers regulated by membrane phospholipids. Changing the composition or packing of phospholipids in mitochondrial membranes can affect membrane dynamics and electron transfer.[13, 55] Mitochondrial membrane potential is indicative of cellular health, as it is critical for generating adenosine triphosphate (ATP) in oxidative phosphorylation. Estrogen also promotes cellular redox homeostasis, targeting and increasing the activities of Complex I and Complex I/II + III in the ETC.[13] Maintaining proper mitochondrial membrane potential is vital to preserving healthy mitochondria, as membrane potential has been identified as a factor in the process of eliminating disabled mitochondria.[56] Thus, further research must investigate how mitochondrial membrane potential may be modulated by estrogens.

Effects of Estrogen Receptors on Mitochondrial DNA

Another important avenue for investigation is mtDNA. The loss of ERα was previously shown to decrease mtDNA content in an E3 ubiquitin ligase parkin-dependent manner while causing mitochondrial dysfunction.[57] Thus, mtDNA levels may be more dependent on ERs than on E2 itself.[57] This degradation of mtDNA concomitantly occurs with reduced mtDNA turnover in skeletal muscle.[58] Additionally, E2 treatment drives ERα to translocate to mitochondria, interacting with 17β-hydroxysteroid dehydrogenase 10, a core subunit of the mitochondrial ribonuclease P complex, to increase mRNA abundance.[59] ERα is also necessary for mitochondrial complex gene expression in osteoblasts that occurs in E2 signaling pathways, principally cyclooxygenase I mRNA expression, which regulates cytochrome-c oxidase activity.[60] Similarly, this has been recapitulated in differentiated myoblastic C2C12 cells, wherein constitutively active ERα has been associated with increased mtDNA content and ATP content.[61] Inversely, antagonists of ERα, inhibit osteoblast maturation, in part due to roles of inhibiting cyclooxygenase I and II.[62] Thus, a central way energy metabolism pathways are disrupted is through the mitochondrial

respiratory chain genes encoded by mtDNA being disrupted by ER expression changes.[63] As previously reviewed, mtDNA depletion results in premature ovarian aging in young mice,[64] suggesting a potential area of research through which ERs are associated with ovarian aging.

Additionally, mtDNA levels can be closely linked to aging through NAD^+ metabolism, as NAD^+ levels are necessary for lifespan and undergo disease and age-related declines (reviewed in the reference 65). For example, Sirtuins, a family of NAD^+-dependent deacetylases, regulate mitochondrial biogenesis and function.[66] As previously reviewed, ovarian aging, which includes fertility in women of advanced maternal age, has been closely linked to a decrease in NAD^+ levels.[67] Notably, past studies have indicated that carcinomas can cause an unconventional prefoldin RPB5 interactor-mediated pathway to result in the inhibition of NAD^+ metabolism in an ER-dependent manner.[68] While NAD^+ metabolism inhibits nuclear DNA synthesis,[68] its results on mtDNA synthesis remain less clear. One study found that in mtDNA depletion syndrome,[69] NAD^+ treatment restores mitochondrial function but independently of mtDNA, while another study found that PARP1, a nuclear protein, restores mtDNA in an NAD^+-dependent manner.[70] Therefore, the interplay between ER expression and NAD^+-mediated regulation of mtDNA remains controversial, which may aid in understanding ovarian aging in the context of ER expression changes. The roles of other ERs in the modulation of mtDNA are less clear. Beyond only mitochondrial count and quality, nucleoids must also be examined. mtDNA is organized and maintained in loci known as nucleoids. Estrogens have been implicated in regulating nucleoid structure and function. Common factors of pathology include decreased nucleoids which typically occur concomitantly with a decline in cristae quality.[71] Notably, the interface of mitochondrial networking fragmentation and nucleoid distribution is an emerging field, with past studies suggesting that fragmentation increases the clustering of nucleoids.[72] However, the functional implications of changes in nucleoid distributions, if they occur, in E2 treatment remains unclear.

Effects of Estrogen Receptor on Mitochondria–Endoplasmic Reticulum Contacts and Calcium Are Not Well Characterized

The communication between the endoplasmic reticulum and mitochondria helps maintain normal physiological homeostasis in the cell.[73] Mitochondria–endoplasmic reticulum contacts (MERCs) are known to play key roles in ion and lipid transfer, cellular signaling, and membrane dynamics.[73] MERCs are involved in Ca^{2+} transfer between the endoplasmic reticulum and mitochondria, through the release of Ca^{2+} by the endoplasmic reticulum and subsequent uptake of Ca^{2+} into the mitochondrial matrix.[74] The mitochondria contain major proteins that then respond to the increase in matrix Ca^{2+} influx.[75] Sites where the mitochondria are constricted by endoplasmic reticulum tubules are the initiation sites of mitochondrial fission,[76] which is essential for cell viability along with mitochondrial fusion.[7] Furthermore, close contact between regions of the endoplasmic reticulum containing cytochrome P450 enzymes and the outer mitochondria membrane aids in the movement of steroidal intermediates from the mitochondria to the endoplasmic reticulum during steroidogenesis, producing steroids that are essential for mammalian life.[77]

E2 has been observed to cause the overexpression of ERα through the PI3K/Akt/mTOR pathway to cause the endoplasmic reticulum stress-associated degradation.[78] This E2-dependent apoptosis occurs in a PERK-dependent manner, with PERK also being known to be enriched in MERC spaces and modulate other pathways, including autophagy, metabolism, and ROS removal.[78] ROS from the intermembrane space of the mitochondria are also known to be delivered to the area of MERCs.[79] However, it remains unclear whether MERCs exhibit localized ROS signaling, despite the presence of numerous ROS targets in the endoplasmic reticulum and mitochondria.[80]

Moreover, in the plasma membrane, E2 binds to GPER where it contributes to many responses including cell proliferation and regulation of glucose metabolism.[81, 82] When GPER is activated in the endoplasmic reticulum, it initiates a response that ultimately results in cell proliferation through the release of Ca^{2+} and activation of the phosphoinositide 3-kinase pathway.[81, 82] While not associated with the mitochondria, GPER affects mitochondrial-induced cell death and mitochondrial function by virtue of its regulation of cellular Ca^{2+} concentration in the aforementioned signaling cascades. Ca^{2+} uptake by the mitochondria induces the opening of the normally closed mitochondrial permeability transition pore, a key regulator of cell death, activating apoptosis.[7] Together, this highlights a potential role of E2 and GPER in apoptosis that must be explored further. Since GPER activation in the endoplasmic reticulum by estrogen induces Ca^{2+} release resulting in cell proliferation,[7] this indirectly impacts mitochondrial function since MERCs are a site of mitochondrial uptake of Ca^{2+}. Increased Ca^{2+} concentration increases mitochondrial respiratory rate and ATP production, and prolonged influx of Ca^{2+} concentration ultimately result in mitochondrial swelling and apoptosis.[83] Activation of GPER by estrogen and its resulting effects on mitochondrial ATP output and possible induction of apoptosis warrants further study.

Looking forward, while the role of MERCs in Ca^{2+} signaling is well studied, less characterized is how other mitochondrial ultrastructure may be involved in Ca^{2+} signaling. For example, nano tunnels are mitochondrial phenotypes associated with molecule transfer that arise in stress states.[84] Nanotunnel frequency is modulated by RyR2 expression, with a calcium imbalance by depression of RyR2 activity resulting in nanotunnel frequency increasing concomitant with Ca^{2+} overload in sarcoplasmic reticulum.[85] Notably, while estrogens play roles in neuroprotective against excessive Ca^{2+}, this role has been found to be dependent on mitochondrial sequestration of Ca^{2+}.[86] This suggests that mitochondrial nanotunnels may serve to rescue Ca^{2+} deficiency, and potentially restore the functions of estrogen, yet studies regarding the modulation of mitochondrial nanotunnel structure and other calcium signaling phenotypes by E2 and concomitant increases in receptors remain poorly elucidated.

Estrogen Receptor-Dependent Remodeling of Mitochondrial Membrane

Recent studies have underscored the importance of studying mitochondrial quality control mechanisms, including mitophagy and the mitochondrial unfolded protein response, beyond only exaiming dysfunction.[42, 43, 87, 88] However, the role of estrogen in regulating these mechanisms remains poorly understood. One area of interest is cholesterol side-chain

cleavage (P450scc), which is located within the inner mitochondrial membrane. Various proteins are required for the transport of steroids from the outer membrane to the inner membrane, which includes voltage-dependent anion channel, TSPO-associated protein 7, and protein kinase A regulatory subunit 1α, as previously reviewed in the reference.[89] If mitochondrial remodeling of membranes can occur, future studies are necessary to understand if such remodeling can affect these transduceosome, thus affecting estrogen synthesis.

One avenue that may affect this is the physical incorporation of estrogens in the mitochondrial membrane. Yasar and colleagues have summarized the plasma membrane-associated signaling pathways which E2 can enact through the activation of ERs,[90] and membrane-localized ERα can serve many functions without the need for transcription, including modulation of cholesterol synthesis.[91] However, contrastingly, the roles of ERα in mitochondrial membrane pathways, if any, may be different from their plasma membrane roles, thus making it an important area to study in the future. While the ERα-independent diffusion of E2 across the mitochondrial membrane to reduce mitochondrial membrane microviscosity has been discussed,[13] the exact mechanisms underlying this translocation process of ERs are not yet fully understood. However, this may involve physical incorporation of E2 in an ER-dependent manner to mitochondrial membranes, as past studies have shown that ERβ binds to PHB2, which is localized in the inner mitochondrial membrane.[92] Additionally, mitochondrial permeability transition pores have recently been highlighted to be regulated by ERβ, possibly explaining sex-dependent differences in permeability in neurodegeneration.[93] Agonists of ERβ similarly have been associated with increased mitochondrial translocase of the inner membrane protein levels, which aid in preserving mitochondrial integrity under ischemia in a murine model.[94] The ratio of ERs may also affect the ability of estrogens to incorporate in membranes, as past studies have indicated that in cell lines with a high ERα-to-ERβ Ratio, there is reduced cardiolipin, a mark of inner mitochondrial membrane quality.[95] Taken together with the fact that mitochondria can have unique membrane potentials, even down to their individual cristae, this highlights the need for future studies to elucidate differential mechanisms of ERs in mitochondria.

Estrogen in Pregnancy

Based on these factors, it is important to consider the current state of pregnancy and consider the roles estrogens play in maternal health. While pregnancy involves numerous signaling pathways estrogen and its receptors play significant roles in development, especially in the context of pregnancy (Figure 2). Estrogen, in general, regulates pregnancy development, including by directly acting on mitochondria and promoting ATP synthase activity.[96] Recent findings demonstrate that, in premature births, E2 treatment can enhance neurodevelopment through an *Arx*-mediated pathway.[97] Notably, estrogen treatments, which parallel levels reached during pregnancy, can confer protection against breast cancer.[98] Estrogens also play numerous pathophysiological roles in other diseases, including tubal ectopic pregnancy,[99] restless leg syndrome,[100] and breast cancer.[101] This suggests that pregnancy can be important to study, as the alterations caused due to estrogens may provide future therapeutic techniques.

However, the exact dependence on estrogen receptors remains slightly controversial in disease states. It is understood that breast cancer is reduced by pregnancy due to estrogen.[98] It has been found that ER-positive breast cancer does not affect pregnancy safety outcomes.[102] Conversely, while increases in estrogen typically cause increased ERs, it was also demonstrated that the increased estrogen from pregnancy did not confer increased resistance against the reoccurrence of breast cancer.[103] This suggests that while pregnancy boosts estrogen levels, this is not a long-term effect that protects against pathophysiology if ERs are already lacking.

Estrogen Receptors in Pregnancy Complications

Estrogen is central to many pregnancy complications, with E2 treatment known to suppress the TLR4 signaling pathway, known to cause chronic low-grade inflammation, and reduce NO- and iNOS-dependent endothelial dysfunction and oxidative stress in murine models of preeclampsia.[104] Generally, ERs are found in reproductive organs and regulate functions including uterine growth, placental function, and the maintenance of pregnancy.[105] Dysregulation of ERs is known to contribute to metabolic syndromes at baseline conditions (reviewed in the reference 106). However, the risk of these metabolic syndromes, such as preeclampsia, may be exacerbated by pregnancy. Pregnancy is marked by dramatically increased production of estrogens, in turn upregulating ERs. After dimerization, ERα is crucial for fertility, while ERβ primarily counteracts unwanted actions of ERα (as discussed in the reference 107). While the direct pregnancy-induced estrogenic action on ERs remains unclear, ERs play distinct roles within the pregnancy, with their dysregulation often associated with pregnancy complications (Figure 3). Aberrant expression or signaling of ERs has been linked to preeclampsia, preterm births, and other dysfunctions of nitricoxide (NO)-mediated vasodilatation, thus impacting uterine quiescence during pregnancy (as previously reviewed in the reference 108). However, ERs show relatively differential roles than estrogen, which may be related to their differential effects on mitochondrial regulation.

The most common role of ERα and ERβ is regulating uterine artery vasodilation, through non-NO-pathways such as hydrogen sulfide (as previously reviewed in the reference 105). This allows fine-tuning of estrogen to disable uterotonic action until term, thus preventing pre-term births. Notably, an important regulator of this is ERα splicing, via hnRNPG and E2, which produces the isoforms of ERΔ7 and ERα46.[109] ERΔ7 specifically is responsible for maintaining myometrial quiescence through inhibition of Connexin 43, until term, at which point myometrial hnRNPG levels decline, mitigating ERα splicing and resulting in myometrial activation.[109] Both ERα and ERβ exhibit isoform formation. The relative impacts of each of these isoforms as well as upstream factors modulating splicing, will continue to be of interest. Of particular interest, the supplementation of hnRNPG, or another splicing factor, to increase ERΔ7 levels may be therapeutic targets in mitigating preterm births.

While ERα has been reported to show lower expression in preeclampsia than in control individuals across several studies, conflicting studies also show unchanged or even elevated expression.[110–114] It may be that these reports of different expressions arise due to varying study sites. For example, gestational diabetes mellitus modifies placental ERα expression

in a cell type-dependent manner, with upregulation of ERα in extravillous trophoblasts but downregulation of ERα in decidual vessels of specifically male fetuses.[115] In any case, changes in ERα expression may increase vulnerability to disease states in several ways. One way is through ER stress. Endoplasmic reticulum stress can cause fetal growth restriction, concomitant with upregulation of estrogen, in response to environmental pollutants.[116] Since in other models, ERα overexpression mitigates endoplasmic reticulum stress through mitochondria,[117] ERα is protective against fetal growth restriction and other unfolded protein response-dependent pregnancy complications (as discussed in the reference 118). Given the roles of ERα in insulin sensitivity in skeletal muscle, its role in the development of preeclampsia remains unclear.[58] Specifically, ERα knockout in murine models of skeletal muscle is linked with impaired mitochondrial health and insulin sensitivity in women.[34] However, it is unclear if these same pathways linking mitochondria and ERs in metabolic homeostasis are also implicated in preeclampsia. Additionally, stress-induced elevations in blood pressure and changes in feeding habits in non-pregnant females have been shown to be affected by ERα acting upon the medial amygdala neurons.[119–121] Thus, it is plausible that preeclampsia develops in part due to neuronal activation of ERα during pregnancy.

There is an emerging role of inflammation changes caused by altered ERα expression contribute to preeclampsia. As extensively reviewed in the reference 105, both E2 stimulation, as well as its downstream effects on ERs, are generally understood to promote the synthesis of NO. NO plays pluralistic roles in both uterine vasodilation and inhibiting interactions between immune and endothelial cells, thus protecting against preeclampsia.[122] However, by shortening the lipopolysaccharide-induced pro-inflammatory phase through cytokine signal molecule IL10, E2 can also act upon ERα to mitigate inflammation in macrophagic cells.[123] Interestingly, in late-term labor, ERα can play roles in inflammation-dependent parturition through a positive feedback loop, by which E2/ERα signaling downregulates miR-181a, enhancing pro-inflammatory signaling and myometrial contractility, and further increasing in ERα.[124, 125] While the potential for dysregulated signaling to lead to pathological-states has not been fully studied, one finding shows that anomalously upregulated pathways of hsa-miR-181a-5p lead to preeclampsia.[126] Otherwise, studies have found that preeclampsia-associated decrease in ERα expressions can occur in decidual macrophages, concomitant with reductions in anti-inflammatory markers.[127] This suggests dualistic roles of ERα expression in inflammation and preeclampsia.

ERβ, in contrast to ERα, is generally reported to consistently have higher expression in preeclampsia.[110, 111, 114] Outside of preeclampsia, ERα and ERβ mRNA expressions are different in the rat uterus, with ERα appearing more closely linked and upregulated close to the labor process.[128] Additionally, ERβ and GPER, but not ERα, are also upregulated in endometriosis.[129] One potential reason for the distinct expression is differential hypoxic reactions. It has been observed that placental hypoxia during early pregnancy may cause preeclampsia in a guinea pig model.[130] In other models, studies have suggested that hypoxia downregulates ERα[131] but upregulates ERβ.[132] Significantly, gestational hypoxia is known to modify ER levels, contributing to preeclampsia, through oxidative stress, calcium signaling, and RyR2 inhibition,[133] suggesting potential involvement of mitochondria and their structure (reviewed in the reference 134). Alternatively, upregulation of ERβ in preeclampsia might represent a compensatory mechanism to counterbalance the pro-

inflammatory and oxidative stress conditions that are prevalent in preeclampsia. Otherwise, research has shown ERβ having distinct roles in several other pregnancy complications. Pregnancy-induced upregulation of ERβ in primary human uterine artery endothelial cells, but not ERα.[135] In turn, ERβ causes AT2R upregulation, which in turn is protective against preeclampsia through increased uterine blood flow.[136] In villous endothelial cells, ERβ has been shown to mediate Cyclooxygenase-2 expression levels in a ligand-independent manner, potentially influencing prostanoid biosynthesis and fetoplacental vasculature.[137] Additionally, in human biopsies, recurrent reproductive failure, including recurrent implantation failure and recurrent pregnancy loss, is associated with significantly lower levels of ERβ, particularly in the vascular endothelium and perivascular areas.[138] Finally, ERβ displays differential localization than ERα; compared to ERα, it is more abundant in the human myometrium and cervix during pregnancy, and the only estrogen receptor present in the endothelium of the endometrium and fetoplacental vasculature, indicating its potential distinct roles in fetoplacental blood flow.[139]

Notably, a deficiency of GPER is associated with preeclampsia.[110, 140] As discussed in the section of “Effects of Estrogen Receptor on Mitochondria-Endoplasmic Reticulum Contacts and Calcium are Not Well Characterized”, GPER has a central role in modulating the mitochondria permeability transition pore opening.[141] It has been suggested that this pore opening, indicative of mitochondrial dysfunction, may contribute to preeclampsia, but it may just as well be a result of existing preeclampsia.[142] Like ERα and ERβ, GPER is important in modulating NO/cGMP pathways for the maintenance of uteroplacental blood flow.[143] However, other pathways through which GPER may be involved in pregnancy complications remain underresearched. In models of breast cancer, GPER can cause fission through the phosphorylation of Drp1.[144] Interestingly, GPER activation during pregnancy in female rodents increases β-cell mass by suppressing miR-338-3p, which promotes β-cell proliferation and protects against apoptosis, but this effect is not observed in human β-cells.[145] While these ERs have distinct roles, they also have confluent roles which cannot be neglected. For example, E2 can be used in the treatment of pelvic organ prolapse by inhibiting mitochondrial fusion protein mitofusin 2, alongside concomitant increases of all ERs (i.e., ERα, ERβ, and GPER).[146] Additionally, E2 enhances mitochondrial function, through PKA, by acting upon both GPER and ERα.[147] cAMP and PKA are important in quiescence by upregulation and downregulation during pregnancy and labor, respectively (reviewed in the reference 148). Their activation is also important in oxytocin receptor expression,[149] thus implicating diminished levels of PKA with preterm labor.[150] While both GPER and ERα expression is increased during pregnancy, due to elevated E2 levels,[145] more studies must consider the co-dependence of these ERs and their impacts on preterm labor in conditional knockouts. Similarly, while upregulation of ERα in extravillous trophoblasts is observed, recent evidence also shows that GPER is associated with placental extravillous trophoblasts invasion, wherein reduced GPER expression in this trophoblasts is associated with preeclampsia, potentially due to impaired estrogen synthesis (as reviewed in the reference 151). Other studies have shown that E2 treatment specifically increases ERα expression only in villous cytotrophoblast cells, again demonstrating the necessity of estrogen in trophoblast differentiation through ERs.[152] Thus, despite the clear presence of ERα, ERβ, and GPER30 in uterine arteries and the placenta, their exact roles in pregnancy

complications, and the influence of mitochondrial dysfunction in enacting these pathological states, remain conflicting.[153]

Notably, ERs contributing to pregnancy complications in the context of aging are poorly elucidated. While recent studies have shown that estrogen-related receptors are modulators of age-related mitochondrial dysfunction and inflammation in kidney aging,[154] it is unclear if this is the case for ERs in the context of pregnancy complications. One recent study found abundant mitochondrially-localized ERβ in ectopic endometriotic tissues, which served crucial bioenergetic roles including through lowering oxidative stress,[11] suggesting ERβ may mitigate therapeutically protect against endometriosis. Another study has found that aging leads to a significant decrease in ERα expression and transcriptional activation in mesangial cells, leading to increased oxidative stress.[155] This study also found that ethidium bromide treatment for mitochondrial depletion reversed some of these effects, suggesting that oxidative stress contributes to the loss of estrogen's protective functions during aging.[155] This has further been recapitulated in C2C12 cells wherein ERα represses hydrogen peroxide generation.[156] Thus, the roles of ER-modulated oxidative stress in the pathology of pregnancy complications, especially across aging, is relevant.

Estrogen Receptors Changing the Pregnancy Microbiome

A further area of research that demands more research is how estrogen affects microbiomes during pregnancy. Notably, the microbiome diversifies in the postpartum period while in healthy pregnancy, there is low diversity with a dominance of *Lactobacillus*, which is concomitant with elevated estrogen levels.[157] Group B *Streptococcus* growth in the lower reproductive tract microbiome is understood to be a key marker of adverse pregnancy outcomes, yet how *Lactobacillus* interacts with it to protect pregnancy remains unclear.[158] While supernatants secreted by *Lactobacillus* can protect against biofilm formation, these were strain-dependent, and bacterial associations resulted in host cell death, in some cases.[158] Beyond this, recent therapeutic techniques for adverse perinatal outcomes have been facilitated by Lactoferrin, which inhibits the biofilm formation of Group B *Streptococcus*.[159] Lactoferrin is an estrogen-inducible protein that interacts with estrogen receptor-related receptor α1.[160] Thus, it is possible that certain adverse outcomes or interactions between these gut microbiomes may be modulated by certain ERs-interactions which aid in explaining the differential development in pregnancy between healthy *Lactobacillus*-dominant environments and the buildup of Group B *Streptococcus*.

Given the alteration in the vaginal microbiome that occurs following the loss of estrogen levels, the role of estrogen in affecting other microbiomes is highlighted. The gut microbiome is also subject to reduced diversity during pregnancy in a progesterone-dependent manner.[161] This lowered diversity may be a defense mechanism against "estrobolomes" which are adverse health outcomes of metabolizing estrogens bacteria that may increase the likelihood of ER–positive breast cancer.[162] Conversely, a key health risk during pregnancy is gingivitis as estrogen can enhance planktonic growth.[163] Notably, clinical studies indicate that salivary estrogen levels are correlated with the likelihood of developing pregnancy gingivitis and gingival inflammation.[164] This suggests that opportunistic infections can utilize elevated estrogen levels during pregnancy, yet estrogen

may serve a modulating role in reducing microbiome biodiversity in certain organ systems to prevent this. Still, the roles of microbiomes in disease states during pregnancy need further investigation to understand these multifaceted roles of estrogen.

Role of Mitochondria in Placental Development

Important in the process of pregnancy is placental development, which remains heavily dependent on both ERs and mitochondrial function and structure, although few studies have looked at the interconnection of these processes. The roles of ERs in placental development and dysfunction are an active field of study. Estrogen related receptor-gamma, which shares DNA-binding domain and the ligand-binding domain with ERα and ERβ, is highly expressed in normal placenta with decreases in placental dysfunctions characteristic of fetal growth restriction (reviewed in the reference 165). The placenta can also play differential roles in binding ERs, with ERβ expression in chorionic villi, particularly in syncytiotrophoblasts, associated with trophoblasts differentiation, while ERα is decreased.[166] Vascular endothelial growth factor expression is important for placental angiogenesis, with E2 often playing regulatory roles.[167] As previously reviewed in the reference 167, vascular endothelial growth factor, and associated angiopoietin-1, expression estrogenic changes caused by are primarily regulated by ERs. However, upregulated ERs expression is not always associated with improved outcomes; the upregulation of expression of ERα and gene polymorphism of ERα in placental tissues were both associated with gestational diabetes mellitus in pregnant women.[168] Placental angiogenesis is also heavily dependent on the action of GPER, with recent findings showing that E2 treatment resulted in GPER-dependent endothelial nitric oxide synthase and Akt signaling, important for maintaining endothelial cell tube formation under conditions of hypoxia and reoxygenation.[169] Additionally, recent findings have shown that E2-dependent increases in GPER activation are responsible for placenta-secreted human chorionic gonadotropin expression, which produces progesterone, trough protein kinase A (PKA)-CREB signaling pathway in human cytotrophoblast cells.[170] Together, this demonstrates differential roles of ERs in placental development, with GPER being of particular interest.

Placenta parallel rapid aging in some ways, as they develop over quick periods. At around ten weeks, blood flow is reduced by trophoblasts resulting in impaired oxygen, associated with decreased respiration and compensatory increased mitochondrial content.[171] Beyond this, numerous pregnancy complications may be linked to alterations in the placenta. As past reviews have emphasized, malnutrition, hypoxia, and obesity may affect placenta mitochondrial function thus causing fetal complications.[172] Additionally, placental trophoblast cells experiencing gestational diabetes mellitus display elevated oxidative stress.[173] However, the exact mechanisms of these complications remain unclear in some contexts. For example, in pig models with excessive back fat, placental dysfunction was noted due to oxidative stress arising from mitochondrial injury.[174] ROS has remained a well-studied topic as a mediator of uteroplacental dysfunction due to the mild oxidative stress caused by pregnancy at baseline which may be exacerbated by certain conditions, as previously reviewed in the reference 153.

However, other aspects of mitochondrial function in placental dysfunction remain poorly elucidated, such as if mtDNA changes in the placenta are implicated in complications is unclear. For example, a hallmark of aging is an accumulation of mtDNA mutations which can result in deleterious heteroplasmy that interferes with mitochondrial function.[175] It is unclear if the short life span of the placenta also causes accelerated mtDNA mutations. Notably, certain conditions such as intrauterine growth restriction are marked by increased mtDNA content.[176] Beyond this, how mitochondrial structure changes across placental aging is unclear. Past results have shown that in the brain, donut-shaped 3D structures are more favorable upon hypoxic conditions,[177] yet it is unclear if certain mitochondrial phenotypes are more favorable to the hypoxic-like conditions that occur at Week ten in placental development. Similarly, several MERC proteins are known to be implicated in the ROS generation,[178] suggesting that these contact sites should be considered as a potential indicator to better understand oxidative stress in the placenta, which has previously been reviewed to be predictive of pregnancy complications.[179]

Given that mitochondrial transplantation is increasingly emerging as an option to potentially restore endometrial injury,[180] this may be a valuable option. As previously reviewed in the references 181 and 182, direct injection, systemic administration, and intranasal administration are all routes of mitochondrial transplantation through which mitochondrial diseases can be mitigated through the replacement of mitochondria. Similarly, mitochondrial replacement therapy via ooplasm injection has been performed for oocyte rejuvenation, but despite successful pregnancies, concerns over mitochondrial DNA heteroplasmy and the risk of mitochondrial diseases led to regulatory restrictions.[183] A better understanding of these dynamics in pregnancy complications and how estrogen affects them in the placenta can be important for precision medicine, especially in future clinical studies aiming to use mitochondrial transplantation or replacement for disease states.

Given the functional requirements of the cholesterol side chain cleavage enzyme P450 in the inner mitochondrial membrane for steroidogenesis, not all cell types are steroidogenic.[89] In the placenta, steroidogenic metabolites and precursors of fetal glands are utilized, emphasizing the relationship and dependency between the placenta and fetus. However, the role of E2 in poor placentation is controversial[184] with some recent cohort studies finding no association and peak E2 levels during ovarian stimulation being independent of birthweight,[185] suggesting that estrogen serum levels alone may not be a primary modulator of mitochondrial function.[186] This again highlights the importance of looking at specific activation of estrogen receptors, as differential recruitment may be a factor in poor placentation.

Future Directions to Understand Estrogen Receptors in Pregnancy

As reviewed in the reference 134, mitochondria dysfunction is well-linked to many pregnancy complications, including preterm birth, stillbirth, and pre-eclampsia. Targeting mitochondria, such as through antioxidants, has been shown to mitigate preeclampsia.[187] Multiple avenues must be explored to further our understanding of how the structural remodeling of mitochondria is altered in response to the modulation of ERs in a tissue-dependent manner, contributing to pregnancy complications. For example, in murine brown

adipocytes, ERα was required for *Drp1*-dependent mitochondrial remodeling, yet it is unclear if this is the case for other tissue types.[57] Beyond only looking at expression levels of associated genes, understanding mitochondrial ultrastructure is important. Further investigation is necessary to better understand the direct effect of estrogen on MERCs. Future directions for the field include determining the relationship between GPER on the endoplasmic reticulum and MERCs with regard to calcium signaling and characterizing MERC activity in response to an estrogen-induced drop in ROS production.

E2 and estrone are widely studied, however, the different roles of estrogen types should further be investigated. For example, equine models express ring B-unsaturated equilin or equilenin which have relatively poorly defined functions.[188] A better understanding of the functional roles of species-specific estrogens can offer insight into the evolutionarily conserved mechanisms that govern estrogens. Beyond the differential roles of estrogens, while this review focused on the different types of ERs, it should also be noted that isoforms may be implicated in pathologies. For example, ERα-LBD, an isoform of ERα is apparent in breast cancer and localizes to mitochondria.[189]

A greater understanding of the intersectionality between estrogens and bacterial microbiomes to both provide negative and positive birth outcomes remains an important avenue to study. Recent studies have indicated that HIV-1 transmission can be prevented in the female genital tract through a combination of elevated E2 levels and lactobacilli,[190] paralleling the results observed in pregnancy. In contrast, E2 degrading bacteria may also naturally exist in the environment and reduce E2 to estrone to reduce stress on certain organisms.[191] While common understanding shows that bacteria reduce E1 to produce ring-cleaved metabolites, the exact mechanism still requires further elucidation.[192] It is understood that different bacteria may reduce E2 levels in varied mechanisms,[193] however, the implications of these varied mechanisms on pregnancy outcomes, mitochondrial structure, and ERs remain poorly elucidated. Given the varied relationships between bacteria and E2, studies on mutualistic interactions of E2 and bacteria on host mitochondria may prove promising. Notably, a study in yeast found that the expression of the bacterial cell division protein FtsZ may lead to mitochondrial fission.[194] This suggests that certain alterations in bacteria may alter mitochondria, and if E2-modulation of its microbiome may also affect mitochondria through poorly explored mechanisms. Beyond only host cell studies, a better understanding of how ERs and E2 interact with bacteria may give insight into their modulation of mitochondrial function given mitochondria's endosymbiotic origin. For example, while binary fission is known in bacteria, it is not clear if, in response to E2, they also use similar fusion and fission pathways to regulate their cell shape and size. Beyond this, how estrogens affect bacterial DNA replication may provide insights into the similar processes that occur in with mtDNA.

Across models, the localization of ERβ in mitochondria is commonly observed, but the localization of ERα differs.[195] Notably, estrogen treatment is associated with increased ERβ localization, which in turn promotes ERE binding to reduce oxidative stress and regulate mtDNA transcription.[5, 195] As a result, ERβ has arisen as a key target for the treatment of Alzheimer's Disease through the inhibition of fission in an AKAP1-dependent manner.[196] However, the potential therapeutic role of targeting ERα and its effects on mitochondrial

structure need further investigation. Even less understood is GPER, which past research has suggested serves protective roles against obesity, glucose intolerance, and increased blood pressure.[197] The roles of the differential ERs in cardiac cells have previously been reviewed,[197] which shows differential roles of the ERs due, in part, to different roles in cardiac Ca^{2+} ion channels.[197]

Limitations

In this narrative review, we sought to synthesize current knowledge on estrogen receptors and mitochondrial function. However much of the existing literature may be fragmented or focused on specific aspects, making it challenging to draw comprehensive conclusions. As this is a narrative review, we sought to highlight areas of perceived interest, but additional systematic reviews are necessary to cover all relevant pathways or conditions.

Conclusions

Given the important role of estrogen in age-related pregnancy and maternal health, it is critical to consider the current state of pregnancy and any concerns about maternal health when evaluating treatments. Recent findings show that in frozen embryo transfer, hormone replacement therapy is associated with an increased risk of preterm birth complications, low birth weight, and hypertensive pregnancies compared to natural cycle-based methods.[198] This indicates that the full effects of pregnancy remain poorly understood and cannot be replicated through E2 treatment alone. There remains a lack of research regarding how the pregnancy process and secretion of estrogen may differ due to certain social determinants and aging. Furthermore, future treatments may consider targeting mitochondria. Also, as opposed to estrogen treatment, it may be interesting to see if it is possible to modulate the binding affinity of the ERs to reduce pregnancy-negative outcomes as a potential therapy. Finally, the role of the microbiome in maternal health, as both modulated by estrogen and as an effector, requires further study. Together, this suggests that each of these receptors plays differential, tissue-dependent roles in mitochondrial function thus conferring risk of pregnancy complications.

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References

1. Santen RJ, Simpson E. History of estrogen: its purification, structure, synthesis, biologic actions, and clinical implications. Endocrinology. 2019;160:605–625. [PubMed: 30566601]

2. Barton M, Filardo EJ, Lolait SJ, Thomas P, Maggiolini M, Prossnitz ER. Twenty years of the G protein-coupled estrogen receptor GPER: Historical and personal perspectives. J Steroid Biochem Mol Biol. 2018;176:4–15. [PubMed: 28347854]

3. Warner M, Fan X, Strom A, Wu W, Gustafsson JÅ. 25 years of ERβ: a personal journey. J Mol Endocrinol. 2021;68:R1–9. [PubMed: 34546964]

4. Cornil CA, Ball GF, Balthazart J. The dual action of estrogen hypothesis. Trends Neurosci. 2015;38:408–416. [PubMed: 26089224]

5. Yager JD, Chen JQ. Mitochondrial estrogen receptors--new insights into specific functions. Trends Endocrinol Metab. 2007;18:89–91. [PubMed: 17324583]

6. Cui J, Shen Y, Li R. Estrogen synthesis and signaling pathways during aging: from periphery to brain. Trends Mol Med. 2013;19:197–209. [PubMed: 23348042]

7. Mooga VP, White CR, Giordano-Mooga S. Estrogen and mitochondrial function in disease. In: Mitochondrial Diseases (IntechOpen); 2018.

8. Paterni I, Granchi C, Katzenellenbogen JA, Minutolo F. Estrogen receptors alpha (ERα) and beta (ERβ): subtype-selective ligands and clinical potential. Steroids. 2014;90:13–29. [PubMed: 24971815]

9. Hill L, Jeganathan V, Chinnasamy P, Grimaldi C, Diamond B. Differential roles of estrogen receptors α and β in control of B-cell maturation and selection. Mol Med. 2011;17:211–220. [PubMed: 21107497]

10. Lin AH, Li RW, Ho EY, et al. Differential ligand binding affinities of human estrogen receptor-α isoforms. PLoS One. 2013;8:e63199. [PubMed: 23646196]

11. Liao TL, Lee YC, Tzeng CR, et al. Mitochondrial translocation of estrogen receptor β affords resistance to oxidative insult-induced apoptosis and contributes to the pathogenesis of endometriosis. Free Radic Biol Med. 2019;134:359–373. [PubMed: 30684560]

12. Klinge CM. Estrogen receptor interaction with co-activators and co-repressors. Steroids. 2000;65:227–251. [PubMed: 10751636]

13. Torres MJ, Kew KA, Ryan TE, et al. 17β-Estradiol directly lowers mitochondrial membrane microviscosity and improves bioenergetic function in skeletal muscle. Cell Metab. 2018;27:167–179.e7. [PubMed: 29103922]

14. Prossnitz ER, Arterburn JB, Sklar LA. GPR30: A G protein-coupled receptor for estrogen. Mol Cell Endocrinol. 2007;265-266:138–142. [PubMed: 17222505]

15. Xu S, Yu S, Dong D, Lee LTO. G protein-coupled estrogen receptor: a potential therapeutic target in cancer. Front Endocrinol (Lausanne). 2019;10:725. [PubMed: 31708873]

16. Duong BN, Elliott S, Frigo DE, et al. AKT regulation of estrogen receptor beta transcriptional activity in breast cancer. Cancer Res. 2006;66:8373–8381. [PubMed: 16951146]

17. Cheng SB, Graeber CT, Quinn JA, Filardo EJ. Retrograde transport of the transmembrane estrogen receptor, G-protein-coupled-receptor-30 (GPR30/GPER) from the plasma membrane towards the nucleus. Steroids. 2011;76:892–896. [PubMed: 21354433]

18. Moreno-Ulloa A, Miranda-Cervantes A, Licea-Navarro A, et al. (−)-Epicatechin stimulates mitochondrial biogenesis and cell growth in C2C12 myotubes via the G-protein coupled estrogen receptor. Eur J Pharmacol. 2018;822:95–107. [PubMed: 29355558]

19. Haas E, Meyer MR, Schurr U, et al. Differential effects of 17beta-estradiol on function and expression of estrogen receptor alpha, estrogen receptor beta, and GPR30 in arteries and veins of patients with atherosclerosis. Hypertension. 2007;49:1358–1363. [PubMed: 17452498]

20. Fuentes N, Silveyra P. Estrogen receptor signaling mechanisms. Adv Protein Chem Struct Biol. 2019;116:135–170. [PubMed: 31036290]

21. Ahn SJ, Yoon MS, Hyuk S, et al. Phospholipase C-protein kinase C mediated phospholipase D activation pathway is involved in tamoxifen induced apoptosis. J Cell Biochem. 2003;89:520–528. [PubMed: 12761885]

22. Kelly MJ, Qiu J, Rønnekleiv OK. Estrogen modulation of G-protein-coupled receptor activation of potassium channels in the central nervous system. Ann N Y Acad Sci. 2003;1007:6–16. [PubMed: 14993035]

23. Pedram A, Razandi M, Wallace DC, Levin ER. Functional estrogen receptors in the mitochondria of breast cancer cells. Mol Biol Cell. 2006;17:2125–2137. [PubMed: 16495339]

24. Kumar A, Xie L, Ta CM, et al. SWELL1 regulates skeletal muscle cell size, intracellular signaling, adiposity and glucose metabolism. Elife. 2020;9:e58941. [PubMed: 32930093]
25. Carracedo A, Pandolfi PP. The PTEN-PI3K pathway: of feedbacks and cross-talks. Oncogene. 2008;27:5527–5541. [PubMed: 18794886]
26. Barros RP, Machado UF, Warner M, Gustafsson JA. Muscle GLUT4 regulation by estrogen receptors ERbeta and ERalpha. Proc Natl Acad Sci U S A. 2006;103:1605–1608. [PubMed: 16423895]
27. Barros RP, Gabbi C, Morani A, Warner M, Gustafsson JA. Participation of ERalpha and ERbeta in glucose homeostasis in skeletal muscle and white adipose tissue. Am J Physiol Endocrinol Metab. 2009;297:E124–133. [PubMed: 19366879]
28. Bryzgalova G, Gao H, Ahren B, et al. Evidence that oestrogen receptor-alpha plays an important role in the regulation of glucose homeostasis in mice: insulin sensitivity in the liver. Diabetologia. 2006;49:588–597. [PubMed: 16463047]
29. Barros RP, Gustafsson JÅ. Estrogen receptors and the metabolic network. Cell Metab. 2011;14:289–299. [PubMed: 21907136]
30. Stubbins RE, Holcomb VB, Hong J, Núñez NP. Estrogen modulates abdominal adiposity and protects female mice from obesity and impaired glucose tolerance. Eur J Nutr. 2012;51:861–870. [PubMed: 22042005]
31. Ohlsson C, Hammarstedt A, Vandenput L, et al. Increased adipose tissue aromatase activity improves insulin sensitivity and reduces adipose tissue inflammation in male mice. Am J Physiol Endocrinol Metab. 2017;313:E450–462. [PubMed: 28655716]
32. Scalzo RL, Foright RM, Hull SE, et al. Breast cancer endocrine therapy promotes weight gain with distinct adipose tissue effects in lean and obese female mice. Endocrinology. 2021;162:bqab174. [PubMed: 34410380]
33. Nilsson M, Dahlman I, Rydén M, et al. Oestrogen receptor alpha gene expression levels are reduced in obese compared to normal weight females. Int J Obes (Lond). 2007;31:900–907. [PubMed: 17224934]
34. Ribas V, Nguyen MT, Henstridge DC, et al. Impaired oxidative metabolism and inflammation are associated with insulin resistance in ERalpha-deficient mice. Am J Physiol Endocrinol Metab. 2010;298:E304–319. [PubMed: 19920214]
35. Ribas V, Drew BG, Le JA, et al. Myeloid-specific estrogen receptor alpha deficiency impairs metabolic homeostasis and accelerates atherosclerotic lesion development. Proc Natl Acad Sci U S A. 2011;108:16457–16462. [PubMed: 21900603]
36. Jia M, Dahlman-Wright K, Gustafsson JÅ. Estrogen receptor alpha and beta in health and disease. Best Pract Res Clin Endocrinol Metab. 2015;29:557–568. [PubMed: 26303083]
37. Pucci G, Alcidi R, Tap L, Battista F, Mattace-Raso F, Schillaci G. Sex- and gender-related prevalence, cardiovascular risk and therapeutic approach in metabolic syndrome: A review of the literature. Pharmacol Res. 2017;120:34–42. [PubMed: 28300617]
38. Zhao L, Fan X, Zuo L, et al. Estrogen receptor 1 gene polymorphisms are associated with metabolic syndrome in postmenopausal women in China. BMC Endocr Disord. 2018;18:65. [PubMed: 30217154]
39. Yang W, Jiang W, Liao W, et al. An estrogen receptor α-derived peptide improves glucose homeostasis during obesity. Nat Commun. 2024;15:3410. [PubMed: 38649684]
40. Klinge CM. Estrogenic control of mitochondrial function. Redox Biol. 2020;31:101435. [PubMed: 32001259]
41. Yoh K, Ikeda K, Horie K, Inoue S. Roles of estrogen, estrogen receptors, and estrogen-related receptors in skeletal muscle: regulation of mitochondrial function. Int J Mol Sci. 2023;24:1853. [PubMed: 36768177]
42. Monzel AS, Enríquez JA, Picard M. Multifaceted mitochondria: moving mitochondrial science beyond function and dysfunction. Nat Metab. 2023;5:546–562. [PubMed: 37100996]
43. Jenkins BC, Neikirk K, Katti P, et al. Mitochondria in disease: changes in shapes and dynamics. Trends Biochem Sci. 2024;49:346–360. [PubMed: 38402097]
44. Cervantes-Silva MP, Cox SL, Curtis AM. Alterations in mitochondrial morphology as a key driver of immunity and host defence. EMBO Rep. 2021;22:e53086. [PubMed: 34337844]

45. Golic I, Velickovic K, Markelic M, et al. Calcium-induced alteration of mitochondrial morphology and mitochondrial-endoplasmic reticulum contacts in rat brown adipocytes. Eur J Histochem. 2014;58:2377. [PubMed: 25308841]
46. Faitg J, Lacefield C, Davey T, et al. 3D neuronal mitochondrial morphology in axons, dendrites, and somata of the aging mouse hippocampus. Cell Rep. 2021;36:109509. [PubMed: 34380033]
47. Saito K, He Y, Yan X, et al. Visualizing estrogen receptor-α-expressing neurons using a new ERα-ZsGreen reporter mouse line. Metabolism. 2016;65:522–532. [PubMed: 26975544]
48. Lam J, Katti P, Biete M, et al. A universal approach to analyzing transmission electron microscopy with ImageJ. Cells. 2021;10:2177. [PubMed: 34571826]
49. Garza-Lopez E, Vue Z, Katti P, et al. Protocols for generating surfaces and measuring 3D organelle morphology using amira. Cells. 2021;11:65. [PubMed: 35011629]
50. Neikirk K, Lopez EG, Marshall AG, et al. Call to action to properly utilize electron microscopy to measure organelles to monitor disease. Eur J Cell Biol. 2023;102:151365. [PubMed: 37864884]
51. Marshall AG, Neikirk K, Stephens DC, et al. Serial block face-scanning electron microscopy as a burgeoning technology. Adv Biol (Weinh). 2023;7:e2300139. [PubMed: 37246236]
52. Denk W, Horstmann H. Serial block-face scanning electron microscopy to reconstruct three-dimensional tissue nanostructure. PLoS Biol. 2004;2:e329. [PubMed: 15514700]
53. Marshall AG, Damo SM, Hinton A Jr. Revisiting focused ion beam scanning electron microscopy. Trends Biochem Sci. 2023;48:585–586. [PubMed: 36990957]
54. Marshall AG, Krystofiak E, Damo SM, Hinton A Jr. Correlative light-electron microscopy: integrating dynamics to structure. Trends Biochem Sci. 2023;48:826–827. [PubMed: 37277285]
55. Shi C, Wu F, Xu J. Incorporation of β-sitosterol into mitochondrial membrane enhances mitochondrial function by promoting inner mitochondrial membrane fluidity. J Bioenerg Biomembr. 2013;45:301–305. [PubMed: 23225137]
56. Zorova LD, Popkov VA, Plotnikov EY, et al. Mitochondrial membrane potential. Anal Biochem. 2018;552:50–59. [PubMed: 28711444]
57. Zhou Z, Moore TM, Drew BG, et al. Estrogen receptor α controls metabolism in white and brown adipocytes by regulating Polg1 and mitochondrial remodeling. Sci Transl Med. 2020;12:eaax8096. [PubMed: 32759275]
58. Ribas V, Drew BG, Zhou Z, et al. Skeletal muscle action of estrogen receptor α is critical for the maintenance of mitochondrial function and metabolic homeostasis in females. Sci Transl Med. 2016;8:334ra54.
59. Sanchez MI, Shearwood AM, Chia T, Davies SM, Rackham O, Filipovska A. Estrogen-mediated regulation of mitochondrial gene expression. Mol Endocrinol. 2015;29:14–27. [PubMed: 25375021]
60. Lin PI, Tai YT, Chan WP, Lin YL, Liao MH, Chen RM. Estrogen/ERα signaling axis participates in osteoblast maturation via upregulating chromosomal and mitochondrial complex gene expressions. Oncotarget. 2017;9:1169–1186. [PubMed: 29416685]
61. Nagai S, Ikeda K, Horie-Inoue K, Takeda S, Inoue S. Estrogen signaling increases nuclear receptor subfamily 4 group A member 1 expression and energy production in skeletal muscle cells. Endocr J. 2018;65:1209–1218. [PubMed: 30333364]
62. Yeh PS, Chen JT, Cherng YG, Yang ST, Tai YT, Chen RM. Methylpiperidinopyrazole attenuates estrogen-induced mitochondrial energy production and subsequent osteoblast maturation via an estrogen receptor alpha-dependent mechanism. Molecules. 2020;25:2876. [PubMed: 32580515]
63. Chen JQ, Brown TR, Russo J. Regulation of energy metabolism pathways by estrogens and estrogenic chemicals and potential implications in obesity associated with increased exposure to endocrine disruptors. Biochim Biophys Acta. 2009;1793:1128–1143. [PubMed: 19348861]
64. Singh KK. Mitochondria as epigenetic regulators of reproductive aging. In: Sex, Gender, and Epigenetics. Elsevier;2023:251–260.
65. Chu X, Raju RP. Regulation of NAD+ metabolism in aging and disease. Metabolism. 2022;126:154923. [PubMed: 34743990]
66. Wu QJ, Zhang TN, Chen HH, et al. The sirtuin family in health and disease. Signal Transduct Target Ther. 2022;7:402. [PubMed: 36581622]

67. Liang J, Huang F, Song Z, Tang R, Zhang P, Chen R. Impact of NAD+ metabolism on ovarian aging. Immun Ageing. 2023;20:70. [PubMed: 38041117]
68. Tummala KS, Gomes AL, Yilmaz M, et al. Inhibition of de novo NAD(+) synthesis by oncogenic URI causes liver tumorigenesis through DNA damage. Cancer Cell. 2014;26:826–839. [PubMed: 25453901]
69. Jing R, Corbett JL, Cai J, et al. A screen using iPSC-derived hepatocytes reveals NAD+ as a potential treatment for mtDNA depletion syndrome. Cell Rep. 2018;25:1469–1484.e5. [PubMed: 30404003]
70. Herrmann GK, Russell WK, Garg NJ, Yin YW. Poly(ADP-ribose) polymerase 1 regulates mitochondrial DNA repair in an NAD-dependent manner. J Biol Chem. 2021;296:100309. [PubMed: 33482196]
71. Ježek P, Dlasková A. Dynamic of mitochondrial network, cristae, and mitochondrial nucleoids in pancreatic β-cells. Mitochondrion. 2019;49:245–258. [PubMed: 31252091]
72. Dlaskova A, Spacek T, Tauber J, et al. Mitochondrial DNA nucleoid redistribution after mitochondrial network fragmentation as visualized by 3D super-resolution biplane fpalm microscopy. Biophys J. 2013;104:657.
73. Giacomello M, Pellegrini L. The coming of age of the mitochondria-ER contact: a matter of thickness. Cell Death Differ. 2016;23:1417–1427. [PubMed: 27341186]
74. Carreras-Sureda A, Jaña F, Urra H, et al. Non-canonical function of IRE1α determines mitochondria-associated endoplasmic reticulum composition to control calcium transfer and bioenergetics. Nat Cell Biol. 2019;21:755–767. [PubMed: 31110288]
75. Csordás G, Weaver D, Hajnóczky G. Endoplasmic reticulum–mitochondrial contactology: structure and signaling functions. Trends Cell Biol. 2018;28:523–540. [PubMed: 29588129]
76. Friedman JR, Lackner LL, West M, DiBenedetto JR, Nunnari J, Voeltz GK. ER tubules mark sites of mitochondrial division. Science. 2011;334:358–362. [PubMed: 21885730]
77. Miller WL. Minireview: regulation of steroidogenesis by electron transfer. Endocrinology. 2005;146:2544–2550.. [PubMed: 15774560]
78. Fan P, Jordan VC. PERK, beyond an unfolded protein response sensor in estrogen-induced apoptosis in endocrine-resistant breast cancer. Mol Cancer Res. 2022;20:193–201. [PubMed: 34728551]
79. Tse G, Yan BP, Chan YW, Tian XY, Huang Y. Reactive oxygen species, endoplasmic reticulum stress and mitochondrial dysfunction: the link with cardiac arrhythmogenesis. Front Physiol. 2016;7:313. [PubMed: 27536244]
80. Csordás G, Hajnóczky G. SR/ER-mitochondrial local communication: calcium and ROS. Biochim Biophys Acta. 2009;1787:1352–1362. [PubMed: 19527680]
81. Su Q, Wang Y, Yang X, et al. Inhibition of endoplasmic reticulum stress apoptosis by estrogen protects human umbilical vein endothelial cells through the PI3 kinase-Akt signaling pathway. J Cell Biochem. 2017;118:4568–4574. [PubMed: 28485890]
82. Schwartz N, Verma A, Bivens CB, Schwartz Z, Boyan BD. Rapid steroid hormone actions via membrane receptors. Biochim Biophys Acta. 2016;1863:2289–2298. [PubMed: 27288742]
83. Santo-Domingo J, Demaurex N. Calcium uptake mechanisms of mitochondria. Biochim Biophys Acta. 2010;1797:907–912. [PubMed: 20079335]
84. Vincent AE, Turnbull DM, Eisner V, Hajnóczky G, Picard M. Mitochondrial nanotunnels. Trends Cell Biol. 2017;27:787–799. [PubMed: 28935166]
85. Lavorato M, Iyer VR, Dewight W, et al. Increased mitochondrial nanotunneling activity, induced by calcium imbalance, affects intermitochondrial matrix exchanges. Proc Natl Acad Sci U S A. 2017;114:E849–858. [PubMed: 28096415]
86. Nilsen J, Diaz Brinton R. Mechanism of estrogen-mediated neuroprotection: regulation of mitochondrial calcium and Bcl-2 expression. Proc Natl Acad Sci U S A. 2003;100:2842–2847. [PubMed: 12604781]
87. Kasai S, Yamazaki H, Tanji K, Engler MJ, Matsumiya T, Itoh K. Role of the ISR-ATF4 pathway and its cross talk with Nrf2 in mitochondrial quality control. J Clin Biochem Nutr. 2019;64:1–12. [PubMed: 30705506]

88. Hinton A Jr, Claypool SM, Neikirk K, et al. Mitochondrial structure and function in human heart failure. Circ Res. 2024;135:372–396. [PubMed: 38963864]

89. Papadopoulos V, Miller WL. Role of mitochondria in steroidogenesis. Best Pract Res Clin Endocrinol Metab. 2012;26:771–790. [PubMed: 23168279]

90. Yaşar P, Ayaz G, User SD, Güpür G, Muyan M. Molecular mechanism of estrogen-estrogen receptor signaling. Reprod Med Biol. 2016;16:4–20. [PubMed: 29259445]

91. Pedram A, Razandi M, O'Mahony F, Harvey H, Harvey BJ, Levin ER. Estrogen reduces lipid content in the liver exclusively from membrane receptor signaling. Sci Signal. 2013;6:ra36. [PubMed: 23695162]

92. Liu G, Wang Y, Zheng Y, et al. PHB2 binds to ERβ to induce the autophagy of porcine ovarian granulosa cells through mTOR phosphorylation. Theriogenology. 2023;198:114–122. [PubMed: 36580849]

93. Burstein SR, Kim HJ, Fels JA, et al. Estrogen receptor beta modulates permeability transition in brain mitochondria. Biochim Biophys Acta Bioenerg. 2018;1859:423–433. [PubMed: 29550215]

94. Schubert C, Raparelli V, Westphal C, et al. Reduction of apoptosis and preservation of mitochondrial integrity under ischemia/reperfusion injury is mediated by estrogen receptor β. Biol Sex Differ. 2016;7:53. [PubMed: 27688871]

95. Martinez-Bernabe T, Sastre-Serra J, Ciobu N, Oliver J, Pons DG, Roca P. Estrogen receptor beta (ERβ) maintains mitochondrial network regulating invasiveness in an obesity-related inflammation condition in breast cancer. Antioxidants (Basel). 2021;10:1371. [PubMed: 34573003]

96. Kim SO, Albrecht ED, Pepe GJ. Estrogen promotes fetal skeletal muscle mitochondrial distribution and ATP synthase activity important for insulin sensitivity in offspring. Endocrine. 2024;85:417–427. [PubMed: 38478198]

97. Panda S, Dohare P, Jain S, et al. Estrogen treatment reverses prematurity-induced disruption in cortical interneuron population. J Neurosci. 2018;38:7378–7391. [PubMed: 30037831]

98. Rajkumar L, Guzman RC, Yang J, Thordarson G, Talamantes F, Nandi S. Short-term exposure to pregnancy levels of estrogen prevents mammary carcinogenesis. Proc Natl Acad Sci U S A. 2001;98:11755–11759. [PubMed: 11573010]

99. Shao R, Feng Y, Zou S, et al. The role of estrogen in the pathophysiology of tubal ectopic pregnancy. Am J Transl Res. 2012;4:269–278. [PubMed: 22937205]

100. Gupta R, Dhyani M, Kendzerska T, et al. Restless legs syndrome and pregnancy: prevalence, possible pathophysiological mechanisms and treatment. Acta Neurol Scand. 2016;133:320–329. [PubMed: 26482928]

101. Ruiz R, Herrero C, Strasser-Weippl K, et al. Epidemiology and pathophysiology of pregnancy-associated breast cancer: A review. Breast. 2017;35:136–141. [PubMed: 28732325]

102. Lambertini M, Kroman N, Ameye L, et al. Long-term safety of pregnancy following breast cancer according to estrogen receptor status. J Natl Cancer Inst. 2018;110:426–429. [PubMed: 29087485]

103. Azim HA Jr, Kroman N, Paesmans M, et al. Prognostic impact of pregnancy after breast cancer according to estrogen receptor status: a multicenter retrospective study. J Clin Oncol. 2013;31:73–79. [PubMed: 23169515]

104. Lin ZH, Jin J, Shan XY. The effects of estradiol on inflammatory and endothelial dysfunction in rats with preeclampsia. Int J Mol Med. 2020;45:825–835. [PubMed: 31985028]

105. Bai J, Qi QR, Li Y, et al. Estrogen receptors and estrogen-induced uterine vasodilation in pregnancy. Int J Mol Sci. 2020;21:4349. [PubMed: 32570961]

106. Hevener AL, Clegg DJ, Mauvais-Jarvis F. Impaired estrogen receptor action in the pathogenesis of the metabolic syndrome. Mol Cell Endocrinol. 2015;418 Pt 3:306–321. [PubMed: 26033249]

107. Parisi F, Fenizia C, Introini A, et al. The pathophysiological role of estrogens in the initial stages of pregnancy: molecular mechanisms and clinical implications for pregnancy outcome from the periconceptional period to end of the first trimester. Hum Reprod Update. 2023;29:699–720. [PubMed: 37353909]

108. Pastore MB, Jobe SO, Ramadoss J, Magness RR. Estrogen receptor-α and estrogen receptor-β in the uterine vascular endothelium during pregnancy: functional implications for regulating uterine blood flow. Semin Reprod Med. 2012;30:46–61. [PubMed: 22271294]

109. Anamthathmakula P, Kyathanahalli C, Ingles J, Hassan SS, Condon JC, Jeyasuria P. Estrogen receptor alpha isoform ERdelta7 in myometrium modulates uterine quiescence during pregnancy. EBioMedicine. 2019;39:520–530. [PubMed: 30502052]
110. Tong C, Feng X, Chen J, et al. G protein-coupled receptor 30 regulates trophoblast invasion and its deficiency is associated with preeclampsia. J Hypertens. 2016;34:710–718. [PubMed: 26848992]
111. Schiessl B, Mylonas I, Hantschmann P, et al. Expression of endothelial NO synthase, inducible NO synthase, and estrogen receptors alpha and beta in placental tissue of normal, preeclamptic, and intrauterine growth-restricted pregnancies. J Histochem Cytochem. 2005;53:1441–1449. [PubMed: 15983116]
112. Park MN, Park KH, Lee JE, et al. The expression and activation of sex steroid receptors in the preeclamptic placenta. Int J Mol Med. 2018;41:2943–2951. [PubMed: 29436602]
113. Yin G, Zhu X, Guo C, et al. Differential expression of estradiol and estrogen receptor α in severe preeclamptic pregnancies compared with normal pregnancies. Mol Med Rep. 2013;7:981–985. [PubMed: 23291833]
114. Lan KC, Lai YJ, Cheng HH, et al. Levels of sex steroid hormones and their receptors in women with preeclampsia. Reprod Biol Endocrinol. 2020;18:12. [PubMed: 32070380]
115. Knabl J, Hiden U, Hüttenbrenner R, et al. GDM alters expression of placental estrogen receptor α in a cell type and gender-specific manner. Reprod Sci. 2015;22:1488–1495. [PubMed: 25947892]
116. Zhang H, Zha X, Zheng Y, et al. Mechanisms underlying the role of endoplasmic reticulum stress in the placental injury and fetal growth restriction in an ovine gestation model. J Anim Sci Biotechnol. 2023;14:117. [PubMed: 37691111]
117. Zhou Z, Ribas V, Rajbhandari P, et al. Estrogen receptor α protects pancreatic β-cells from apoptosis by preserving mitochondrial function and suppressing endoplasmic reticulum stress. J Biol Chem. 2018;293:4735–4751. [PubMed: 29378845]
118. Guzel E, Arlier S, Guzeloglu-Kayisli O, et al. Endoplasmic reticulum stress and homeostasis in reproductive physiology and pathology. Int J Mol Sci. 2017;18:792. [PubMed: 28397763]
119. Hinton AO Jr, He Y, Xia Y, et al. Estrogen receptor-α in the medial amygdala prevents stress-induced elevations in blood pressure in females. Hypertension. 2016;67:1321–1330. [PubMed: 27091896]
120. Hinton AO Jr., Xu P, Yan X, Reynolds CL, Xu Y. Estrogen-responsive neurons in the medial amygdala prevent stress-induced hypertension. FASEB J. 2013;27:654.11–654.11.
121. Xu P, Cao X, He Y, et al. Estrogen receptor-α in medial amygdala neurons regulates body weight. J Clin Invest. 2015;125:2861–2876. [PubMed: 26098212]
122. Meher S, Duley L. Nitric oxide for preventing pre-eclampsia and its complications. Cochrane Database Syst Rev. 2007;2007:CD006490. [PubMed: 17443623]
123. Villa A, Rizzi N, Vegeto E, Ciana P, Maggi A. Estrogen accelerates the resolution of inflammation in macrophagic cells. Sci Rep. 2015;5:15224. [PubMed: 26477569]
124. Gao L, Wang G, Liu WN, Kinser H, Franco HL, Mendelson CR. Reciprocal feedback between miR-181a and E2/ERα in myometrium enhances inflammation leading to labor. J Clin Endocrinol Metab. 2016;101:3646–3656. [PubMed: 27459534]
125. Foley HB, Howe CG, Eckel SP, et al. Extracellular vesicle-enriched miRNA profiles across pregnancy in the MADRES cohort. PLoS One. 2021;16:e0251259. [PubMed: 33979365]
126. Huang X, Wu L, Zhang G, Tang R, Zhou X. Elevated microRNA-181a-5p contributes to trophoblast dysfunction and preeclampsia. Reprod Sci. 2019;26:1121–1129. [PubMed: 30376765]
127. Vishnyakova P, Poltavets A, Nikitina M, et al. Expression of estrogen receptor α by decidual macrophages in preeclampsia. Biomedicines. 2021;9:191. [PubMed: 33672970]
128. Murata T, Narita K, Honda K, Matsukawa S, Higuchi T. Differential regulation of estrogen receptor alpha and beta mRNAs in the rat uterus during pregnancy and labor: possible involvement of estrogen receptors in oxytocin receptor regulation. Endocr J. 2003;50:579–587. [PubMed: 14614214]

129. Kobayashi H, Kimura M, Maruyama S, Nagayasu M, Imanaka S. Revisiting estrogen-dependent signaling pathways in endometriosis: Potential targets for non-hormonal therapeutics. Eur J Obstet Gynecol Reprod Biol. 2021;258:103–110. [PubMed: 33421806]
130. Thompson LP, Pence L, Pinkas G, Song H, Telugu BP. Placental hypoxia during early pregnancy causes maternal hypertension and placental insufficiency in the hypoxic guinea pig model. Biol Reprod. 2016;95:128. [PubMed: 27806942]
131. Whitman NA, Lin ZW, Kenney RM, Albertini L, Lockett MR. Hypoxia differentially regulates estrogen receptor alpha in 2D and 3D culture formats. Arch Biochem Biophys. 2019;671:8–17. [PubMed: 31163125]
132. Frump AL, Selej M, Wood JA, et al. Hypoxia upregulates estrogen receptor β in pulmonary artery endothelial cells in a HIF-1α-dependent manner. Am J Respir Cell Mol Biol. 2018;59:114–126. [PubMed: 29394091]
133. Hu XQ, Song R, Romero M, et al. Gestational hypoxia inhibits pregnancy-induced upregulation of Ca2+ sparks and spontaneous transient outward currents in uterine arteries via heightened endoplasmic reticulum/oxidative stress. Hypertension. 2020;76:930–942. [PubMed: 32683903]
134. Marín R, Chiarello DI, Abad C, Rojas D, Toledo F, Sobrevia L. Oxidative stress and mitochondrial dysfunction in early-onset and late-onset preeclampsia. Biochim Biophys Acta Mol Basis Dis. 2020;1866:165961. [PubMed: 32916282]
135. Mishra JS, Te Riele GM, Qi QR, et al. Estrogen receptor-β mediates estradiol-induced pregnancy-specific uterine artery endothelial cell angiotensin type-2 receptor expression. Hypertension. 2019;74:967–974. [PubMed: 31378106]
136. Mishra JS, Kumar S. Activation of angiotensin type 2 receptor attenuates testosterone-induced hypertension and uterine vascular resistance in pregnant rats†. Biol Reprod. 2021;105:192–203. [PubMed: 33739377]
137. Su EJ, Lin ZH, Zeine R, et al. Estrogen receptor-beta mediates cyclooxygenase-2 expression and vascular prostanoid levels in human placental villous endothelial cells. Am J Obstet Gynecol. 2009;200:427.e1–8.
138. Al-Lamee H, Ellison A, Drury J, et al. Altered endometrial oestrogen-responsiveness and recurrent reproductive failure. Reprod Fertil. 2022;3:30–38. [PubMed: 35350653]
139. Su EJ, Xin H, Monsivais D. The emerging role of estrogen receptor-β in human reproduction. Semin Reprod Med. 2012;30:62–70. [PubMed: 22271295]
140. Feng X, Zhou L, Mao X, et al. Association of a reduction of G-protein coupled receptor 30 expression and the pathogenesis of preeclampsia. Mol Med Rep. 2017;16:5997–6003. [PubMed: 28849224]
141. Bopassa JC, Eghbali M, Toro L, Stefani E. A novel estrogen receptor GPER inhibits mitochondria permeability transition pore opening and protects the heart against ischemia-reperfusion injury. Am J Physiol Heart Circ Physiol. 2010;298:H16–23. [PubMed: 19880667]
142. Smith AN, Wang X, Thomas DG, Tatum RE, Booz GW, Cunningham MW. The role of mitochondrial dysfunction in preeclampsia: causative factor or collateral damage? Am J Hypertens. 2021;34:442–452. [PubMed: 33417666]
143. Tropea T, De Francesco EM, Rigiracciolo D, et al. Pregnancy augments G protein estrogen receptor (GPER) induced vasodilation in rat uterine arteries via the nitric oxide - cGMP signaling pathway. PLoS One. 2015;10:e0141997. [PubMed: 26536245]
144. Rekha P, Gupta A, Goud KS, et al. GPER induces mitochondrial fission through p44/42 MAPK - Drp1 pathway in breast cancer cells. Biochem Biophys Res Commun. 2023;643:16–23. [PubMed: 36584588]
145. Sharma G, Mauvais-Jarvis F, Prossnitz ER. Roles of G protein-coupled estrogen receptor GPER in metabolic regulation. J Steroid Biochem Mol Biol. 2018;176:31–37. [PubMed: 28223150]
146. Wang XQ, He RJ, Xiao BB, Lu Y. Therapeutic effects of 17β-estradiol on pelvic organ prolapse by inhibiting Mfn2 expression: an in vitro study. Front Endocrinol (Lausanne). 2020;11:586242. [PubMed: 33324344]
147. Bauzá-Thorbrügge M, Rodríuez-Cuenca S, Vidal-Puig A, et al. GPER and ERα mediate estradiol enhancement of mitochondrial function in inflamed adipocytes through a PKA dependent mechanism. J Steroid Biochem Mol Biol. 2019;185:256–267. [PubMed: 30253224]

148. Li JKH, Lai PF, Tribe RM, Johnson MR. Transcription factors regulated by cAMP in smooth muscle of the myometrium at human parturition. Biochem Soc Trans. 2021;49:997–1011. [PubMed: 33860781]

149. Yulia A, Singh N, Lei K, Sooranna SR, Johnson MR. Cyclic AMP effectors regulate myometrial oxytocin receptor expression. Endocrinology. 2016;157:4411–4422. [PubMed: 27673556]

150. Yulia A, Varley AJ, Singh N, Lei K, Tribe R, Johnson MR. Changes in cAMP effector predominance are associated with increased oxytocin receptor expression in twin but not infection-associated or idiopathic preterm labour. PLoS One. 2020;15:e0240325. [PubMed: 33253216]

151. Alencar AKN, Swan KF, Pridjian G, Lindsey SH, Bayer CL. Connecting G protein-coupled estrogen receptor biomolecular mechanisms with the pathophysiology of preeclampsia: a review. Reprod Biol Endocrinol. 2023;21:60. [PubMed: 37393260]

152. Bukovsky A, Cekanova M, Caudle MR, et al. Expression and localization of estrogen receptor-alpha protein in normal and abnormal term placentae and stimulation of trophoblast differentiation by estradiol. Reprod Biol Endocrinol. 2003;1:13. [PubMed: 12646062]

153. Hu XQ, Song R, Zhang L. Effect of oxidative stress on the estrogen-NOS-NO-KCa channel pathway in uteroplacental dysfunction: its implication in pregnancy complications. Oxid Med Cell Longev. 2019;2019:9194269. [PubMed: 30881600]

154. Wang XX, Myakala K, Libby AE, et al. Estrogen-Related Receptor Agonism Reverses Mitochondrial Dysfunction and Inflammation in the Aging Kidney. Am J Pathol. 2023;193:1969–1987. [PubMed: 37717940]

155. Pereira-Simon S, Xia X, Catanuto P, Elliot S. Oxidant stress and mitochondrial signaling regulate reversible changes of ERα expression and apoptosis in aging mouse glomeruli and mesangial cells. Endocrinology. 2012;153:5491–5499. [PubMed: 23027807]

156. Long X, Gao Y, Liu W, et al. Natural flavonoid silibinin promotes the migration and myogenic differentiation of murine C2C12 myoblasts via modulation of ROS generation and down-regulation of estrogen receptor α expression. Mol Cell Biochem. 2020;474:243–261. [PubMed: 32789659]

157. Nunn KL, Witkin SS, Schneider GM, et al. Changes in the vaginal microbiome during the pregnancy to postpartum transition. Reprod Sci. 2021;28:1996–2005. [PubMed: 33432532]

158. Shiroda M, Aronoff DM, Gaddy JA, Manning SD. The impact of Lactobacillus on group B streptococcal interactions with cells of the extraplacental membranes. Microb Pathog. 2020;148:104463. [PubMed: 32828901]

159. Talbert JA, Lu J, Spicer SK, Moore RE, Townsend SD, Gaddy JA. Ameliorating adverse perinatal outcomes with Lactoferrin: An intriguing chemotherapeutic intervention. Bioorg Med Chem. 2022;74:117037. [PubMed: 36215812]

160. Zhang Z, Teng CT. Estrogen receptor-related receptor alpha 1 interacts with coactivator and constitutively activates the estrogen response elements of the human lactoferrin gene. J Biol Chem. 2000;275:20837–20846. [PubMed: 10779508]

161. Mallott EK, Borries C, Koenig A, Amato KR, Lu A. Reproductive hormones mediate changes in the gut microbiome during pregnancy and lactation in Phayre's leaf monkeys. Sci Rep. 2020;10:9961. [PubMed: 32561791]

162. Kwa M, Plottel CS, Blaser MJ, Adams S. The intestinal microbiome and estrogen receptor-positive female breast cancer. J Natl Cancer Inst. 2016;108:djw029. [PubMed: 27107051]

163. Fteita D, Könönen E, Söderling E, Gürsoy UK. Effect of estradiol on planktonic growth, coaggregation, and biofilm formation of the Prevotella intermedia group bacteria. Anaerobe. 2014;27:7–13. [PubMed: 24594108]

164. Gürsoy M, Gürsoy UK, Sorsa T, Pajukanta R, Könönen E. High salivary estrogen and risk of developing pregnancy gingivitis. J Periodontol. 2013;84:1281–1289. [PubMed: 23237582]

165. Zou Z, Forbes K, Harris LK, Heazell AEP. The potential role of the E SRRG pathway in placental dysfunction. Reproduction. 2021;161:R45–60. [PubMed: 33361468]

166. Bukovsky A, Caudle MR, Cekanova M, et al. Placental expression of estrogen receptor beta and its hormone binding variant--comparison with estrogen receptor alpha and a role for estrogen

receptors in asymmetric division and differentiation of estrogen-dependent cells. Reprod Biol Endocrinol. 2003;1:36. [PubMed: 12740031]

167. Albrecht ED, Pepe GJ. Estrogen regulation of placental angiogenesis and fetal ovarian development during primate pregnancy. Int J Dev Biol. 2010;54:397–408. [PubMed: 19876841]
168. Li C, Qiao B, Zhou Y, Qi W, Ma C, Zheng L. Association of estrogen receptor α gene polymorphism and its expression with gestational diabetes mellitus. Gynecol Obstet Invest. 2020;85:26–33. [PubMed: 31466066]
169. Zhou L, Chen H, Mao X, Qi H, Baker PN, Zhang H. G-protein-coupled receptor 30 mediates the effects of estrogen on endothelial cell tube formation in vitro. Int J Mol Med. 2017;39:1461–1467. [PubMed: 28440394]
170. Yang S, Jia Y, Wu Z, et al. Activation of G protein-coupled estrogen receptor stimulates placental human chorionic gonadotropin expression through PKA-CREB signaling. Mol Cell Endocrinol. 2023;577:112033. [PubMed: 37506871]
171. Holland OJ, Hickey AJR, Alvsaker A, et al. Changes in mitochondrial respiration in the human placenta over gestation. Placenta. 2017;57:102–112. [PubMed: 28863998]
172. Lu M, Sferruzzi-Perri AN. Placental mitochondrial function in response to gestational exposures. Placenta. 2021;104:124–137. [PubMed: 33338764]
173. Fisher JJ, Vanderpeet CL, Bartho LA, et al. Mitochondrial dysfunction in placental trophoblast cells experiencing gestational diabetes mellitus. J Physiol. 2021;599:1291–1305. [PubMed: 33135816]
174. Tian L, Huang J, Wen A, Yan P. Impaired mitochondrial function results from oxidative stress in the full-term placenta of sows with excessive back-fat. Animals (Basel). 2020;10:360. [PubMed: 32102192]
175. Elorza AA, Soffia JP. mtDNA heteroplasmy at the core of aging-associated heart failure. An integrative view of OXPHOS and mitochondrial life cycle in cardiac mitochondrial physiology. Front Cell Dev Biol. 2021;9:625020. [PubMed: 33692999]
176. Lattuada D, Colleoni F, Martinelli A, et al. Higher mitochondrial DNA content in human IUGR placenta. Placenta. 2008;29:1029–33. [PubMed: 19007984]
177. Liu X, Hajnóczky G. Altered fusion dynamics underlie unique morphological changes in mitochondria during hypoxia-reoxygenation stress. Cell Death Differ. 2011;18:1561–1572. [PubMed: 21372848]
178. Resende R, Fernandes T, Pereira AC, Marques AP, Pereira CF. Endoplasmic reticulum-mitochondria contacts modulate reactive oxygen species-mediated signaling and oxidative stress in brain disorders: the key role of sigma-1 receptor. Antioxid Redox Signal. 2022;37:758–780. [PubMed: 35369731]
179. Schoots MH, Gordijn SJ, Scherjon SA, van Goor H, Hillebrands JL. Oxidative stress in placental pathology. Placenta. 2018;69:153–161. [PubMed: 29622278]
180. Kshersagar J, Pulgam L, Damle MN, Tardalkar K, Sharma R, Joshi MG. Transplantation of human placenta derived mitochondria promotes cell communication in endometrium in a murine model of disturbed endometrium. Stem Cell Rev Rep. 2023;19:1384–1401. [PubMed: 36856954]
181. Neikirk K, Stephens DC, Beasley HK, et al. Considerations for developing mitochondrial transplantation techniques for individualized medicine. Biotechniques. 2024;76:125–134. [PubMed: 38420889]
182. Joshi MG, Damle MN, Sharma RK. Application route of mitochondrial transplantation. In: Mitochondrial Transplantation and Transfer. Elsevier; 2024:231–280.
183. Chiang JL, Shukla P, Pagidas K, et al. Mitochondria in ovarian aging and reproductive longevity. Ageing Res Rev. 2020;63:101168. [PubMed: 32896666]
184. Ganer Herman H, Volodarsky-Perel A, Ton Nu TN, et al. The effect of higher estradiol levels during stimulation on pregnancy complications and placental histology. Placenta. 2022;126:114–118. [PubMed: 35796062]
185. Huang J, Lu X, Lin J, et al. Association between peak serum estradiol level during controlled ovarian stimulation and neonatal birthweight in freeze-all cycles: a retrospective study of 8501 singleton live births. Hum Reprod. 2020;35:424–433. [PubMed: 32078675]

186. Dunne C, Cho K, Shan A, Hutcheon J, Durland US, Seethram K, Havelock JC. Peak serum estradiol level during controlled ovarian stimulation is not associated with lower levels of pregnancy-associated plasma protein-a or small for gestational age infants: a cohort study. J Obstet Gynaecol Can. 2017;39:870–879. [PubMed: 28606451]

187. Long J, Huang Y, Tang Z, et al. Mitochondria targeted antioxidant significantly alleviates preeclampsia caused by 11β-HSD2 dysfunction via OPA1 and mtDNA maintenance. Antioxidants (Basel). 2022;11:1505. [PubMed: 36009224]

188. Esteller-Vico A, Ball BA, Troedsson MHT, Squires EL. Endocrine changes, fetal growth, and uterine artery hemodynamics after chronic estrogen suppression during the last trimester of equine pregnancy. Biol Reprod. 2017;96:414–423. [PubMed: 28203724]

189. Strillacci A, Sansone P, Rajasekhar VK, et al. ERα-LBD, an isoform of estrogen receptor alpha, promotes breast cancer proliferation and endocrine resistance. NPJ Breast Cancer. 2022;8:96. [PubMed: 35999225]

190. Dizzell S, Nazli A, Reid G, Kaushic C. Protective effect of probiotic bacteria and estrogen in preventing HIV-1-mediated impairment of epithelial barrier integrity in female genital tract. Cells. 2019;8:1120. [PubMed: 31546582]

191. Zhang Q, Xue C, Owens G, Chen Z. Isolation and identification of 17β-estradiol degrading bacteria and its degradation pathway. J Hazard Mater. 2022;423:127185. [PubMed: 34537637]

192. Chen YL, Yu CP, Lee TH, et al. Biochemical mechanisms and catabolic enzymes involved in bacterial estrogen degradation pathways. Cell Chem Biol. 2017;24:712–724.e7. [PubMed: 28552583]

193. Ke J, Zhuang W, Gin KY, Reinhard M, Hoon LT, Tay JH. Characterization of estrogen-degrading bacteria isolated from an artificial sandy aquifer with ultrafiltered secondary effluent as the medium. Appl Microbiol Biotechnol. 2007;75:1163–1171. [PubMed: 17396255]

194. Spier A, Sachse M, Tham NT, Matondo M, Cossart P, Stavru F. Bacterial FtsZ induces mitochondrial fission in human cells. bioRxiv [Preprint]. 2020. doi:10.1101/2020.01.24.917146.

195. Solakidi S, Psarra AM, Sekeris CE. Differential subcellular distribution of estrogen receptor isoforms: localization of ERalpha in the nucleoli and ERbeta in the mitochondria of human osteosarcoma SaOS-2 and hepatocarcinoma HepG2 cell lines. Biochim Biophys Acta. 2005;1745:382–392. [PubMed: 15993498]

196. Sarkar S, Jun S, Simpkins JW. Estrogen amelioration of Aβ-induced defects in mitochondria is mediated by mitochondrial signaling pathway involving ERβ, AKAP and Drp1. Brain Res. 2015;1616:101–111. [PubMed: 25964165]

197. Mahmoodzadeh S, Dworatzek E. The role of 17β-estradiol and estrogen receptors in regulation of Ca2+ channels and mitochondrial function in cardiomyocytes. Front Endocrinol (Lausanne). 2019;10:310. [PubMed: 31156557]

198. Zong L, Liu P, Zhou L, Wei D, Ding L, Qin Y. Increased risk of maternal and neonatal complications in hormone replacement therapy cycles in frozen embryo transfer. Reprod Biol Endocrinol. 2020;18:36. [PubMed: 32366332]

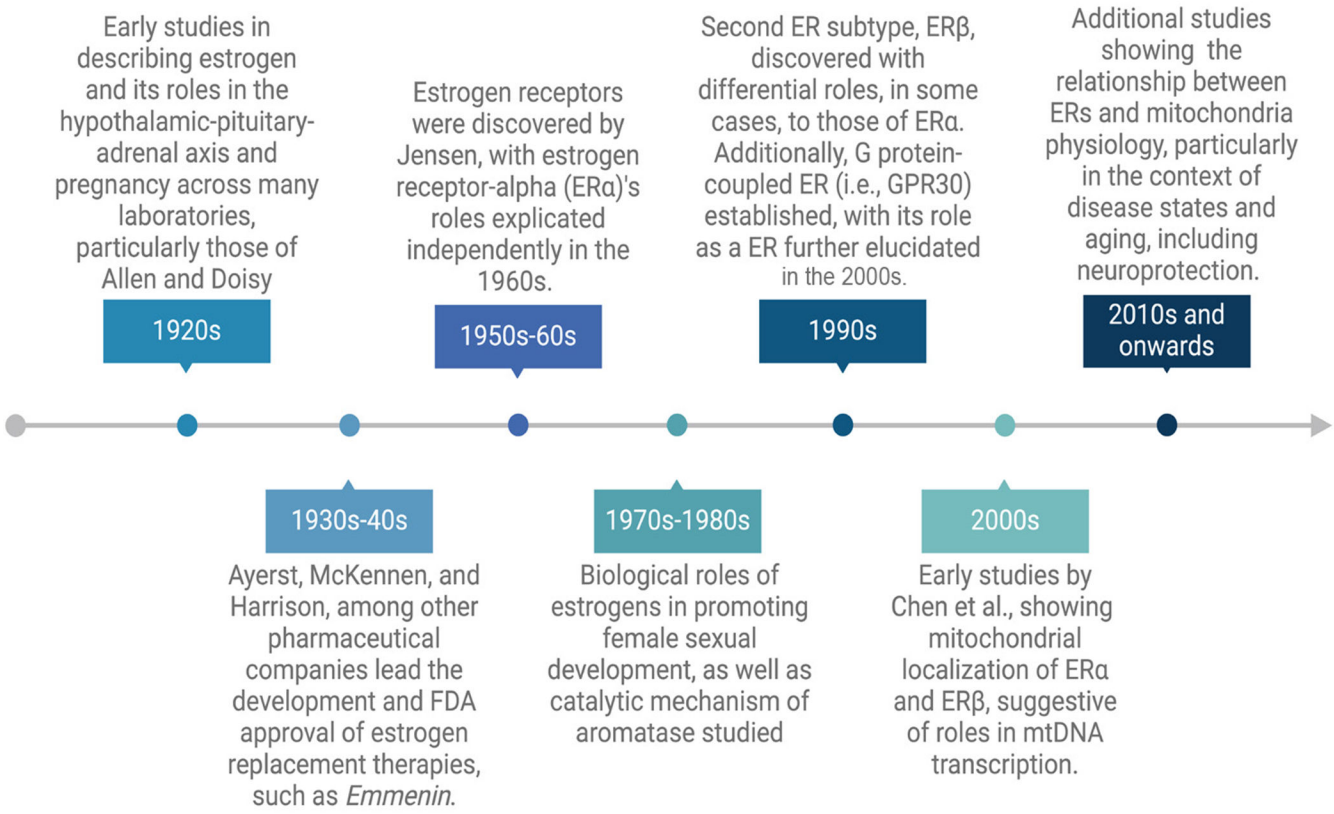

Figure 1: A brief timeline of the discovery of estrogen and its receptors, as well as an emerging understanding of their roles in mitochondria, particularly in the context of age-related disease states.

Based on previous reviews.[1–3] Created with BioRender.com. mtDNA: Mitochondrial DNA.

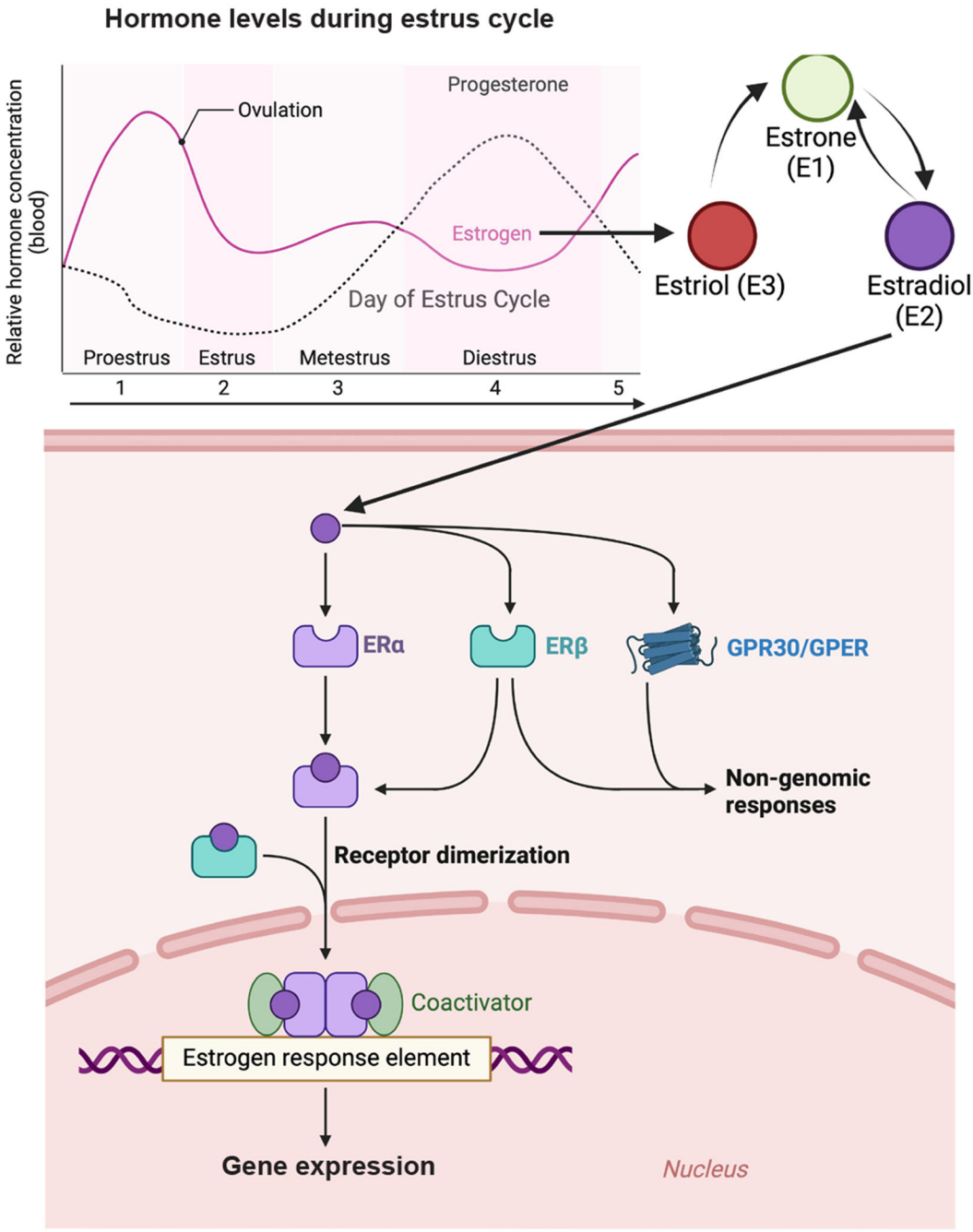

Figure 2: Estrogen receptors (ERs) which regulate genomic and non-genomic signaling pathways during pregnancy.

During pregnancy, estrogen levels show an uptick, with various levels, typically inverse to progesterone. Estriol (E3), the form of estrogen which is synthesized by the placenta, is the most plentiful. Since E3 is not biologically activated, it can be converted to the ovarian estrogen form estradiol (E2), which is biologically active. Thus, circulating E2 levels are relatively high throughout pregnancy. E2 can then bind to ERs (ERα and ERβ) and G-protein-coupled receptor 30 (GPR30/GPER). They undergo genomic effects, in the case of ERα and ERβ, or rapid, non-genomic signaling pathways, which commonly occur with

GPR30. ERα is widely expressed in reproductive tissues, such as the uterus, placenta, and breast. While ERβ is also expressed in ovary, uterus, and placenta, its expression is generally lower than ERα. Created with BioRender.com.

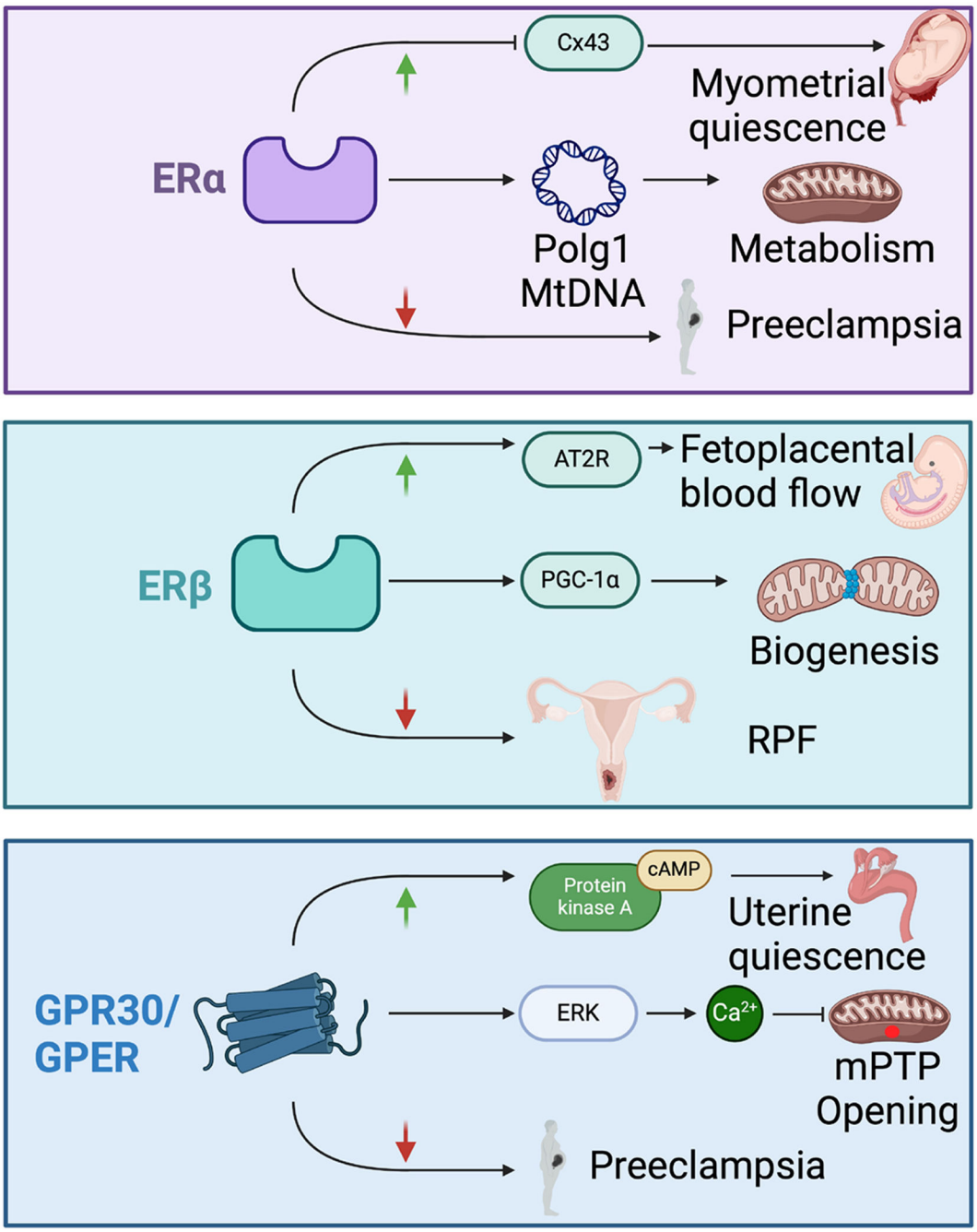

Figure 3: Roles of estrogen receptors (ERs) in mitochondrial function and pregnancy.

Roles in pregnancy of ERs (ERα and ERβ) and G-protein-coupled receptor 30 (GPR30/GPER) during their upregulation (top, green arrow) or downregulation (bottom, red arrow). Involved mitochondrial roles are in the middle. ERα: (Top) ERα isoforms, namely ERΔ7 whose splicing is determined by hnRNPG, downregulate connexin-43 for myometrial quiescence. Thus, the continual hormonally regulated splicing of ERα into isoforms, and subsequent inhibition of splicing, is necessary to prevent preterm labor then incur labor. (Middle) While ERα has multiple mitochondrial roles, a central one is regulating Polg1,

in turn affecting mitochondrial DNA (mtDNA) and regulating mitochondrial metabolism. (Bottom) Lower levels of ERα are observed in women with preeclampsia. ERβ: (Top) ERβ upregulates angiotensin type 2 receptor expression (AT2R) and increases uterine artery blood flow. However, higher levels of ERβ are observed in women with preeclampsia. (Middle) ERβ has central roles in upregulating Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), which is a central regulator of mitochondrial biogenesis. (Bottom) Lower levels of perivascular and vascular endothelium ERβ are associated with recurrent reproductive failure (RRF), including recurrent implantation failure (RIF) and recurrent pregnancy loss (RPL). GPR30/GPER: (Top) GPR30/GPER can increase Gs alpha subunit protein, which in turn raises cyclic adenosine monophosphate (cAMP), activating Protein kinase A. Protein kinase A in turn maintains uterine quiescence. (Middle) A principal mitochondrial function of GPR30 is activation of extracellular signal-regulated kinase (ERK), resulting in the mobilization of intracellular Ca^{2+} concentration. This prevents the opening of the mitochondrial permeability transition pore (mPTP), a pathological channel that is Ca^{2+}-dependent. (Bottom) Rapid, non-genomic vasodilatory actions via transmembrane GPR30 may be disturbed in preeclampsia, as levels in GPR30 are lower in the placentae from women with preeclampsia. Created with BioRender.com.