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# Gender Dimorphisms in Progenitor and Stem Cell Function in Cardiovascular Disease

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## Abstract

Differences in cardiovascular disease outcomes between men and women have long been recognized and attributed, in part, to gender and sex steroids. Gender dimorphisms also exist with respect to the roles of progenitor and stem cells in post-ischemic myocardial and endothelial repair and regeneration. Understanding how these cells are influenced by donor gender and the recipient hormonal milieu may enable researchers to further account for the gender-related disparities in clinical outcomes as well as utilize the beneficial effects of these hormones to optimize transplanted cell function and survival. This review discusses (1) the cardiovascular effects of sex steroids (specifically estradiol and testosterone); (2) the therapeutic potentials of endothelial progenitor cells, mesenchymal stem cells, and embryonic stem cells; and (3) the direct effect of sex steroids on these cell types.

### Keywords

Progenitor Cells; Stem Cell Therapy; Sex Steroids; Gender Differences; Cardiovascular Disease

## Introduction

Gender differences exist in numerous types of injuries and disease states including renal ischemia/reperfusion (I/R) injury [1], trauma/hemorrhage [2], sepsis [3], intestinal ischemia-induced organ injury, cardiovascular disease [4], and myocardial inflammation [5]. With regard to cardiovascular disease, premenopausal women are at lower risk for atherosclerosis, coronary artery disease, and myocardial infarction compared to postmenopausal women and age-matched men [4,6]. Such gender dimorphisms have been linked at least in part to differences in sex steroid expression, specifically estrogen.

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Within the last decade, stem cell and progenitor cell-based therapies have been applied to the injured heart with promising initial results. Clinical trials have utilized bone marrow cell populations that include endothelial progenitor cells (EPCs) and mesenchymal stem cells (MSCs). As a circulating population, EPCs may home to areas of tissue injury and augment endothelial protection and repair [7]. MSCs in addition to other bone marrow-derived mononuclear cells may also be mobilized from bone marrow and traffic to injured myocardium or may be therapeutically applied in a more directed fashion [8]. In vitro and animal models have also investigated the similar application of embryonic stem cells (ESCs) [9].

Therefore, it is not surprising that as estrogen has been observed in multiple models to confer direct protective on the vasculature, its effect on the function of progenitor and stem cells has also drawn increasing interest. It is becoming increasingly important to understand how these cells are affected by endogenous and exogenous influences including sex steroids as well as how their function may be optimized prior to their therapeutic application. To this purpose, we review the cardiovascular effects of sex steroids; the therapeutic potentials of EPCs, MSCs, and ESCs; and the direct effect of sex steroids on these cell types.

## Roles of Sex Steroids in Cardiovascular Disease

Estrogen, specifically 17β-estradiol (E2), has been demonstrated to exert multiple cardiovascular protective effects in animal models [10]. Many of these effects are exerted directly on the vasculature and involve modulation of atherogenic and vasoreactive mechanisms (Table 1). The further recognition of the role of endothelial dysfunction in atherosclerosis and cardiovascular disease has also guided investigation into how estrogen protects and repairs damaged endothelium.

Estrogen functions primarily by signaling via estrogen receptors (ER)α and β which belong to the steroid/thyroid superfamily of nuclear receptors [11,12]. These receptors are expressed by a wide variety of cells including vascular smooth muscle cells, endothelial cells, EPCs, MSCs, and other progenitor and stem cells. Following ligand binding, ERs mediate their effects through either genomic or non-genomic mechanisms. Genomic mechanisms include regulation of gene transcription through the direct binding of the nuclear estrogen receptor to estrogen response elements or other transcriptional regulator sequences (Fig. 1) [13]. Consequently, estrogen may suppress pro-atherogenic genes and induce athero-protective genes, downregulate interleukin (IL)-6 expression [14], and increase production of protective growth factors including vascular endothelial growth factor (VEGF) and insulin-like growth factor-1 (IGF-1) [15,16]. E2 has also been shown to upregulate suppressor of cytokine signaling (SOCS) protein expression with resultant resistance to deleterious tumor necrosis factor-α (TNF-α) signaling in females [17,18]. Non-genomic effects involve the direct action of estrogen on the vasculature including the rapid activation of endothelial nitric oxide synthetase (eNOS) and vasodilation which may augment tissue perfusion [19,20].

Evidence supporting the protective role of E2 in the setting of vascular injury includes the observations that E2 increased re-endothelialization, increased endothelial functional recovery (increased nitric oxide production), and decreased neointimal formation in a dose-dependent fashion in ovariectomized (OVX) mice following carotid artery injury [21]. This E2-induced re-endothelialization appears to be mediated by ERα [22,23]. ERβ, on the other hand, has separately been shown to mediate vasculoprotective effects in reproductive organs [24] and myocardial protection during ischemia/reperfusion injury via upregulation of PI3K/Akt and decreased cardiomyocyte apoptosis [25]. Interestingly, E2 may also protect the vasculature in the absence of ERα or ERβ as shown in mouse knockout models [26,27]. Specifically, early atheroprotection has recently been shown to occur independently of ERα in OVX ERα $^{-/-}$ mice

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treated with exogenous E2 [12]. Thus, while ERα and ERβ are important mediators of E2-induced vasculoprotection, other receptors or signaling pathways are likely involved.

The promising results of these early animal studies have not been fully realized in clinical trials, however. In the Heart and Estrogen/Progestin Replacement Study that included menopausal women with documented coronary artery disease, there was no reduction in cardiovascular events with exogenous hormone therapy [28,29]. In addition, hormonal therapy was associated with an increased risk of early coronary events and venous thrombo-embolic events. The Women's Health Initiative Estrogen/Progesterone Study was also stopped early due to increased risks of breast cancer, coronary events, and stroke [30]. Similarly, the unopposed estrogen arm of this study was stopped due to an increased risk of stroke without any change in heart disease risk [31]. Further research is warranted to explain these discrepancies between the results of the animal studies and the clinical outcomes following estrogen therapy.

The evidence that men have a greater incidence of coronary artery disease (CAD) and myocardial infarction (MI) than age-matched women also raised the hypothesis that testosterone (T) negatively affects the cardiovascular system. As demonstrated in a rat model of I/R injury, T exhibits deleterious effects on the myocardium specifically by downregulating signal transducer and activator of transcription 3 (STAT3) and suppressor of cytokine signaling 3 (SOCS3) expression during acute I/R [32]. However, other evidence suggests that T may actually possess vasculoprotective properties as well. Exogenous T was shown to inhibit aortic atherosclerosis in castrated male rabbits [33]. In addition, reduced plasma T was associated with increased arterial stiffness in men [34,35], and the oral administration of T in men with CAD improved brachial artery vasoreactivity [36]. Furthermore, the acute administration of T in men with CAD had beneficial effects on exercise-induced myocardial ischemia [37].

## Endothelial Progenitor Cells

Bone marrow-derived endothelial progenitor cells are self-renewing and capable of differentiating into mature endothelial cells. As a circulating pool, EPCs offer an immediate mechanism of repair of endothelial damage [7], and it was this ability to home to ischemic sites and differentiate into mature endothelial cells that led to their discovery [38]. Given the protective endothelial effects of E2 as well as the eventually recognized role of EPCs in maintaining endothelial integrity, it was further hypothesized that E2 may confer some of its vasculoprotective effects through regulation of EPC function.

Ischemic/injured tissue recruits EPCs through the local release of growth factors and cytokines. Both endogenous and exogenous granulocyte colony-stimulating factor and granulocyte colony-stimulating factor have been directly shown to enhance EPC mobilization and migration [39,40]. Hypoxia-inducible factors, which are activated under low oxygen levels, induce expression of stromal cell-derived factor-1 (SDF-1) in endothelial cells with subsequent increase in the adhesion, migration, and homing of circulating EPC to ischemic areas via the CXCR4 receptor [41]. In a rat model of MI, skeletal myoblasts over-expressing SDF-1 produced an endogenous gradient that facilitated stem and progenitor cell home migration to areas of infarction and increased angiogenesis [42]. EPC migration has also been shown to be mediated by VEGF receptor 1 (flt1) and 2 (flk1) in an eNOS-dependent fashion [43,44]. These receptors may also facilitate the increased levels of circulating EPCs following acute MI and vascular trauma [45,46] and in relation to plasma VEGF levels [47].

EPCs have demonstrated therapeutic efficacy for ischemic disease in early animal and human studies. Mice hindlimb ischemia models have shown that endogenously mobilized and exogenously delivered EPCs improve tissue perfusion and limb recovery [40,48]. Post-ischemic injection of EPCs resulted in improved myocardial capillary density and function 28 days later in a rat coronary artery ligation model [49]. Clinical trials investigating the use of

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EPC mobilization as well as exogenous intracoronary infusion or direct intramyocardial injection in patients with acute and chronic myocardial ischemia have shown promising preliminary results with improvements in left ventricular (LV) function and myocardial neovascularization following these directed therapies (reviewed in [50]). While one study ended early due to a greater-than-expected rate of in-stent restenosis in patients who received cell therapy [51], phase I and II trials are on-going.

In addition to their therapeutic potential, EPCs may also serve as predictors of cardiovascular disease outcomes. Hill et al. [52] found EPCs to be more predictive of vascular reactivity compared to conventional cardiac risk factors. In the same study, EPCs from high-risk subjects exhibited greater in vitro senescence compared to low-risk subjects. Werner et al. [53] observed that increased levels of EPCs were associated with a reduced risk of adverse cardiovascular disease outcomes but were not predictive of MI or death from all causes. Similarly, levels of circulating EPCs and function have also inversely correlated with age-related decreases in VEGF [54], diabetes [55,56], cardiac allograft vasculopathy [57], unstable angina [58], chronic renal failure [59], essential hypertension [60], and acute stroke patients [61].

## Sex Steroids and Endothelial Progenitor Cells

Investigations into the effect of E2 on EPCs have focused primarily on EPC mobilization, survival, and promotion of re-endothelialization. Numerous studies have demonstrated either a positive association between levels of circulating EPCs and E2 or an ability of E2 to directly stimulate mobilization of these cells (Fig. 2) [62,63]. E2 mediates this action via ERα and ERβ; however, ERα may play a more significant role [15]. Following ER ligand binding, activation of the PI3K pathway, eNOS induction, and fibroblast growth factor (FGF)-2 production appear to be critical steps in bone marrow EPC mobilization [62,64–66]. E2-induced EPC mobilization is also associated with accelerated re-endothelialization following vascular injury [62,63] and direct incorporation into areas of myocardial ischemia with subsequently reduced LV scarring and increased LV function [65].

E2 also affects EPC survival and growth kinetics. Specifically, E2 inhibits apoptosis via attenuation of caspase 8 activity [62]. It also inhibits EPC senescence onset in culture, increases telomerase activity, increases mitogenic activity, and activates the Akt survival pathway [60].

Hormonal status may be an important determinant of in vivo EPC function. Levels of circulating EPCs are greater in premenopausal women compared to postmenopausal women [67]. EPC levels also correlate with the level of circulating E2 during the menstrual cycle [68,69] and are higher in fertile women than in young men or postmenopausal women. However, there is no difference in levels of EPCs between postmenopausal women and age-matched men [68]. In addition, EPCs from middle-aged women exhibit greater colony-forming capacity and migratory activity in vitro compared to those from age-matched men, providing further evidence of the effect of endogenous E2 on EPC function [70]. Finally, linear regression analysis indicates that gender and age correlate with EPC levels independent of other cardiovascular parameters.

While EPCs express androgen receptors (AR), there are more limited data regarding the effect of androgens on EPCs [71]. In one study, hypogonadal males had decreased basal circulating EPCs which increased following T treatment [71]. However, in this study, both T and estrogen levels increased following T treatment, and the peripheral conversion of T to estrogen could not be excluded as a cause of the increased EPC levels. As a follow-up study, EPCs were treated with a synthetic nonaromatizable androgen in vitro and demonstrated increased migration and proliferation by an AR-mediated mechanism [72].

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The effects of androgens on EPC mobilization have recently been challenged. By separately isolating early and late EPCs, Fadini et al. [73] found that androgen stimulation had no effect on late EPC expansion and adhesion in vitro. Castration decreased levels of circulating EPCs, but this effect was not reversed with exogenous T administration. In a sample of healthy middle-aged men, the level of circulating EPCs more closely correlated with E2 than with T. Pro-survival and growth-stimulatory effects of T may also be restricted to more mature progenitor cells [74]. Furthermore, in an analysis of plasma steroid levels in males with inflammatory bowel disease, EPC levels did not correlate with T levels [75]. Thus, while the data are conflicting regarding the full role of androgens in EPC proliferation and function, T appears to be less influential than E2 in this capacity

## Mesenchymal Stem Cells

MSCs derived from bone marrow are self-renewing cells capable of differentiating into multiple cell types including osteoblasts, adipocytes, chondrocytes, endothelial cells, and potentially cardiomyocytes [76,77]. In addition to their potential for differentiation, MSCs may protect ischemic tissue via the paracrine release of growth factors and anti-inflammatory cytokines that mitigate the ischemia-induced local inflammatory response [78]. MSCs also exhibit unique immunologic characteristics in that allogeneic MSCs may be able to evade the host immune system and potentially even suppress local activation of host T lymphocytes [79]. These latter properties in particular have spurred interest in developing the cells as a readily available source of cells for the treatment of various tissue injuries.

Several randomized and nonrandomized clinical trials have evaluated the use of autologous bone marrow-derived mononuclear cells for the treatment of acute and chronic myocardial ischemia [80,81]. While this population of cells represents both hematopoietic and mesenchymal progenitor and stem cells, the cumulative beneficial effects of transplantation of these cells has promoted further investigation into the use of the individual cell types for myocardial and peripheral ischemia.

## Sex Steroids and Mesenchymal Stem Cells

MSCs exhibit gender-related differences in paracrine function [82]. In vitro, female MSCs as compared to male MSCs produced more VEGF and less TNF-α in response to stress stimuli such as lipopolysaccharide and hypoxia. Since VEGF is an important paracrine factor in MSC-mediated myocardial protection following ischemia [83], this suggested that source gender may be an important determinant of MSC function and potential cardioprotection. In addition, the decreased production of TNF-α, a pro-inflammatory mediator of I/R-related myocardial dysfunction and apoptosis, suggests that female MSCs are more resistant to certain injurious stimuli [84]. Using an ex vivo model of isolated heart perfusion, intra-coronary infusion of female MSCs was associated with greater post-ischemic myocardial functional recovery compared to male MSCs [85]. Given that these cells were removed during the in vivo estrous cycle, these observed responses may reflect the inherent chronic effects of estrogen or estrogen-independent functions. Exogenous administration E2 may also play a role on MSC function as E2 stimulates male MSC production of VEGF in vitro [86]. Moreover, hearts infused with E2-treated MSCs exhibited greater functional recovery after I/R compared to those infused with untreated MSCs [86]. Cumulatively, these data suggest that exposure to estrogen may augment MSC function and potentially MSC-mediated cardioprotection.

The roles of ERα and ERβ in MSC function have also been evaluated. Our lab observed that E2 as well as ERα but not ERβ agonism can stimulate production of VEGF and hypoxia-inducible factor-1α (HIF-1α) as well as activate STAT3 in vitro (Fig. 2) [87]. These effects were abolished in ERα and STAT3 but not ERβ-KO MSCs. The HIF-1 family of cytokines is upregulated in response to reduced tissue oxygenation and may mediate growth factor

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production including VEGF [88]. Following induction by cellular stress and growth factors, STAT3 participates in several cell functions including cell survival/apoptosis, proliferation, inflammation, and angiogenesis [89,90]. STAT3 functions as a direct transcriptional activator of VEGF in MSCs and other cell types [89,91], a process that is in part mediated by HIF-1α [92]. In addition, STAT3 is a direct target gene of E2 as evidenced by its upregulation in response to E2 or ERα agonism [93]. E2-stimulated MSC production of HIF-1α and VEGF occurs also via protein kinase C, PI3K, Akt, SAPK/JNK, and ERK-mediated mechanisms [94]. E2 also stimulates MSC production of bone morphogenetic protein-2 (BMP-2) via both ERα and ERβ, although with greater reliance on ERα [95].

Following the demonstration that TNFR1 plays a detrimental role in the myocardium during I/R injury [96], attention was given to the potential roles of TNFR1 and TNFR2 in MSC function. MSCs from male TNFR1-KO mice produce more VEGF and less TNF-α and IL-6. In vitro, we observed that male TNFR1KO MSCs underwent less apoptosis in vitro compared to wild-type MSCs and were equal to that of wild-type female MSCs [97]. Ablation of TNFR1 in female MSCs also resulted in improved paracrine function. Similar investigation into the role of TNFR2 signaling in gender-related MSC functional differences demonstrated that TNFR2 is a more significant regulator of VEGF and IGF-1 production in male but not female MSCs [98].

In addition to enhancing MSC paracrine functions, E2 has been shown to increase MSC proliferation [94,99] and differentiation [100]. The osteogenic differentiation of murine MSCs appears to be promoted by ERα and possibly inhibited by ERβ in response [101]. Lastly, E2 may induce MSC telomerase activity via ERα and delay the onset of senescence [102].

The role of androgens in MSC function is less clear. In vitro, MSCs from castrated male rats showed increased VEGF production compared to MSCs from normal rats, and exogenous T decreased VEGF production by female MSCs, thereby suggesting that T may have an inhibitory effect [103]. The full effect of T on MSCs including its mechanisms of action remains to be elucidated.

## Embryonic Stem Cells

ESCs are undifferentiated, totipotent cells obtained from the inner cell mass of blastocysts that exhibit an extensive capacity for differentiation. This potential has led to the investigation of their use in the regenerative therapy for a wide range of pathologies. Early animal studies in which undifferentiated murine ESCs were transplanted into ischemic myocardium resulted in cardiac recovery following ischemia primarily via the paracrine release of growth factors [9, 104]. However, undifferentiated ESCs also possess potential for disorganized growth and induction of intramyocardial immune reactions [105,106], and the former may be due to a lesser capability of the native myocardial environment to drive cardiogenic transformation of ESCs compared to the embryo itself [107]. One strategy for overcoming this limitation involves preprogramming ESCs to cardiac-specific differentiation through treatment with TNF-α [108]. Transplantation of these preprogrammed cells resulted in no teratoma formation at any cell load in contrast to the observed 70% rate of teratoma formation following delivery of equivalent ESC loads. In addition, these preprogrammed stem cells also promoted recovery of cardiac function following LAD ligation. Alternatively, more differentiated, ESC-derived cardiomyocytes obtained from postnatal tissue exhibit similar functional and molecular characteristics as mature cardiomyocytes but lack the adverse growth characteristics of undifferentiated ESCs [109,110]. Similarly, Nelson et al. [111] recently demonstrated that fibroblasts can be reprogrammed with the human stemness factors OCT3/4, SOX2, KLF4, and c-MYC and exhibit ESC characteristics. Importantly, these induced pleuripotent stem cells

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demonstrated the ability to engraft into the native myocardium in an organized fashion and to restore post-ischemic myocardial function.

ESCs express ERα and ERβ during early embryonic development [112]. E2 has been shown to stimulate ESC expression of ERα and ERβ expression; increase mRNA expression of the proto-oncogenes c-fos, c-jun, and c-myc; and increase proliferation in part via ERK [113]. Little is known regarding the effect of E2 on the paracrine properties of ESCs, however.

ESCs also express androgen receptors [114], and treatment with nilutamide, a nonsteroidal antiandrogen, stimulates their growth and Akt expression. However, ESC proliferation is unaffected by androgen treatment. Treatment of murine ESCs with T in vitro resulted in the development of a cardiomyocyte-like phenotype with spontaneous contractility and expression of cardiac markers [115]. This effect was inhibited with the addition of flutamide, providing further evidence for the role of the AR-mediated signaling in this cell population. Interestingly, these ESCs were also found to produce T at levels similar to unstimulated Leydig cells, suggesting a possible autocrine role of T.

The recent development of proteomics is enabling investigators to conduct large-scale analyses of paracrine factors involved in cell differentiation as well as characterization of intracellular secretory processes (secretome) [116]. In addition, transcriptome investigation including systems expression profiling with bioinformatic network analysis is facilitating the investigation of spatiotemporal expression patterns of surface biomarkers and cardiogenic genes which may allow for the selection of specific progenitor and stem cell subpopulations that possess the greatest potential for organized cardiac differentiation [117]. These techniques will undoubtedly play important roles in the investigation of the role of sex hormones in stem cell differentiation and function as well as the development of strategies for optimizing the therapeutic efficacy of these cells.

## Conclusion

Source gender may be a significant determinant of progenitor and stem cell function via direct actions of sex steroids on these cells. Understanding these mechanisms may enable us to further understand the apparent discrepancy in cardiovascular disease outcomes between men and women. In addition, by utilizing the beneficial effects of E2 on stem and progenitor cell function, these cells may be optimized during ex vivo expansion prior to their therapeutic use in order to improve post-transplantation function and survival.

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## Abbreviations

| | |
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| AR | Androgen receptor |
| BMP-2 | Bone morphogenetic protein-2 |
| CAD | Coronary artery disease |
| E2 | 17β-estradiol |
| eNOS | Endothelial nitric oxide synthase |
| ER | Estrogen receptor |
| EPC | Endothelial progenitor cell |

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|---|---|
| ESC | Embryonic stem cell |
| FGF | Fibroblast growth factor |
| HIF | Hypoxia-inducible factor |
| IGF-1 | Insulin-like growth factor-1 |
| IL | Interleukin |
| I/R | Ischemia/reperfusion |
| MI | Myocardial infarction |
| MSC | Mesenchymal stem cell |
| OVX | Ovariectomized |
| SDF-1 | Stromal cell-derived factor-1 |
| SOCS/3 | Suppressor of cytokine signaling |
| STAT3 | Signal transducer and activator of transcription |
| T | Testosterone |
| TNF | Tumor necrosis factor |
| TNFR | Tumor necrosis factor receptor |
| VEGF | Vascular endothelial growth factor |
| VEGR | Vascular endothelial growth factor receptor |

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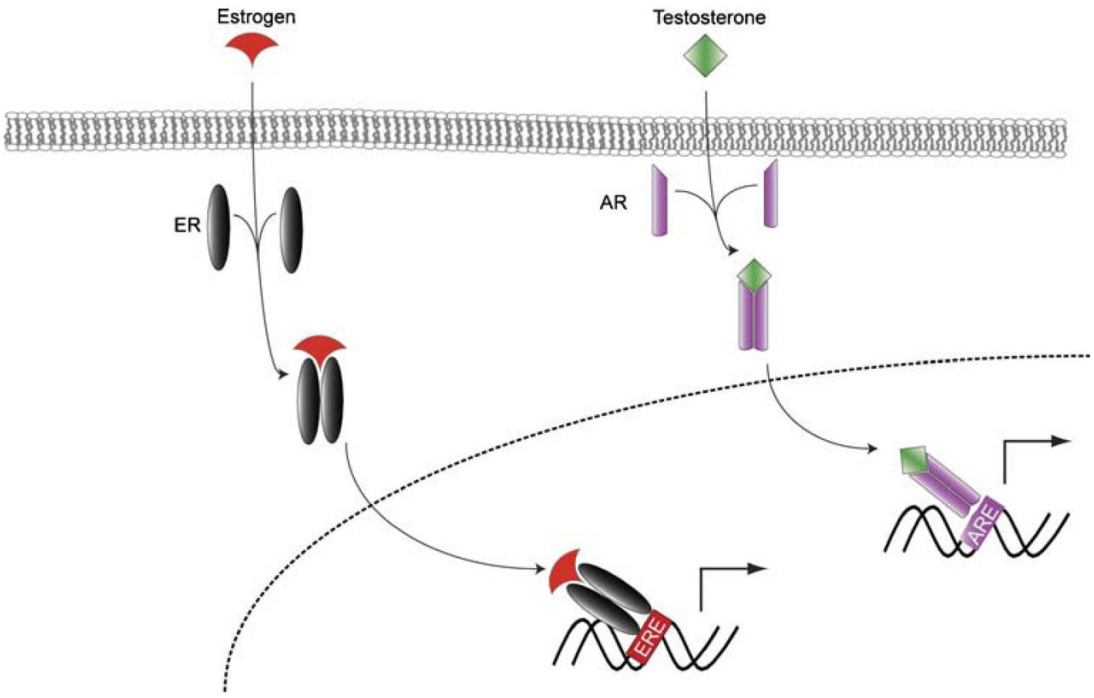


**Fig. 1.**

After entering the cytoplasmic space, estrogen and androgen bind to and induce dimerization of their respective receptors. The dimers then translocate to the nucleus where they engage either estrogen response elements (*ERE*) or androgen response elements (*ARE*) to regulate gene transcription. *ER* estrogen receptor, *AR* androgen receptor

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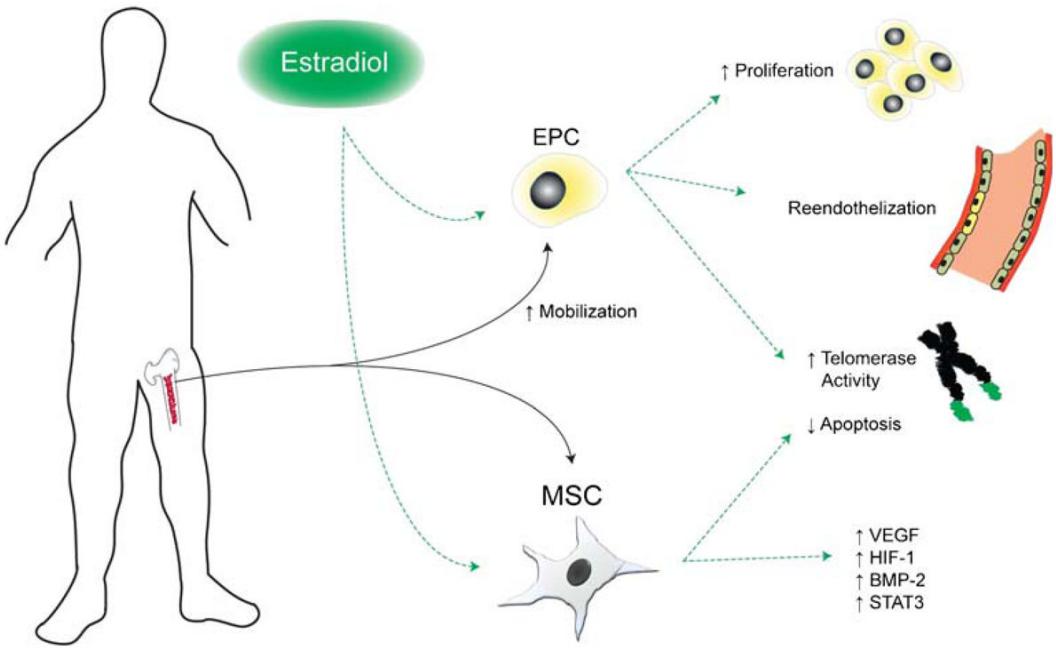


**Fig. 2.**

Effects of estradiol on endothelial progenitor cell and mesenchymal stem cell function. *EPC* endothelial progenitor cell, *MSC* mesenchymal stem cell, *VEGF* vascular endothelial growth factor, *HIF-1* hypoxia-inducible factor-1, *BMP-2* bone morphogenetic protein-2

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**Table 1**

Protective effects of estrogen (17β-estradiol) on the vasculature

| Site of action | Effect | Reference |
|---|---|---|
| Vascular smooth muscle | ↓ Vasoreactivity | [118] |
| | ↓ LDL oxidation | |
| | ↓ Proliferation | |
| Tunica intima | ↓ Atherosclerosis | [119,120] |
| | ↑ NO synthesis | |
| Vascular endothelium | ↓ Macrophage and platelet adhesion | [121–123] |
| | ↓ Reactive oxygen species | |
| | ↑ Post-injury re-endothelialization | |
| Pulmonary vasculature | ↓ Hypoxia-induced vasoreactivity | [124] |