HHS Public Access

Author manuscript

Clin Sci (Lond). Author manuscript; available in PMC 2022 June 20.

Published in final edited form as:

Clin Sci (Lond). 2020 December 23; 134(24): 3237–3257. doi:10.1042/CS20201023.

Hypertension and reproductive dysfunction: a possible role of inflammation and inflammation-associated lymphangiogenesis in gonads

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Abstract

Hypertension is one of the most prevalent diseases that leads to end organ damage especially affecting the heart, kidney, brain, and eyes. Numerous studies have evaluated the association between hypertension and impaired sexual health, in both men and women. The detrimental effects of hypertension in men includes erectile dysfunction, decrease in semen volume, sperm count and motility, and abnormal sperm morphology. Similarly, hypertensive females exhibit decreased vaginal lubrication, reduced orgasm, and several complications in pregnancy leading to fetal and maternal morbidity and mortality. The adverse effect of hypertension on male and female fertility is attributed to hormonal imbalance and changes in the gonadal vasculature. However, mechanistic studies investigating the impact of hypertension on gonads in more detail on a molecular basis remain scarce. Hence, the aim of the current review is to address and summarize the effects of hypertension on reproductive health, and highlight the importance of research on the effects of hypertension on gonadal inflammation and lymphatics.

Introduction

Reproductive health is vital for human flourishing and survival of the species, and includes the sexual wellness of both men and women along with maternal and infant health. A male's ability to produce sperm in the testes coupled with a female's release of matured eggs from the ovaries are necessary for successful reproduction. Human reproduction is affected by lifestyle, genetics, and environmental factors. It is also affected by physiological abnormalities like obesity, dyslipidemia, insulin resistance, and hypertension [1,2]. Hypertension, present in almost half of the population, is a well-known factor increasing cardiovascular disease risk by altering the macro- and micro-vasculature in

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Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

target organs like the brain, heart, and kidney. However, hypertension also vastly affects reproductive health in both men and women [3–7]. While the roles that the primary male gonadal hormone testosterone (T) and the primary female gonadal hormone estrogen play in cardiovascular disease have been studied extensively, the mechanisms by which hypertension affects gonads are less known. In this review, we discuss how hypertension impacts fertility with a focus on the effects on male and female gonadal function. How hypertension influences gonadal inflammation and what roles lymphatic vessels might play in these tissues are highlighted to bring attention to novel findings that impact fertility.

Hypertension and reproductive health

The major public health epidemic of hypertension can result in target-organ injury and is associated with inflammation and sexual dysfunction in both men and women [3–7]. For example, the prevalence of erectile dysfunction (ED) is higher in hypertensive men than normotensive men [8,9]. Similarly, hypertensive women exhibit decreased vaginal lubrication, less frequent orgasm, and more frequent genital pain than normotensive women [4,10]. It is known that hypertension affects the reproductive ability of both men and women (Figure 1), but the mechanisms by which hypertension impacts reproductive health and function remain to be fully unraveled.

Hypertension and male fertility

Hypertension and spermatogenesis—There are several studies on hypertensive men that established an inverse relationship between blood pressure and total serum T [11,12], free T [13–15], and the sex hormone-binding globulin[15]. Nevertheless, only a few studies are available to date that relate hypertension and male reproductive function. A study involving 110 newly diagnosed, never treated hypertensive men and 110 normotensive men attributed a reduction in sexual activity reported in hypertensive men to a decrease in T levels [12]. Using a rat model exhibiting renovascular hypertension, diminished sexual behavior and impaired spermatogenesis were attributed to attenuated levels of prolactin, T, and follicle stimulating hormone [16]. Another group evaluated the quality of ejaculated spermatozoa in a group of 25 normotensive and 25 hypertensive subjects by measuring (i) the levels of clusterin, a glycoprotein associated with abnormal sperm morphology, and (ii) sperm DNA damage. There was a significant increase in clusterin levels and sperm DNA damage in hypertensive subjects that established a strong relationship between hypertension and poor sperm quality [17]. Guo et al. [18] explored the association between hypertension and semen quality utilizing collected data from the Stanford Reproductive Endocrinology and Infertility Center. The study cohort included male patients who visited the infertility center between 1994 and 2011 who had been diagnosed with hypertension prior to or within one year after semen analysis. Abnormal semen parameters were characterized based on the World Health Organization Manual on Semen Analyses [19]. Hypertensive males were found to have decreased semen volume, sperm motility, total sperm count, and motile sperm count with respect to normotensive males [18,20]. Eisenberg et al. [21] also revealed higher rates of semen abnormalities, including reduced sperm count and motility, in hypertensive men. These studies reinforce a strong association between hypertension and impaired semen and

sperm quality. As correlations, however, these studies lack thorough investigation and a direct end-organ effect of hypertension on the testes.

Hypertension and erectile dysfunction—There are several studies that link hypertension with reproductive dysfunction based primarily on hormonal imbalance and/or ED. It is well established that hypertensive men have an increased risk to develop ED [22–24]. ED-associated erectile tissue morphological changes including smooth muscle tissue hypertrophy and stenosis in the corpus cavernosum correlate positively with hypertension [25–27]. One of the plausible mechanisms behind the morphological changes in erectile tissue may be the oxidative stress caused by chronic hypertension, leading to endothelial dysfunction resulting in inefficient dilation of the arteries of the corpus cavernosum [28]. Spontaneously hypertensive rats that are stroke prone (SHRSP) demonstrated a significant reduction in erectile response to ganglionic stimulation. However, this improved after a single intracavernous injection of drug Y-27632 [29]. This drug binds to the ATP-binding site on Rho-kinase, preventing the phosphorylation and subsequent inactivation of myosin light chain (MLC) phosphatase, thereby promoting cavernosal smooth muscle relaxation leading to erection [29–34]. This demonstrates the RhoA/Rho-kinase pathway as a critical regulator controlling the erectile process by regulating the phosphorylation of MLC phosphatase. A similar trend was observed using the mineralocorticoid-salt model of hypertension, where treatment with Y-27632 improved the erectile response [29]. Nitric oxide (NO) induces vasodilation in cavernosal tissues leading to erection by inhibiting RhoA activity [32–34]. Sildenafil, a commonly prescribed ED drug, acts by promoting NO-mediated activation of guanylyl cyclase and eventual accumulation of cGMP leading to relaxation of the corpus cavernosum and subsequent erection [35,36]. This drug slows down the process of apoptosis in corpora cavernosa and ameliorate spermatogenesis, eventually improving the microcirculation in men with ED [36]. Few studies have reported that antihypertensive drugs (e.g. β-blockers and diuretics) themselves are associated with ED [37], while others had controversial reports [38,39]. Despite these sometimes-conflicting reports, evidence suggests a relationship between hypertension and male fertility that warrants further mechanistic investigations.

Hypertension and testes—Despite strong evidence for the association between hypertension, sperm quality, and male fertility [2,9,12,16–18,20,21], the target organ testes have not been studied extensively. Rats made hypertensive using a deoxycorticosterone acetate treatment protocol exhibited reduced testis weight in addition to a reduction in sexual behavior, seminal vesicle weight, and T levels when compared with the uninephrectomized controls [40,41]. SHRs demonstrated testicular hypertrophy and ED [42]. A study in the SHRSP model reported the hypertensive changes in intra-testicular arterioles resulted in the impairment of Sertoli cell (SC) function with subsequent loss of spermatogenic cells [43]. Another study using the same model confirmed the marked alterations in intra-testicular arteries including increased intimal thickness, fibrinoid necrosis, and hyalinization [44]. These alterations in testicular arterial vasculature induce ischemia and a significant reduction in the nutritional supply to the organ [45–47]. This study also identified an impairment in spermatogenesis likely due to atrophic seminiferous tubules with reduced numbers of spermatids in hypertensive rats [44]. Due to the presence of the blood–testis barrier

(BTB), germ cells (GCs) depend on SCs for nutrients [48]. Hence, a reduced blood supply to SCs in testes resulted in impaired spermatogenesis. Akagashi et al. [45] measured a significant reduction in the concentration of transferrin in SHRSP rats that coincides with impaired spermatogenesis. Treatment with manidipine (a long-lasting calcium channel blocker, CCB) to improve blood flow led to improvement in spermatogenesis. Atanassova et al. [49], in support of the previous studies, demonstrated deleterious testicular changes with GC depletion in seminiferous tubules of SHRs. Elevations in the local expression of testicular angiotensin-converting enzyme (ACE) in SHRs occurred earlier in the stage of spermiogenesis and correlated with decreased fertility when compared with age-matched normotensive rats. Increased ACE activity is known to be involved in vascular remodeling. Therefore, the compromise in fertility observed in SHR may be attributed in part to the dysfunctional intra-testicular vasculature [49].

In a study with SHRs, enalapril (an ACE inhibitor) treatment restored the morphological changes in the testes and normalized spermatozoid production by preventing vascular remodeling in the testes [50]. A recent study in hypertensive rats identified increased testicular weight and altered testicular morphology resulting from arterial alterations (Figure 2) and impaired testicular vasomotion [51]. These rats had a reduction in sperm concentration and DNA integrity, and increased percentages of sperm with dysfunctional mitochondria, intracellular superoxide anion activity, and abnormal morphology. Histological studies showed perturbed spermatogenesis with immature GCs in the tubular lumen and tubular necrosis. The hypertensive rats also displayed increased arteriolar adventitia in the testicular microvasculature. The authors also reported elevated hypoxia-inducible factor-1α gene expression in the testes, suggesting an inadequate oxygen supply to the tissue in hypertensive animals. Increased levels of vascular endothelial growth factor (VEGF) were also observed in the testes of hypertensive animals due to testicular hypoxia-induced protein expression [51]. In total, these studies help us to have a better understanding of the mechanisms implicated under hypertensive conditions with respect to male fertility.

Hypertension and female fertility

Hypertension and sexual health—The prevalence of hypertension in women of reproductive age is relatively less compared with age-matched males [52,53], but should not be ignored in women as it is one of the most prevalent risk factors for cardiovascular disease and can also greatly complicate pregnancy and offspring health. Women with hypertension may show sexual dysfunction due to alterations in clitoral and vaginal vasculature, reduced blood flow in the pelvic region, and thinning of the vaginal wall and clitoral smooth muscles resulting in vaginal dryness [54,55]. Subsequent complications include pain during sexual intercourse, lack of orgasm, adverse effects of the arousal phase, and sexual reluctance in the woman have also been reported [54]. Hypertension can lead to fibrosis of the clitoris and the vaginal wall due to lower NO levels and reduced blood flow in the pelvic region [56,57]. A reduction in blood flow with inefficient vasocongestion during sexual arousal results in decreased lubrication and finally dyspareunia in hypertensive women [58,59].

Hypertension and pregnancy—It is reported that out of the estimated 4 million pregnancies in the United States each year, around 5% of women face complications due to hypertension [60], leading to maternal and fetal mortality [60–62]. Women with chronic hypertension are highly susceptible to preeclampsia [63–65]. In hypertensive women, the chances of placental abruption [63–65], maternal stroke [66–68], renal failure, pulmonary edema, and death [67,68] are tremendously increased. The adverse effects of chronic hypertension on fetal growth can lead to perinatal and maternal morbidity and mortality through premature delivery; intrauterine growth restriction [64,65]; hemolysis, elevated liver enzymes, low platelet count (HELLP) syndrome; perinatal death; and maternal convulsion or eclampsia [64,67,69–71]. A retrospective study conducted using medical records at Mettu Karl Referral Hospital, Mettu, Ethopia, for the period January 1, 2010 to December 1, 2013 reported increased rates of fetal death, low birth weight, low APGAR score, abortion, preterm delivery, and HELLP syndrome in pregnant women with hypertensive disorders [72]. Khosravi et al. [73] reported a higher prevalence of hypertensive disorders among pregnant women who were admitted to a tertiary center in Tehran for delivery. Zhou et al. [74] reported higher risk of pregnancy loss in hypertensive women among 2940 women attempting pregnancy in Anhui, China. Several clinical studies have associated both preconception and early pregnancy blood pressures with risk of pregnancy loss [71,72,74,75]. While clearly linked, the actual underlying mechanisms of sexual dysfunction and pregnancy loss in hypertensive women remains obscure.

Hypertension and ovaries—Possibly due to the lower prevalence of hypertension in young reproductive women than their male counterparts, studies on the effects of hypertension on the ovarian vasculature and folliculogenesis have been neglected. To date, there are only a few studies that have specifically focused on the mechanisms by which hypertension affects female fertility. Studies on female SHRs demonstrated an association between hypertension and sexual dysfunction with reduced ovulation and morphological changes in the clitoris [76]. In female rats with renovascular hypertension, there was a delay in re-establishing estrous cyclicity. Resumed hypertensive rats demonstrated a significant decrease in lordosis quotient (a measure of sexual posturing) and oocyte number, demonstrating a reduction in sexual behavior and ovulation, respectively [77]. Although many studies have identified a higher prevalence of sexual dysfunction in hypertensive women when compared to normotensive women [78–80], further studies are needed to identify the exact pathophysiological mechanisms underlying the adverse effects on reproduction. With the profound effects that hypertension has on the male testis vasculature, it seems likely that the ovaries may demonstrate similar changes, but this area of research remains unexplored.

Antihypertensive drugs and reproduction

The antihypertensive drugs are mainly classified into five categories namely β-blockers, CCBs, ACE inhibitors, angiotensin II receptor blockers, and diuretics [18]. The effect of antihypertensive drugs on sexual function has always remained a topic of debate. CCBs have been reported to attenuate hormone levels viz., T, luteinizing hormone, and follicular stimulating hormone, leading to impaired spermatogenesis and sperm parameters [81,82].

Many *in vitro* studies have demonstrated the negative impact of CCBs on reproductive function by hampering spermatozoa–oocyte interaction and the fertilizing capability of sperm [83–86]. These effects are obvious since it is well known that Ca^{2+} is crucial for spermatogenesis, sperm motility, capacitation, acrosome reaction, and fertilization [83–92]. Clonidine, a selective agonist of α_2-adrenoceptors, induced desensitization of functional α2-adrenoceptors and elicited contractions of the rat testicular capsule affecting the proper transport of spermatozoa out of the testes [93]. It was reported that rats treated with lisinopril, an ACE inhibitor, exhibit decreased sperm density and motility along with a decrease in sperm acrosomal reaction [94]. β-Blockers hamper the relaxation of smooth muscles in the corpora cavernosum by blocking the β2 adrenergic receptors and cause a reduction in plasma T levels [38,95–100]. In contrast with these findings, a few studies elucidated beneficial aspects of these drugs on sperm parameters and sexual activity [50,101,102].

ACE inhibitors or angiotensin II receptor blockers are teratogenic and have been shown to increase the likelihood of congenital anomalies; hence they are generally not recommended to women who are planning to get pregnant [103–106]. Methyldopa has been considered safe even in the early trimester, with no potential harm to the growth and development of the fetus [107,108]. β-Blockers have been implicated in intrauterine growth restriction and premature birth [109]. CCBs such as nifedipine have been reported to reduce blood pressure in pregnant women without compromising fetal health and prevent premature labor [110]. Further extensive research is required in this field to choose the appropriate treatment and improve the impact of hypertension treatment on the patient's reproductive health. Since it is difficult to delineate the effect of hypertension and antihypertensive drugs on reproductive function, a better understanding of the direct effects of hypertension on reproductive organs can pave the way for alternative treatments.

It is well known that hypertension promotes inflammation, immune cell trafficking, and inflammation-associated lymphangiogenesis in several organs like the skin, intestine, heart, kidney, and airway tract [111–113], but the impact of hypertension on gonadal lymphatics remains obscure. In the following sections, we will focus on the development of gonadal lymphatic vasculature and inflammation-associated lymphangiogenesis in gonads under several pathological conditions to emphasize the need for extensive research in this area.

Gonads and lymphatics

The lymphatic system plays a crucial role in the maintenance of tissue fluid homeostasis by recirculating the interstitial fluid, formed by blood vessel extravasation, and in the transportation of antigen-presenting cells and lymphocytes from tissues to lymph nodes [114–116]. These roles are particularly important during inflammation, when blood vascular permeability is enhanced and immune cell numbers and activation increase in the tissue.

The lymphatic network in testes originates 9.5 days post coitum in the mouse and its distribution varies across mammalian species [117–119]. The lymphatic network arises on the surface of the testes spreading across the tunica albuginea in both large animals and rodents [118–121]. Using immunolabeling with lymphatic vessel endothelial hyaluronan

receptor 1 (LYVE-1) antibodies or Prox-1-EGFP transgenic lymphatic reporter mice to identify lymphatics, it is reported that testicular lymphatics are found only on the surface and do not penetrate into the interstitium (Figures 3 and 4) [118,119,122]. Similarly, lymphatic vessels were also absent in the interstitium in rat testes [123]. In contrast, lymphatic capillaries were detectable within the testicular interstitium of large animals; these vessels drain into the tunica albuginea via fibrous septa and finally into larger collecting lymphatic vessels in the mediastinum testis [117,120,121,124]. In large animals, these large lymphatics carrying testicular lymph drain into the latero- or para-aortic lymph node groups (Figure 5) [122,125,126]. In rats, collecting lymphatics from three regions (superior, middle, inferior) combine to form a large testicular lymphatic trunk [127]. The testicular lymphatics in rats may drain to either the renal or lumbar lymph nodes, but in certain cases bypass lymph nodes and lead directly to the thoracic duct [128]. Interestingly, there are distinct routes of lymphatic drainage of testes from those of the scrotum (that drains to the inguinal lymph node) (Figure 5) [129]. It was concluded in studies using rams that testicular lymphatics do not play an important role in the return of T into the blood due to both the lower concentration of the hormone in lymph and lower flow rate of lymph when compared to venous blood [130]. Nevertheless, studies on pigs and horses have demonstrated a comparatively higher concentration of conjugated steroids like estrone sulfate and dehydroepiandrosterone sulfate in testicular lymph than in the testicular venous blood accounting for the contribution in returning these to the circulation [131,132]. Hence, the role of testicular lymphatics in hormone transport and distribution may be species- and molecule-dependent [122].

Ovaries secrete hormones and produce ova for the maintenance of female fertility. Unlike testes, ovaries possess a dense network of lymphatics (Figure 6) and the distribution of lymphatic networks are consistent among many mammalian species where the lymphatic vessels reside in the thecal layer surrounding the developing follicles and the periphery of the corpus luteum [119,121,133–136]. The density and size of the lymphatic network depends on the different stages of the estrous cycle in pigs and sheep [121]. The formation and degeneration of the lymphatic network corresponds to the follicular development and regression of the corpus luteum, respectively [137–139]. The first appearance of ovarian lymphatics in mice occurs after birth around post-natal day 10 and starts expanding along the length of the uterine horn [119,136]. These lymphatics undergo remodeling corresponding to folliculogenesis, attributed to the increased expression of VEGF-C, VEGF-D, and vascular endothelial growth factor receptor-3 (VEGFR-3) genes in adult mice [136]. The ovarian lymphatic network combines at the hilum into a dense plexus and eventually into 4–6 larger lymphatic vessels anastomosing with other lymphatics from the uterus and Fallopian tube and finally drains into the lumbo-aortic nodes [125,140–142]. A recent study suggested three lymphatic drainage paths occurring in the human ovary (Figure 7) [143]. The first major route arises from the cranial side of the ovary, accompanying the ovarian artery in the infundibulopelvic ligament towards the para-aortic and paracaval nodes [143]. The second arises from the caudal side of the ovary that travels along the ovarian artery that anastomoses to the uterine artery in the ovarian ligament, ultimately draining to internal iliac lymph nodes [143]. The third route appears to be minor and involves sparse lymphatic vessels following the round ligament to the inguinal nodes [122,143].

A higher concentration of ovarian steroid hormones is detected in the draining lymph from ovaries compared with peripheral blood, but these concentrations are comparatively lower than in sampled ovarian venous blood [144,145]. These ovarian hormones are transported back to the ovarian arteries through retrograde transfer, revealing the potential role of lymphatics in the feedback mechanism in hormonal regulation and thereby female reproductive health [146,147].

Testicular inflammation and lymphangiogenesis

The predominant process underlying male infertility is the disrupted or disturbed spermatogenesis. A few studies have correlated impaired spermatogenesis with inflammatory and/or immunological factors by demonstrating immunoglobulin and complement reactivity on the thickened basement membrane of seminiferous tubules in testis biopsy specimens from infertile men [148–152]. The testis is an immunologically privileged organ that protects the auto-immunogenic spermatids from the male's immune system through the BTB between SCs [153]. However, testes are still vulnerable to immune activation leading to inflammatory reactions. Testicular inflammation, or orchitis, can occur due to bacterial, viral, or other pathogenic infections as well as exposure to drugs and heavy metals [154]. Autoimmune orchitis can lead to male infertility [153,155–157]. This condition is characterized by an increased infiltration of immune cells (macrophages, dendritic cells, and subsets of T cells) that results in elevated proinflammatory cytokines levels [158–160], leading to degeneration and apoptosis of GCs. It is known that resident macrophages and mast cells, as well as SCs, can produce an array of cytokines, including both pro- and anti-inflammatory molecules such as interleukin −1β (IL-1β), IL-6, tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), members of the transforming growth factor-β (TGF-β) family, and IL-10 [161–164]. Increased levels of mRNAs of proinflammatory cytokines, such as IL-1β, TNF, and IFN-γ were linked to disturbed spermatogenesis and inflammatory lesions in human testicular biopsies showing GC neoplasia [165]. Stimulation with TNF-α and IL-1α resulted in upregulation of IL-6 in cultured SCs [166]. In an experimental autoimmune orchitis (EAO) model, peritubular and intratubular immune cell infiltration was reported that was attributed to chemotactic gradients established in the testes due to up-regulation of cell adhesion molecules (CD31, CD44, CD106), chemokines [monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory proteins (MIP) 1α and 1β] and chemokine receptors (CCR2, CCR5) [161,167]. There was an increase in the expression of CCR7 in dendritic cells isolated from EAO rat testes [168]. TNF-α has also be shown to upregulate MCP-1, IL-6, and cyclooxygenase-2 in cultured human testicular peritubular cells [169].

Spiess et al. [170] reported an increased mRNA expression of high-affinity IgE receptor and the mast cell-related fractalkine receptor in a cross-sectional microarray analysis study involving testicular biopsies with spermatogenic failure. A similar study reported an increase in transcript levels associated with inflammatory activity in human testicular biopsies [171]. This is in accordance with earlier studies that pointed out increased numbers of mast cells in testicular biopsies from infertile men with impaired spermatogenesis [165,172,173]. Mast cells, in addition to secreting proinflammatory cytokines like TNF and IL-6, also produce serine protease tryptase that enhances the synthesis of collagen and subsequently tubular

fibrosis by exerting its mitogenic effect on fibroblasts and peritubular cells [174–177]. Mast cell tryptase activates proteinase-activated receptor-2 on isolated peritubular cells *in vitro* leading to up-regulation of inflammatory molecules such as MCP-1, cyclooxygenase-2, and TGF-β2 [177]. Translocation of high-mobility group box protein-1 from the nuclei in EAO rat testes has been shown to regulate inflammatory responses. A similar result was found in testes of infertile men with impaired spermatogenesis along with lymphocytic infiltrates [178]. Galectin-1, activins, and inhibin have also been reported to play a crucial role in the development of testicular immunopathology [179–182].

Immune cell infiltration has been associated with disruption of the BTB in seminiferous tubules [183,184]. IL-6 has proved to be an essential factor in the development of testicular inflammatory responses, which is found to disrupt the integrity of the BTB in rats, thereby attacking the immunological barrier in testes [185]. IL-6 inhibits protein degradation and activates phosphorylated ERK in SCs [186]. IL-6 also interferes with GC differentiation or degeneration by acting through the transcription factor Zfp637 on spermatogonia. TNF/TNFR1, Fas/FasL, and Bax/Bcl-2 systems have all been implicated along with IL-6 and its receptor in GC apoptosis in the rat EAO model [164,168,187]. Oh et al. [188] reported attenuated mRNA expression of SC tight junction proteins such as Claudin-11 (Cldn11), Occludin, and Zona occludens-1 in varicocele testes when compared with normal testes. Immunolocalization showed that Cldn11 was found in the cytoplasm rather than in the periphery of the seminiferous tubule suggesting impaired subcellular localization. Increased mRNA levels of proinflammatory cytokines (*Tnfa, Il1a*, and *Il6*), leukocyte marker (*Cd45*), and T-cell markers (*Cd3g* and *Cd3d*) were observed in varicocele testes, indicating immune cell infiltration. This might have deregulated *Cldn11* expression in SCs in varicocele testes, thereby attenuating the permeability of the BTB and finally resulting in impaired spermatogenesis [188]. Another study using rats with EAO reported an increase in IL-6 along with a decrease in Occludin, and delocalization of Cldn 11 and Zona occludens-1 leading to a disrupted BTB and membrane permeability [185]. Hence, it is very clear from the available literature that impaired spermatogenesis is associated with testicular inflammation and immune cell infiltration (Table 1).

Expansion of the local lymphatic vasculature, lymphangiogenesis, is common under inflammatory conditions and generally considered to be a beneficial process in restoring tissue homeostasis [111–113,189–191]. Several tissue cells and infiltrating immune cells secrete the predominant lymphangiogenic proteins VEGF-C and VEGF-D during inflammation. It was also reported that TNF-α, VEGF-A, VEGF-C, and VEGF-D are produced by macrophages and induced angiogenesis and lymphangiogenesis [192–198]. Few studies have directly examined lymphangiogenesis in orchitis. It is reported that seminiferous tubules are immersed in lymph and the tissue fluid is drained through lymphatic capillaries extended beneath the tunica albuginea but not within the testicular interstitium [118]. Naito et al. [199] demonstrated an expanded interstitium area in the EAO model that was restored under post-inflammatory condition. The increased lymphatics observed around the inflammatory lesions point out the importance of interstitial fluid and infiltrated immune cell clearance. Hirai and colleagues [200] identified a significant increase in VEGF-D in the testes of EAO mice. Quantifying inflammation-associated lymphangiogenesis is complicated in some tissues because LYVE-1, a commonly used

marker to identify lymphatic endothelial cells, is expressed on a subset of macrophages under inflammatory conditions [196,201,202]. LYVE-1+ cells were identified in the interstitium proper of inflamed testes; however, all of these cells were F4/80+ and CD31- indicating that these LYVE-1+ cells were mature macrophages and not true lymphatic endothelial cells. An incorporation of LYVE-1+ macrophages into the wall of lymphatic capillaries under the tunica albuginea was also identified in this model. This indicates that F4/80+ macrophages expressing LYVE-1 may play a role in promoting testicular lymphangiogenesis during inflammation [200]. Whether gonadal lymphangiogenesis occurs in hypertension has not yet been described.

Ovarian inflammation, endomteriosis, and lymphangiogenesis

Autoimmune ovarian disease (AOD), a chronic inflammatory disease, is linked to lymphocytic infiltration in the ovarian follicle in women with premature ovarian failure [158]. Researchers have established a rodent model of AOD by immunization of animals with zona pellucida (ZP) antigens [203–206]. ZP is an extracellular glycoprotein found around the oocytes and aids follicular development, spermatozoa–oocyte interaction, and fertilization [206]. Immunization with ZP led to the release of anti-ZP autoantibodies and activation of autoreactive T cells eventually resulting in autoimmune oophoritis, a condition identified by inflammation in the ovarian interstitium and organized monocytic granulomata [203–207]. Adoptive transfer of ZP peptide-specific T cells into näıve mice caused granulomatous oophoritis and up-regulation of proinflammatory markers including IL-1, TNF-α, and IFN-γ, but did not alter ovarian function or fertility [207]. Ovarian granulosa cells had ectopic expression of major histocompatibility complex (MHC) II in the site of inflammation, induced by IFNγ [208]. Similar results were observed with cynomolgus macaques where pZP3 (zona pellucida 3 peptide) immunization showed co-localization of T cell clusters with MHC-II+ macrophages in the ovarian interstitium [205]. Increased level of chemokines such as MIP-1α, IL8, eotaxin-1, and interferon inducible protein-10, as well as the pro-lymphangiogenic factor VEGF-D, was reported to be associated with an early stage of premature ovarian insufficiency [209]. Beyond the ovary, an increase in the production of chemokines and macrophage recruitment have been identified in the uterine tissues of women with endometriosis [210–212]. Increased production of intercellular adhesion molecule-1, insulin-like growth factor-I, IL-1, IL-6, IL-8, IL-12, MCP-1 (CCL2), MIP-1α (CCL3), RANTES (CCL5), eotaxin (CCL11), VEGF, and TNFα were associated with endometriosis [211,213]. It is to be noted that inflammation in the ovaries and uterus are associated with reproductive dysfunction in females (Table 2).

Lymphangiogenesis occurs in the ovaries and uterus under normal physiological conditions with respect to reproductive cycles and pregnancy [139,214]. While studied somewhat extensively in ovarian cancer due to lymphatics serving as a route of metastases, lymphangiogenesis in the ovary during inflammation has been understudied. Studies have reported that proinflammatory cytokines like IL-6 and TNF-α might be involved in pathologic lymphangiogenesis in ovarian cancer-related inflammation [215,216]. Reichelt et al. [217] demonstrated lymphangiogenesis in peritoneal endometriosis with up-regulation of VEGF-C and VEGF-D expression. The pathological lymphangio-genesis observed may be ascribed to the chronic inflammatory responses during endometriosis induced by VEGF-

C and VEGF-D expressing macrophages [217]. Recently, it was reported that VEGFR1 signaling is responsible for the induction of lymphangiogenesis in endometrial tissues and contributes to the pathogenesis of endometriosis in a mouse model [218]. Whether hypertension causes inflammation-associated lymphangiogenesis in female reproductive organs and whether this is beneficial or detrimental remains an area to be explored.

Future perspectives and conclusion

To conclude, studies conducted so far demonstrate that hypertension has a deleterious effect on male and female fertility. It is also now appreciated that hypertension induces inflammation, immune cell trafficking, and inflammation-associated lymphangiogenesis in various organs, but it remains largely unknown whether hypertension induces inflammation and inflammation-associated lymphangiogenesis in the gonads (Figure 8). Moreover, while limited in reports thus far, inflammation-associated lymphangiogenesis occurs in gonads in certain other pathological conditions (Figure 8). Hence, identifying how and to what extent inflammation and lymphangiogenesis occurs as a result of hypertension in reproductive tissues is a question of keen interest to our group. Preliminary results from our lab demonstrate up-regulation of lymphatic vessel markers, pro-lymphangiogenic growth factors, their respective receptors, and proinflammatory cytokines and chemokines in the testes of hypertensive mice (Figure 9). Studies are underway to examine how hypertension may induce inflammation-associated lymphangiogenesis in the gonads of hypertensive male and female mice. Whether enhancing lymphatic density in gonads may help in combating the inflammatory response and its consequences on reproductive function are key questions to consider. Further studies along these lines are required to support our hypothesis that may enlighten the mechanisms behind the deleterious effect of hypertension on fertility.

Acknowledgements

All authors have read the journal’s authorship statement and agree that this article is original and has not been presented in any form previously or is under consideration elsewhere.

Funding

This work was supported by the National Institutes of Health [grant number DK120493 (to B.M.M.)].

Abbreviations

AOD	autoimmune ovarian disease
BTB	blood–testis barrier
CCL	C-C motif chemokine ligand
Cldn11	Claudin-11
EAO	experimental autoimmune orchitis
ED	erectile dysfunction
GC	germ cell

IFN-γ	interferon-γ
IL	interleukin
L-NAME	L-arginine methyl ester hydrochloride
LYVE-1	lymphatic vessel endothelial hyaluronan receptor-1
MCP-1	monocyte chemoattractant protein-1
MHC-II	major histocompatibility complex-II
MIP-1α	macrophage inflammatory protein-1α
MLC	myosin light chain
NO	nitric oxide
SC	Sertoli cell
SHRSP	spontaneously hypertensive rats that are stroke prone
SHR	spontaneously hypertensive rat
T	testosterone
TNF-α	tumor necrosis factor-α
VEGF	vascular endothelial growth factor
VEGFR	vascular endothelial growth factor receptor
ZP	Zona pellucida

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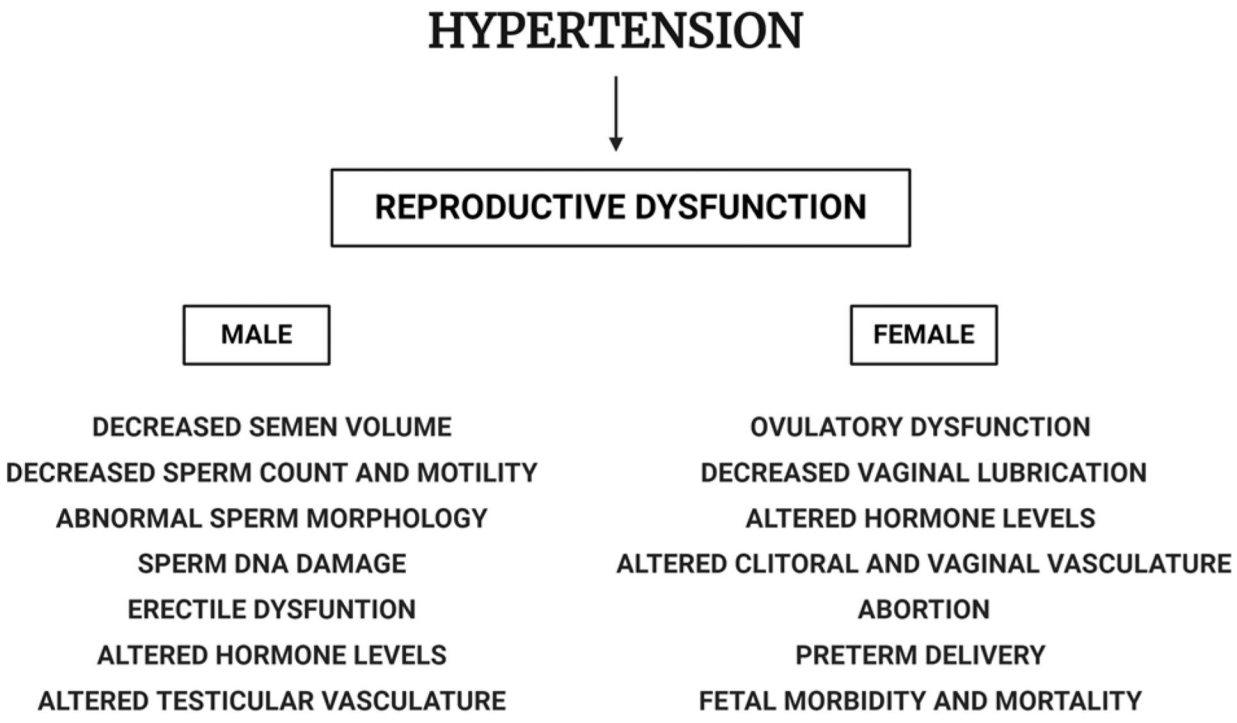

Figure 1. Effect of hypertension on gonadal function

Hypertension affects reproductive health by altering hormone levels and reproductive tissue vasculature in both males and females, thereby disturbing spermatogenesis and oogenesis, respectively. Created with BioRender.com.

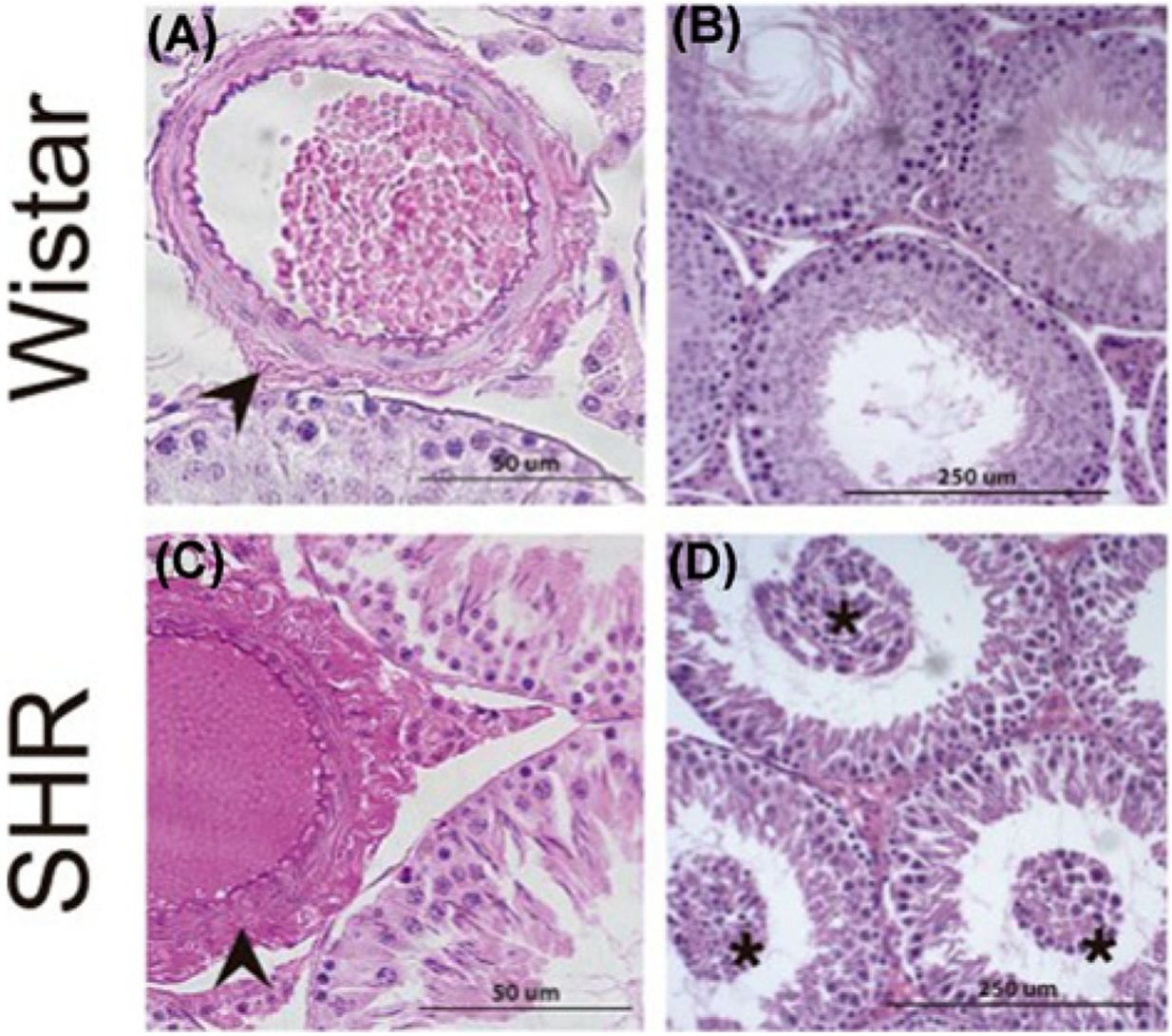

Figure 2. Hematoxylin and eosin staining of testis from normal and spontaneously hypertensive rats

(**A**) Normal histoarchitecture of testes from 26-week-old Wistar rats. Arrowheads indicate arteriolar adventitia. (**B**) Testes from 26-week-old Wistar rats showing intact seminiferous tubules and interstitial compartment. (**C**) A significant increase in adventitial layers in testicular arterioles (arrowheads) independent of their diameter in spontaneously hypertensive rats (SHR). (**D**) Testes from SHR showing immature germ cells in the tubular lumen (asterisk). The images are reproduced from the reference [51] with permission.

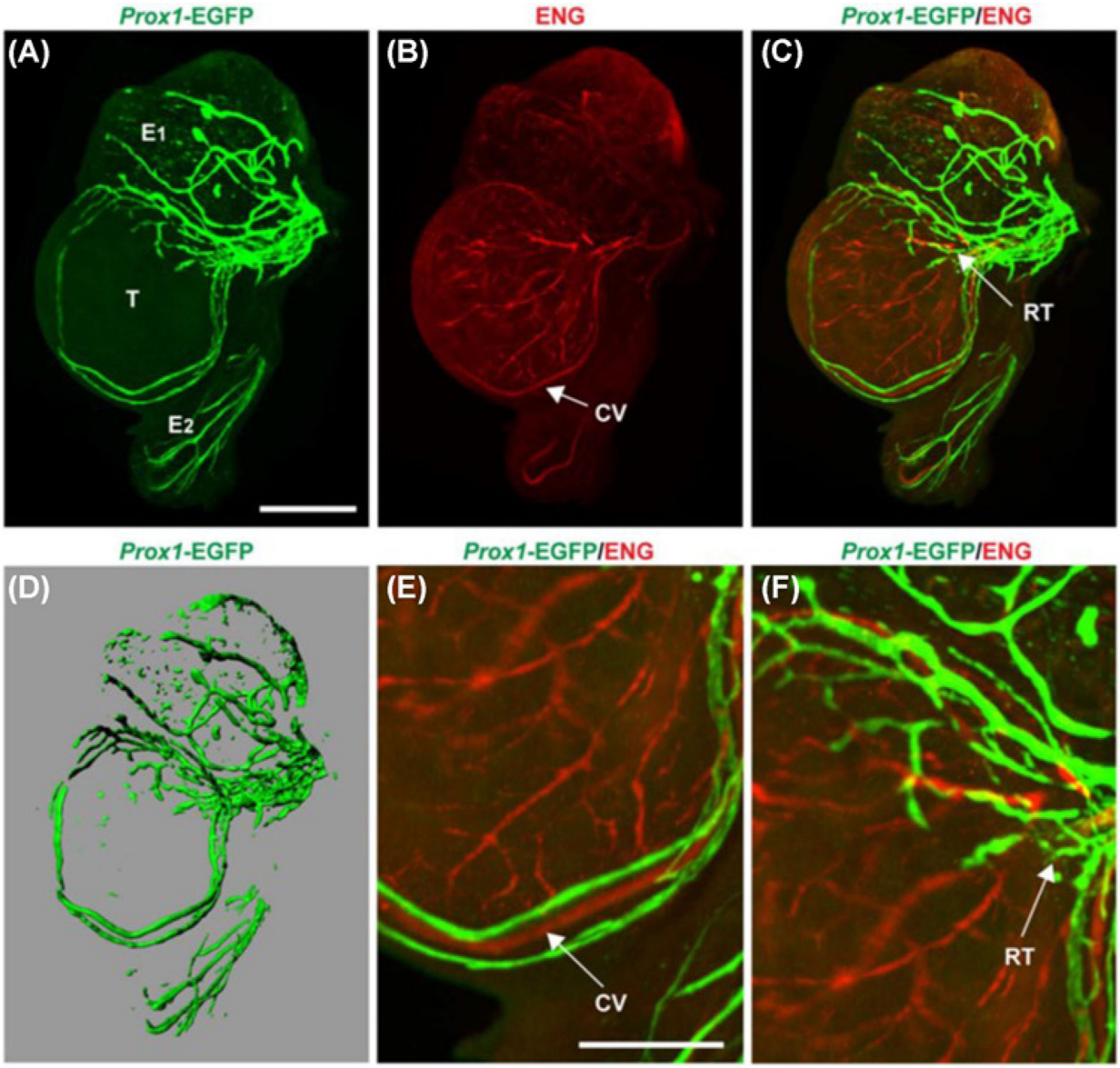

Figure 3. Distribution of lymphatic vasculature in the prenatal testes of Prox1-EGFP reporter mice

(**A**) During embryonic development (E17.5), EGFP-positive lymphatic vessels begin to grow from the spermatic cord across the surface of the testis (T) but does not cross the testis cap. Lymphatics are also established on the epididymal head (E1) and tail (E2). (**B**) Endoglin (ENG) staining was done to view the distribution of blood vessels in fetal testes. (**C**) The lymphatics (Prox 1-EGFP-positive) and blood vessels (ENG labelled) did not colocalize. Regions in yellow represent overlapping of both green and red signals from different planes. (**D**) A 3-D representative image of the lymphatics in fetal testes. (**E**) Magnified image of fetal testes illustrating the lymphatic vessels running alongside the coelomic vessel (CV). (**F**) Magnified view of the rete testes (RT). Scale bar for panels (A–D) = 500 μm; (E and F) = 250 μm. The images are reproduced from the reference [119] with permission.

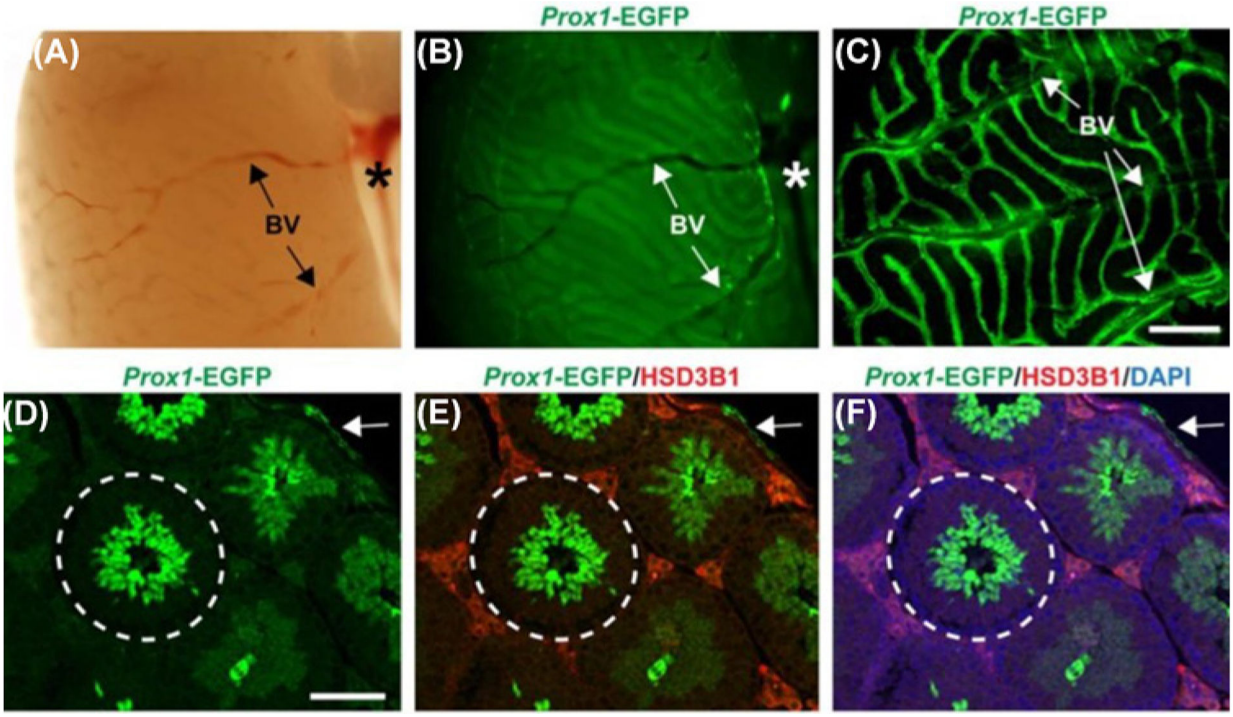

Figure 4. Distribution of the lymphatic vasculature in the adult testes of Prox1-EGFP reporter mice

(**A**) A brightfield image of the surface of adult mouse testes exhibiting blood vessels (BV) and the spermatic cord (asterisk). (**B**) Prox 1-EGFP-positive signals were observed from the same surface view. (**C**) A confocal image showing the Prox1-EGFP-positive lymphatic network across the tunica albuginea originating from the spermatic cord. (**D**–**F**) Prox 1-EGFP positive lymphatics were confined within the tunica albuginea (arrow) but was not found inside the seminiferous tubule (encircled) when co-stained with the Leydig cell marker HSD3B1 and counterstained with DAPI. Within the seminiferous tubules, EGFP expression was observed in spermatids that are in close proximity to the lumen. Scale bar for panel (C) = 600 mm, (D) = 100 mm. The images are reproduced from the reference [119] with permission.

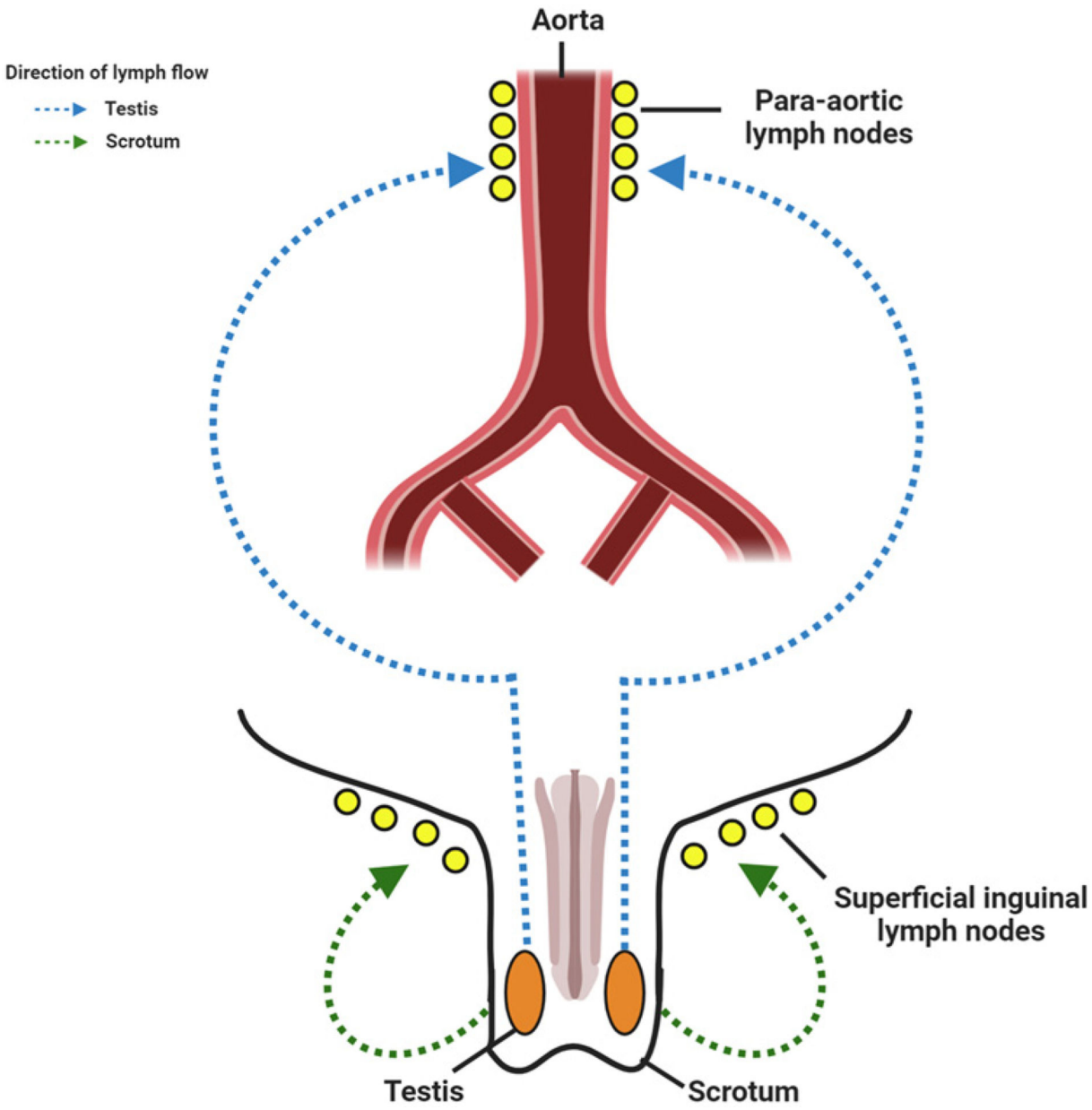

Figure 5. Lymphatic drainage of the testes and scrotum

Lymphatics from the testes drain into the para-aortic lymph nodes, whereas the lymphatics from the scrotum drain into the superficial inguinal lymph nodes. Created with BioRender.com.

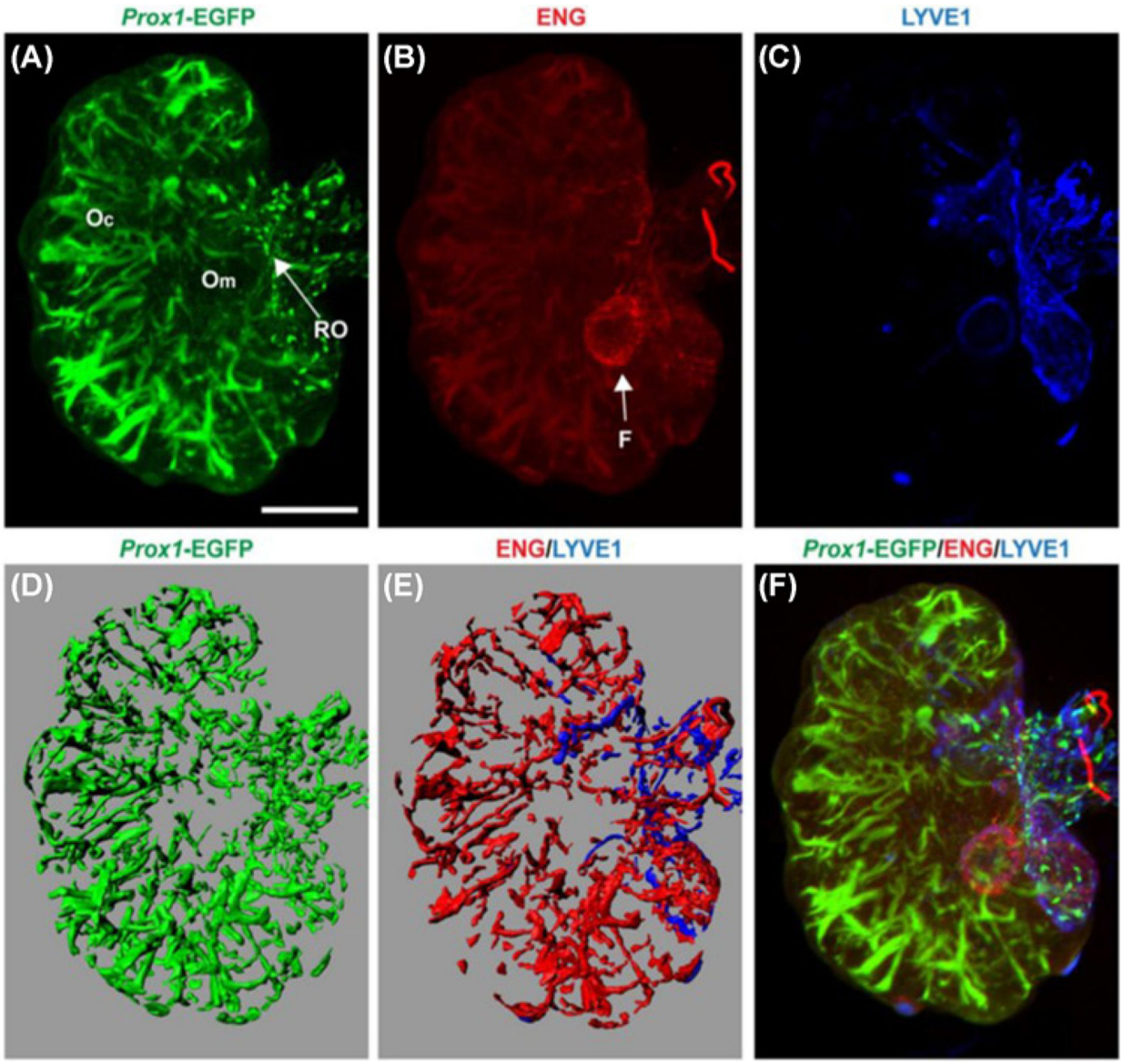

Figure 6. Distribution of the lymphatic vasculature in the adult ovary of Prox1-EGFP reporter mice

The adult ovary possesses a rich lymphatic network largely overlapping with the blood vasculature. (**A**) Prox 1-EGFP-positive lymphatics were observed throughout the ovary, originating from the rete ovarii (RO) (arrow), and extending into the ovarian medulla (Om) and ovarian cortex (Oc). (**B**) Endoglin (ENG) labeling showed an extensive network of blood vessels in the ovary with the follicle (F) indicated by an arrow. (**C**) Lyve-1 positive lymphatic vessels were confined to the ovarian and extraovarian rete. (**D**) A 3-D representative image showing Prox1-EGFP-positive lymphatics. (**E**) Lyve1- positive lymphatic vessels and ENG-positive blood vessels. (**F**) Combined image showed some colocalization of Prox1, Lyve1, and ENG and a distinct pattern of the blood and lymphatic network in the adult ovary; scale bar = 1 mm. The images are reproduced from the reference [119] with permission.

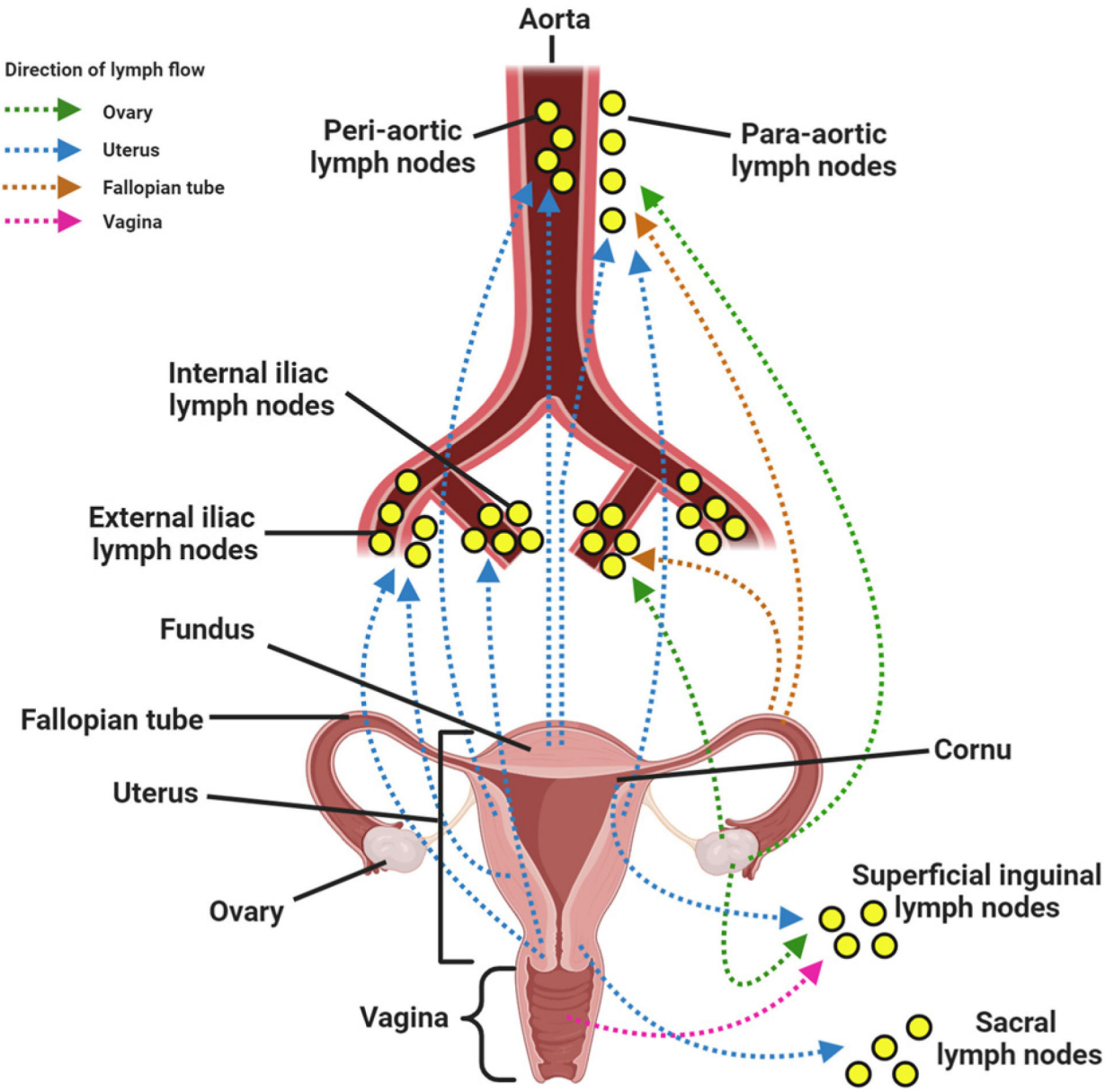

Figure 7. Lymphatic drainage of the ovary and uterus

(**A**) The lymphatic vessels from ovaries drain into (i) the para-aortic lymph nodes, (ii) internal iliac lymph nodes, and (iii) inguinal lymph nodes. (**B**) Lymphatics from the fallopian tube drain into para-aortic and internal iliac lymph nodes. (**C**) Lymphatic vessels from the (i) fundus and superior uterine body drain into pre-aortic and para-aortic lymph nodes, cornu into the superficial inguinal lymph nodes, (ii) middle uterine body into the external iliac nodes, and (iii) the lower portion (cervix) into the internal iliac, external iliac, and sacral lymph nodes. (**D**) Lymphatics from the vagina drain into the superficial inguinal lymph nodes. Created with BioRender.com.

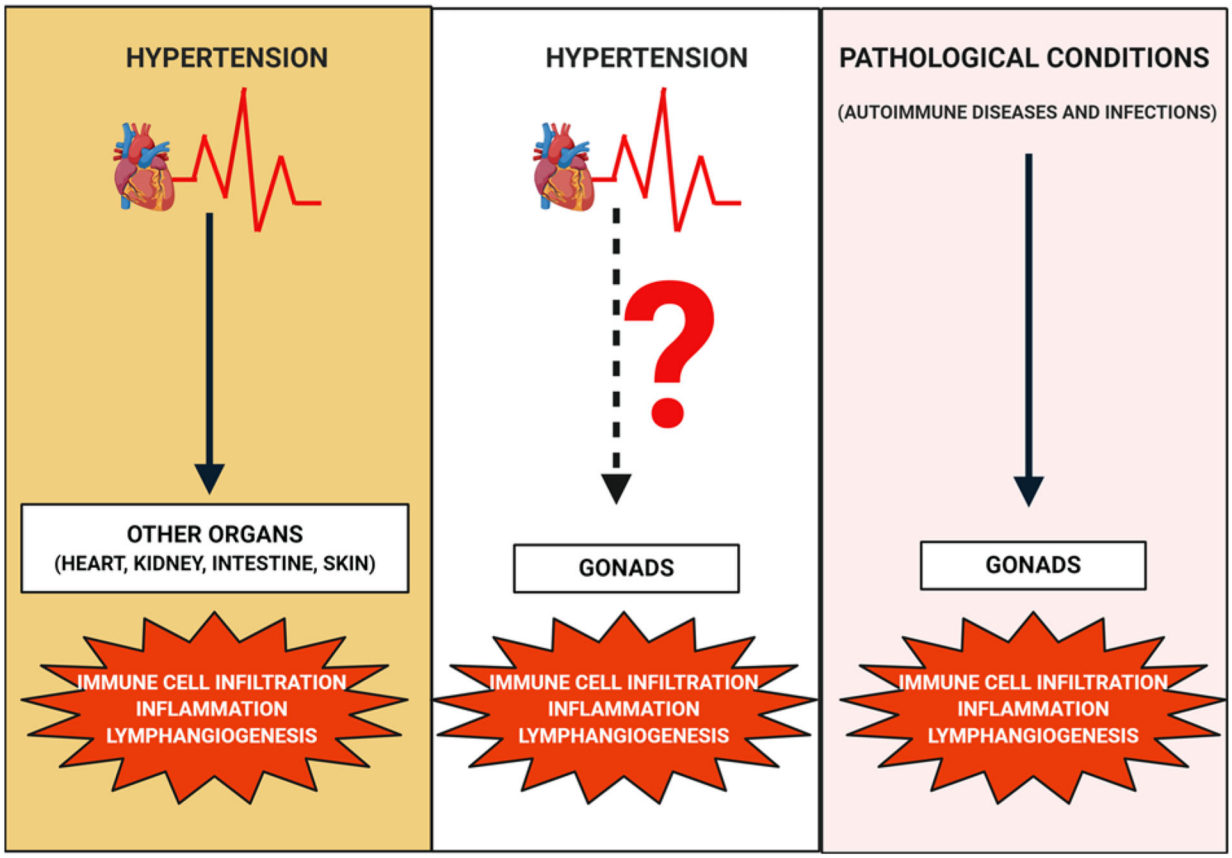

Figure 8. Graphical overview of the effects of hypertension on gonadal function and the plausible mechanisms behind these effects

Hypertension is known to promote immune cell infiltration, inflammation, and inflammation-associated lymphangiogenesis in several organs like the heart, kidneys, intestine, and skin. Similarly, immune cell infiltration, inflammation, and lymphangiogenesis have been observed in gonads under certain pathological conditions. However, the exact molecular mechanisms by which hypertension affects gonads are still unclear. Hence, one of the plausible mechanisms might be immune cell infiltration and inflammation in gonads thereby leading to reproductive dysfunction. Inflammation-associated lymphangiogenesis may occur in a compensatory manner and its role in gonadal function in hypertension is unknown. Created with BioRender.com.

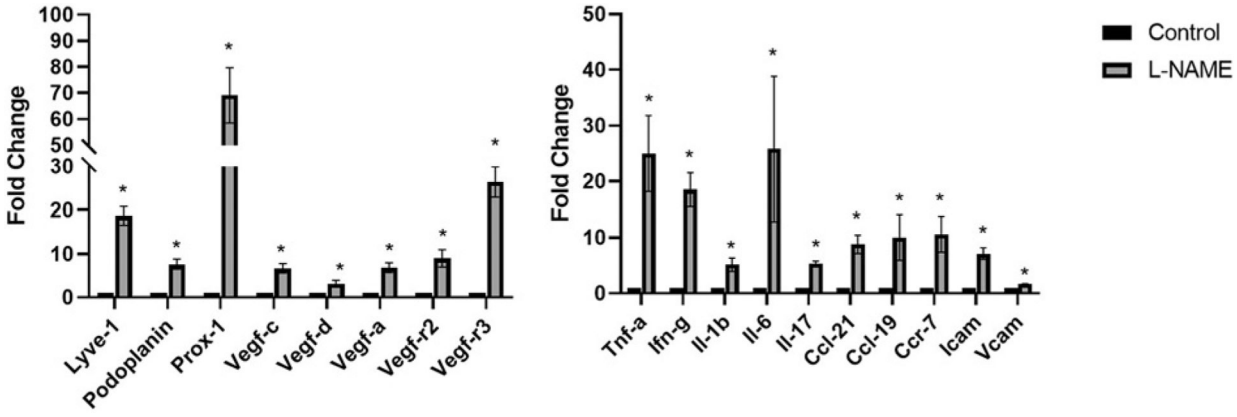

Figure 9. Testes from L-NAME induced hypertensive mice had a significant increase in gene expression of lymphatic markers, proinflammatory cytokines, chemokines, and adhesion molecules

Results are expressed as mean ±SEM (n=4 per group), and statistical analyses were performed with a Student's t test. *P<0.05 vs. Control mice.

Table 1

Inflammatory markers altered in testis during inflammation

S.No.	Inflammation model	Findings of the study	References
1.	Experimental autoimmune orchitis model	Increased infiltration of macrophages, dendritic cells and subsets of T cells, elevated pro-inflammatory cytokines levels IL-6, TNF- α, up-regulation of cell adhesion molecules (CD31, CD44, CD106), chemokines (MCP-1, MIP 1 α and 1 β) and chemokine receptors (CCR2, CCR5 and CCR7) Increased levels of IL-6 disrupted the integrity of the BTB by down-regulating the expression of Occludin, and delocalization of Claudin 11 and Zona occludens-1 in the testes, IL-6 also induces GC apoptosis	[153,158–162,164,167,168,185,187]
2.	Testicular cancer biopsies	Increased levels of mRNAs of pro-inflammatory cytokines, such as IL-1β, TNF-α, and IFN-γ	[165]
3.	Testicular biopsies from patients with spermatogenic failure	Increased mRNA expression of high-affinity IgE receptor and the mast cell-related fractalkine receptor revealing increased inflammatory activity in the testis	[170,171]
4.	Lipopolysaccharide-induced inflammation	Up-regulation of IL-1β and IL-6 in the testis	[163]
5.	Varicocele testes model	Increased mRNA levels of proinflammatory cytokines (*Tnfa*, *//1a, and* //6), leukocyte marker *(Cd45)*, and T-cell markers *(Cd3g* and *Cd3d*), attenuated mRNA expression of SC tight junction proteins such as Claudin-11, Occludin and Zona occludens-1 in varicocele testes	[188]

Table 2

Inflammatory markers altered in ovaries and uterus during inflammation

S.No.	Inflammation Model	Findings of the study	References
1.	Autoimmune ovarian disease model	Up-regulation of proinflammatory markers such as IL-1, TNF-α, and IFN-γ	[207]
2.	Ovarian biopsies from women with premature autoimmune ovarian failure.	Ovarian granulosa cells had ectopic expression of MHC-II in the site of inflammation.	[208]
3.	Autoimmune ovarian disease model (Cynomolgus macaques)	Co-localization of T cell clusters with MHC-II+ macrophages in the ovarian interstitium.	[205]
4.	Females with premature ovarian insufficiency	Increased level of chemokines such as MIP-1α, IL-8, eotaxin-1 and interferon inducible protein-10 as well as lymphangiogenic factor VEGF-D in follicular fluid.	[209]
5.	Endometriosis	Elevated levels of intercellular adhesion molecule-1, insulin-like growth factor-I, IL-1, IL-6, IL-8, IL-12, MCP-1, MIP-1α, RANTES, eotaxin, VEGF and TNFα were associated with endometriosis.	(Explained in detail in the cited reviews) [211,213]