SUPPLEMENTARY MATERIAL

Title

Hypertension Induces Gonadal Macrophage Imbalance, Inflammation, Lymphangiogenesis, and Dysfunction

Authors and Affiliations

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Supplementary Tables

Table S1. Flow cytometry panel description for mouse gonads.

Fluorochrome	BV421	FITC	APC	PE-Cy7	BV785
Antigen	CD45.2	CD11b	CD11c	F4/80	CD206
Clone	104	M1/70	N418	BM8	C068C2

Abbreviations: BV=Brilliant Violet; FITC=fluorescein isothiocyanate; APC=allophycocyanin; PE-Cy7=R-phycoerythrin cyanine 7

Table S2. Primer sequences for quantitative RT-PCR analysis of murine gonadal tissue.

Target	**Forward (5' to 3')**	**Reverse (5' to 3')**
Il1b	GCCACCTTTTGACAGTGATGAG	GACAGCCCAGGTCAAAGGTT
Il6	GAGGATACCACTCCCAACAGACC	AAGTGCATCATCGTTGTTCATA
Il17	TTTAACTCCCTTGGCGCAAAA	CTTTCCCTCCGCATTGACAC
Tnfa	GAGAAAGTCAACCTCCTCTCTG	GAAGACTCCTCCCAGGTATATG
Ifng	TCAAGTGGCATAGATGTGGAAGAA	TGGCTCTGCAGGATTTTCATG
Nos2	GTTCTCAGCCCAACAATACAAGA	GTGGACGGGTCGATGTCAC
Lyve1	CTGACAAGCAGTTTCAGGCTTGGT	TTCAGCCCACACTCCGCTATACAT
Pdpn	ACCGTGCCAGTGTTGTTCTG	AGCACCTGTGGTTGTTATTTTGT
Prox1	CTCTTGCCTCGCTATCCCC	CACAGTCCCACTGACGTACC
Vegfc	CAGTGTCAGGCAGCTAACAAG	GAAGGTCCACAGACATCATGGAA
Vegfd	TGGCAAGACTTTTGAGCTTCAA	AAATCGCGCACTCTGAGGA
Vegfr2	GCCCTGCTGTGGTCTCACTAC	CAAAGCATTGCCCATTCGAT
Vegfr3	ATCAGAAGATCGGGCGCTGTTGTA	TGTGTCATGTCCGCCCTTCAGTTA
Icam	GTGATGCTCAGGTATCCATCCA	CACAGTTCTCAAAGCACAGCG
Vcam	AGTTGGGGATTCGGTTGTTCT	CCCCTCATTCCTTACCACCC
Ccl19	GGGGTGCTAATGATGCGGAA	CCTTAGTGTGGTGAACACAACA
Ccl21	CCCTGCTTCAACCATTACATCTGC	CCTGCTGTCTCCTTCCTCATTCC
Ccr7	TGTACGAGTCGGTGTGCTTC	GGTAGGTATCCGTCATGGTCTTG
Star	GAACGGGGACGAAGTGCTAA	TGGTCTACCACCACCTCCAA
Hsd3b1	CAGGAGAAAGAACTGCAGGAGGTC	GCACACTTGCTTGAACACAGGC
Hsd17b1	AATTGAACGCTGTGGGTGCT	GAATGGCAGTCCCATCAAGC
Cyp11a1	GGGGACAGTATGCTGGCTAA	ACGTAGGGCTCAGGAAAGGT
Cyp17a1	TGGAGGCCACTATCCGAGAA	CACATGTGTGTCCTTCGGGA
Cyp19a1	TCACTCTACTAACTCAAGGGCG	GGGAGGCTCAGGTTCTGTTC
Ar	CCCTGAGGCCGCTAACATAG	GGGCTTGAGGAGAACCATCC
Era	AATTCTGACAATCGACGCCAG	GTGCTTCAACATTCTCCCTCCTC
Erb	TCTTTGCTCCAGACCTCGTTC	GGGACAGCACTCTTCGTCTG
Fshr	GGTCTATTCCCTGCCCAACC	AGGGAGCTTTTTCAAGCGGT
Lhr	ACGAGACGCTTCATCACTCTG	GATGGCATGTCTCAGCCTCA
Inhba	AAATCAGAACGCCTCCGCTA	TCCCGAGTGTAGAGTTCGGT
Inhbb	AGGCCAGCGGATCAGTTTTA	CAGGCCACTCGAAGGATTGT
Scgb1b24	GCTCCTGCATTCAGGGGTAT	ACATACTCTTCTGAGGTCCTGTG
Tfr	AGAACCGCTGGTTGGAACAT	GCGCAGCCTTGACTGAAAAA
Cldn11	TTGCTCTTTCCTCGGGCATT	CCCAATCCACACCCAAGTCA
Ocln	CCCCTCTTTCCTTAGGCGACA	AGGCTCCCAAGATAAGCGAAC

Tjp1	AGACGCCCGAGGGTGTAG	TGGGACAAAAGTCCGGGAAG
F11r	GCAGATGCCAAGAAAACCCG	TCTGGGCCTGGCAGTAGTAT
Ubc	GCCCAGTGTTACCACCAAGAAG	GCTCTTTTTAGATACTGTGGTGAG

All sequences were verified through National Center for Biotechnology Information Primer-BLAST and single products were confirmed with a melting point dissociation step post amplification.

Il-1b, Interleukin 1b; Il-6, Interleukin 6; Il-17, Interleukin 17; Tnf-a, tumor necrosis factor alpha; Ifn-g, Interferon gamma; Nos2, nitric oxide synthase 2; Lyve-1, lymphatic vessel endothelial hyaluronan receptor 1; Prox-1, prospero homeobox 1; Vegf-c, vascular endothelial growth factor C; Vegf-d, vascular endothelial growth factor D; Vegfr-2, vascular enodotheilal growth factor receptor 2;Vegfr-3, vascular enodotheilal growth factor receptor 3; Icam, intercellular adhesion molecule; Vcam, vascular cell adhesion molecule; Ccl-19, chemokine ligand 19; Ccl-21, chemokine ligand 21; Ccr7, C-C chemokine receptor type 7;Star, steroidogenic acute regulatory; Hsd3b1, 3-beta hydroxysteroid dehydrogenase; Hsd17b1, 17-beta hydroxysteroid dehydrogenase; Cyp11a1, Cytochrome P450 side chain cleavage; Cyp17a1, Cytochrome P450 17α-hydroxylase; Cyp19a1, Cytochrome P450 aromatase; Ar, Androgen receptor; Era, Estrogen receptor alpha; Erb, Estrogen receptor beta; Fshr, Follicle stimulating hormone receptor; Lhr, Luteinizing hormone receptor; Inhba, Inhibin beta a subunit; Inhbb, Inhibin beta b subunit; Scgb1b24, Secretoglobin, family 1B, member 24/ Androgen binding protein; Tfr, Transferrin; Cldn11, Claudin 11; Ocln, Occludin; Tjp1, Tight junction protein-1; F11r, Junctional adhesion molecule A; Ubc, Ubiquitin.

Table S3. Body and testis/ovary weights.

	Body Weight (BW) (g)	Testis/Ovary Weight (mg/BW)
CON (Male)	31.71± 1.03	3.42±0.08
SSHTN (Male)	31.87±0.45	4.14±0.10*
CON (Female)	23.80±0.59	0.22±0.01
SSHTN (Female)	23.48±0.51	0.21±0.01

CON=C57 control mice, SSHTN=salt-sensitive hypertension; *n*=6,*P<0.05

Table S4. Body and testis/ovary weights.

	Body Weight (BW) (g)	Testis/Ovary Weight (mg/BW)
CON (Male)	26.62±0.86	3.66±0.11
LHTN (Male)	25.65±0.43	4.12±0.03*
CON (Female)	20.23±0.75	0.21±0.01
LHTN (Female)	19.52±0.57	0.23±0.01

CON=C57 control mice, LHTN=L-NAME-induced hypertension; *n*=6,*P<0.05

Table S5. Quantification of LYVE-1 positive lymphatic vessels in CUBIC cleared gonads.

Sample	LYVE-1 + vessel volume relative to total gonadal volume (%)	
	Testes	Ovaries
Control	0.002798 ± 0.0002	0.003604 ± 0.0001
SSHTN	0.006826 ± 0.0003*	0.005900 ± 0.0005*
LHTN	0.005596 ± 0.0007*	0.006698 ± 0.0005*

SSHTN=salt-sensitive hypertension; LHTN=L-NAME-induced hypertension, (n=3,*P<0.05)

Supplementary Figures

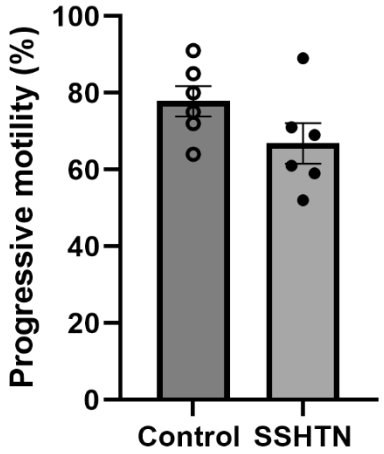

Figure S1. Progressive motility of spermatozoa collected from control and mice administered nitro-l-arginine methyl ester hydrochloride (L-NAME) in the drinking water for 2 weeks, then 2 weeks of tap water washout, then 3 weeks of 4% salt diet (SSHTN). Results are expressed as mean ± SEM, and statistical analysis consisted of a Student's *t* test. *n*=6, **P*<0.05 vs control.

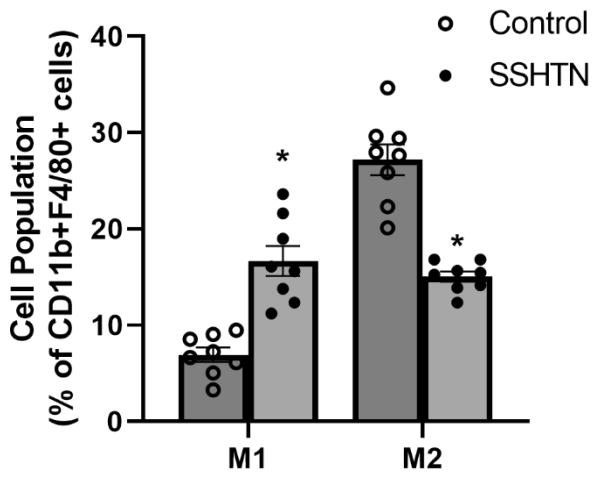

Figure S2. Macrophage (M1 and M2) populations expressed as percentage of CD11b+F4/80+ cells as determined by flow cytometry in testes from control and mice administered nitro-l-arginine methyl ester hydrochloride (L-NAME; 1.5 mg/ml) in the drinking water for 4 weeks, then 1 week of tap water washout, then a subsequent 3 weeks of 4% salt diet (SSHTN). Results are expressed as mean ± SEM, and statistical analysis consisted of a Student's *t* test. *n*=8, **P*<0.05 vs control.

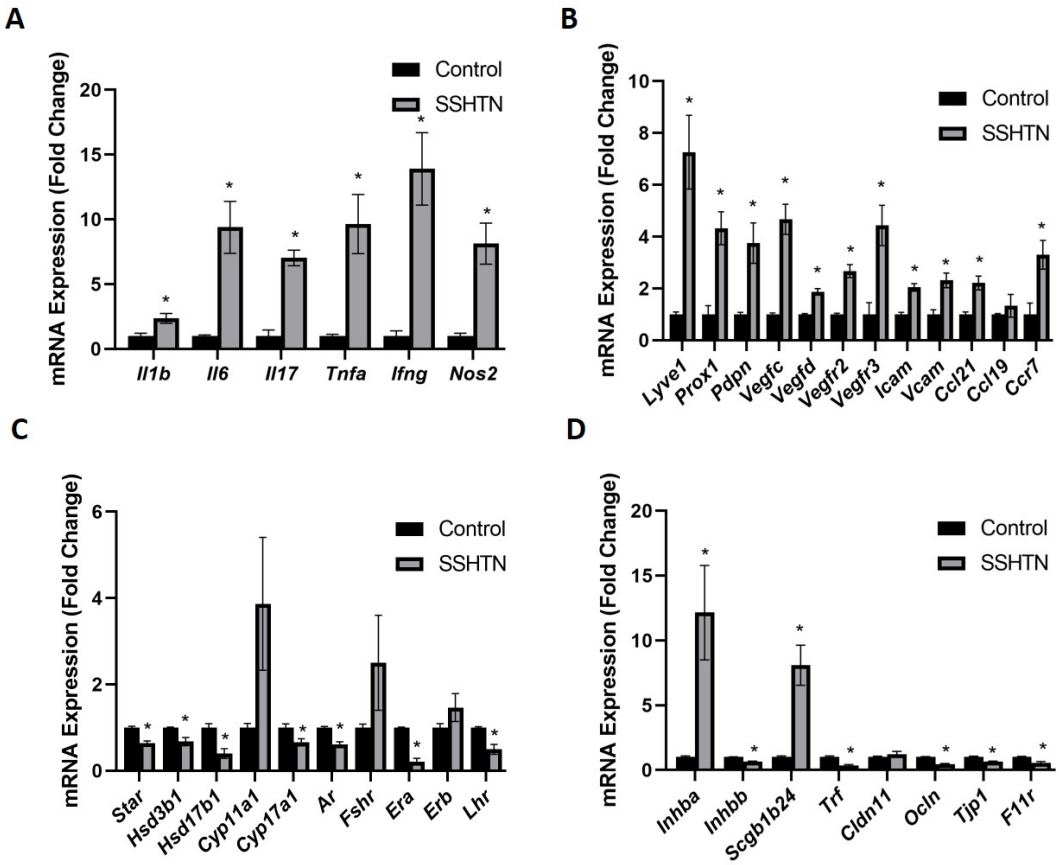

Figure S3. Gene expression of **(A)** pro-inflammatory mediators, **(B)** lymphatic markers, **(C)** steroidogenic pathway genes and hormone receptors, and **(D)** secretory proteins and tight junction proteins in the testes of control and mice administered nitro-l-arginine methyl ester hydrochloride (L-NAME; 1.5 mg/ml) in the drinking water for 4 weeks, then 1 week of tap water washout, then a subsequent 3 weeks of 4% salt diet (SSHTN). Results are expressed as mean ± SEM. Statistical analyses were performed using Student's *t* test. *n*=6, **P*<0.05 vs control.

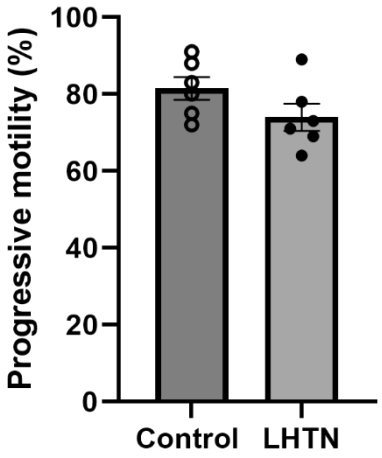

Figure S4. Progressive motility of spermatozoa collected from control and mice administered nitro-l-arginine methyl ester hydrochloride (L-NAME) in the drinking water for 3 weeks (LHTN). Results are expressed as mean ± SEM, and statistical analysis consisted of a Student's *t* test. n=6, *P<0.05 vs control.

Legends for Video Files

Supplement video 1: CUBIC processed testis from control mice showing immunostaining for the lymphatic marker LYVE-1.

Supplement video 2: CUBIC processed testis from salt-sensitive hypertension (SSHTN) mice showing immunostaining for the lymphatic marker LYVE-1.

Supplement video 3: CUBIC processed ovary from control mice showing immunostaining for the lymphatic marker LYVE-1.

Supplement video 4: CUBIC processed ovary from salt-sensitive hypertension (SSHTN) mice showing immunostaining for the lymphatic marker LYVE-1.

Supplement video 5: CUBIC processed testis from L-NAME-induced hypertension (LHTN) mice showing immunostaining for the lymphatic marker LYVE-1.

Supplement video 6: CUBIC processed ovary from L-NAME-induced hypertension (LHTN) mice showing immunostaining for the lymphatic marker LYVE-1.