

Role of Androgen on Blood Flow and Capillary Structure in Rat Seminal Vesicles

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ONO, Y., SUZUKI, K., KASHIWAGI, B., SHIBATA, Y., ITO, K., FUKABORI, Y. and YAMANAKA, H. *Role of Androgen on Blood Flow and Capillary Structure in Rat Seminal Vesicles*. Tohoku J. Exp. Med., 2004, **202** (3), 193-201 — To clarify the effect of androgen on the microcirculation in seminal vesicles of adult Wistar rats, we investigated the organ blood flow and morphological features in the capillaries after castration and subsequent testosterone supplementation. Testosterone (T) was subcutaneously injected every 12 hours after castration and its doses were set to 10^{-2} , 10^{-1} , 10^0 and 10^1 mg/kg-body weight (T10⁻², T10⁻¹, T10⁰ and T10¹ groups, respectively). Organ blood flow was measured using laser Doppler flowmetry, and the subepithelial capillaries were analyzed using transmission electron microscopy. The capillaries were morphologically classified into 3 types; oval opened (type 1), intermediate (type 2) and collapsed (type 3), and their luminal areas were measured using a computed image analyzer. The organ blood flow was significantly reduced from 36.3 ± 5.1 to 21.9 ± 2.7 ml · min⁻¹/100 g tissue, and the luminal area of the capillaries was significantly reduced from 9.02 ± 1.28 to 4.85 ± 0.82 μm² with the shift of the type 1 and type 2 to type 3 after castration. The reduction of the luminal area and the blood flow reduction, and shift of the capillary type were significantly protected by graded testosterone supplementation. These results indicate that the maintenance of the blood flow and morphological profiles in capillaries depend on androgen-supplementation levels in seminal vesicles. ——— seminal vesicles; blood flow; capillary; transmission electron microscopy

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Androgen has important role on differentiation, development and maintenance of epithelial cells in seminal vesicles (SV) as well as in the prostate (Mata 1995). The stroma in rat SV depend on both androgen and estrogen in its maintenance and proliferation, and this hormonal dependency is similar to that of canine and human prostates (Partin and Rodriguez 2002). Synergistic stimulation of estrogen and androgen induces development of prostatic hypertrophy more rapidly in their stroma and gland in the canine models than androgen supplementation alone (Walsh and Wilson 1976; Coffy and Walsh 1990). In humans as well, although the pathogenesis of benign prostatic hyperplasia (BPH) is still unclear, the synergistic effect of estrogen and androgen may be one of the essential factors in the pathogenesis of BPH with stromal hyperplasia (Coffy and Walsh 1990; Suzuki et al. 1995). In contrast, this hormonal effect does not occur in rat prostate, because they contain small amounts of stromal materials, including fibroblast, smooth muscle and extracellular matrix, with or without small amounts of estrogen receptors in rat prostatic stroma (Ehrlichman et al. 1981). Therefore, SV may be better models to investigate the stromal-epithelial interactions via sex hormones and peptides of growth factor families which are transported or mediated by microvasculatures than the prostate in rat models.

The androgen-dependent blood flow control system has not been fully investigated in SV, but it has been extensively investigated in the prostate. Castration reduces the blood flow in the prostate, and testosterone administration resumes this blood flow to normal levels (Lissbrant et al. 1998; Shabsigh et al. 1999a). Nitrogen oxide and vascular endothelial growth factors were suggested as blood flow control factors related to androgen (Häggström et al. 1998; Hayek et al. 1999; Burchardt et al. 2000). We also have reported that androgen-dependent ultrastructural changes in the capillaries occurred in relation to the blood flow changes in rat prostate using transmission electron microscopy (TEM) and laser Doppler

flowmetry (Ono et al. 2004). In addition, we have preliminarily confirmed that castration significantly reduced organ blood flow in SV as well, but not in the liver, spleen, kidney or penis using laser Doppler flowmetry (Ono et al. 2004). The purpose of this study was to clarify the androgen-dependent blood flow changes and morphological features of the subepithelial capillaries in rat SV by similar methodology using in the prostate.

MATERIALS AND METHODS

Experimental animals and treatments

Adult male Wistar rats (8 weeks of age, 230~300 g body weight) were purchased from SLC Japan, Ltd. (Hamamatsu, Shizuoka) and castration was done under ether anesthesia. Organ blood flow was measured via a low abdominal mid-incision under subcutaneous injection of pentobarbiturate (40 mg/kg-body weight) before castration, and 1, 2, 3 and 7 days after castration. Testosterone (T) was subcutaneously injected every 12 hours beginning immediately after castration. The T doses were set to 10^{-2} , 10^{-1} , 10^0 and 10^1 mg/kg-body weight, and organ blood flow was measured and SV were removed for TEM at day 2 under subcutaneous injection of pentobarbiturate ($n=4$). This experimental study was performed with the approval of the Animal Care and Experimental Committee, Gunma University School of Medicine, Showa Campus.

Measurement of organ blood flow by laser Doppler flowmetry

Laser Doppler flowmetry (ALF-21R, Advance Co., Ltd., Tokyo), which was designed to measure the blood flow within a radius of 1 mm around the tip of a laser probe and to automatically compute the organ blood flow ($\text{ml} \cdot \text{min}^{-1} / 100 \text{ g}$ of organ weight), was used as in the previous report (Ono et al. 2004). Each organ blood flow measurement was repeated three times and statistically analyzed.

Transmission electron microscopy, and measurement and classification of the capillary lumen

SV were removed and immediately fixed in 2.5% glutaraldehyde with 0.1 M phosphate buffer and cut into 1 mm blocks. The blocks were fixed in osmium acid and embedded in Quetol 812 (Nissin EM Co., Ltd., Tokyo) after dehydration. Ultra thin sections of $6 \times 10^{-2} \mu\text{m}$ were made and double staining with lead and uranyl acetate was performed. The specimens of SV were observed using a JEM 1010 CX TEM (JEOL Ltd., Tokyo) at 80kV power. We analyzed the subepithelial capillaries that were adjacent to the epithelial cells in peripheral acini of the SV. TEM images of $\times 4000$ magnification were captured by a digital image scanner. The lumens of the capillaries were traced manually using Photoshop™ 4.0J (Adobe Systems Incorporated, Sun Jose, CA, USA) and the area of the lumen was measured by NIH Image™ ver 1.52 (public domain by Wayne Rasband, NIH, Bethesda, MD, USA).

We defined three types of the capillaries by the morphological features of their lumens. Type 1: Oval opened type. The lumen was smooth, oval and widely opened (Figs. 1A and 1B). Many capillaries of this type contained red blood cells in their lumens and some of them were absent of red blood cells. This type of capillary likely maintained good blood flow. Type 2: Intermediate type. The lumen was partially depressed and closed and the luminal wall was irregular (Fig. 1C). Some of them contained red blood cells in their lumen. Type 3: Collapsed type. The lumen was collapsed and closed (Fig. 1D). Red blood cells could not be seen. This type of capillary likely had interrupted blood flow. The capillaries were classified into one of these 3 types and the ratio of each type was calculated in each experimental group. These methods followed the methods of the previous study (Ono et al. 2004).

Statistical analysis

Values of the organ blood flow were presented as means \pm standard deviation (s.d.) and

those of the capillary lumen were presented as means \pm standard error of the means (s.e.). These values were analyzed by Mann-Whitney's U-test. Capillary type in each group was compared using Fisher's exact test. A *p*-value less than 0.05 was considered statistically significant.

RESULTS

Ultrastructural findings of the subepithelial capillaries of SV

The subepithelial capillaries were clearly identified as being closely adjacent to the epithelial cells (Fig. 1). These capillaries consisted of 2~4 endothelial cells connected by junctional complex with a tight junction and were surrounded by stromal matrix including collagen fibers. Endothelial cells contained micropinocytotic vesicles and the number of vesicles in an endothelial cell varied widely. The outer layer of these capillaries was made of smooth muscle cells and myoepithelial cells.

Blood flow changes after castration

The organ blood flow was markedly reduced after castration, however, organs at day 7 after castration were involuted and the size was too small to acquire stable measurements (Fig. 2). In this study, it was difficult to measure blood flow in SV under about 60~70 mg of organ weight using this laser Doppler flowmetry system.

Blood flow, luminal size and morphological features after castration with T-supplementation

Organ weight of SV (mg) comparing with 100 g of body weight (relative organ weight: mg/100 g) was revealed in Fig. 3A. In this series, T supplementation level that reached to normal biological activity was suggested to range between the T10⁻¹ and T10⁰ group. The reduction of blood flow was protected in the T10⁻¹ (*p*<0.05), and in the T10⁰ and T10¹ groups (*p*<0.001), (Fig. 3B). The luminal area of the capillaries was markedly reduced at day 2 from $9.02 \pm 1.28 \mu\text{m}^2$ to $4.85 \pm 0.82 \mu\text{m}^2$ (*p*<0.001, Fig. 3C). The reduction

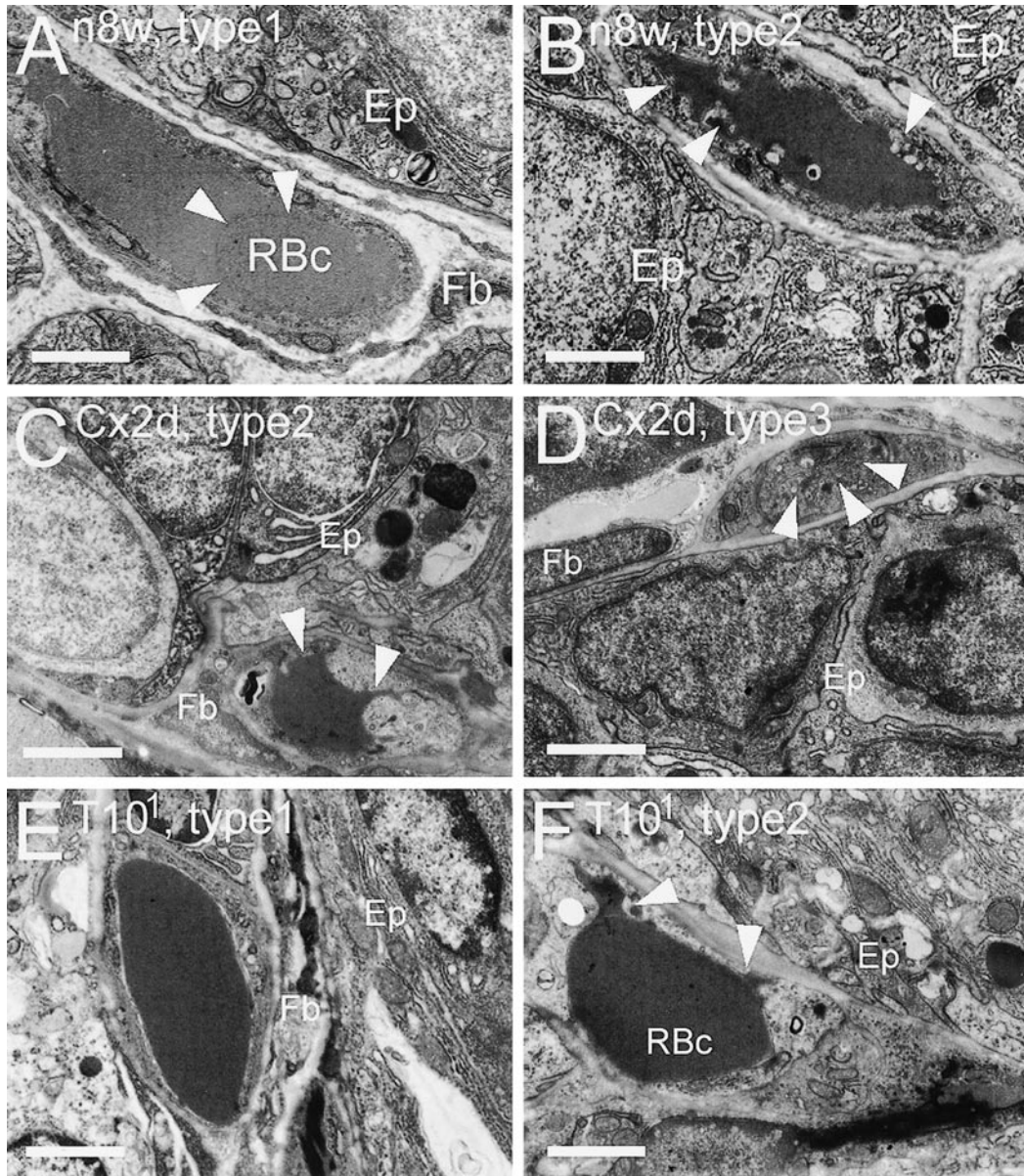


Fig. 1. TEM images of the SV. A: A subepithelial capillary (oval opened type) at 8 weeks of age adjacent to the epithelial cells. This type of capillary has a round lumen with red blood cells, which is classified as type 1 (oval opened type). B: A type 2 (intermediate type) subepithelial capillary at 8 weeks of age. The lumen is irregular (arrowheads). C: A type 2 subepithelial capillary on day 2 after castration. The lumen is irregular, partially collapsed (arrowheads). D: A type 3 (collapsed type) subepithelial capillary on day 2 after castration. The lumen is completely closed (arrowheads). E: A type 1 subepithelial capillary on day 2 after castration with T supplementation of 10^1 mg/kg ($T10^1$). F: A type 2 subepithelial capillary in $T10^1$. The lumen is widely opened but is partially closed (arrowheads).

n8w, normal rat 8 weeks of age; Cx, castration; $T10^1$, castration with T supplementation of 10^1 mg/kg; Ep, epithelial cell; Fb, fibroblast; RBC, red blood cell. The bar is $2\ \mu\text{m}$.

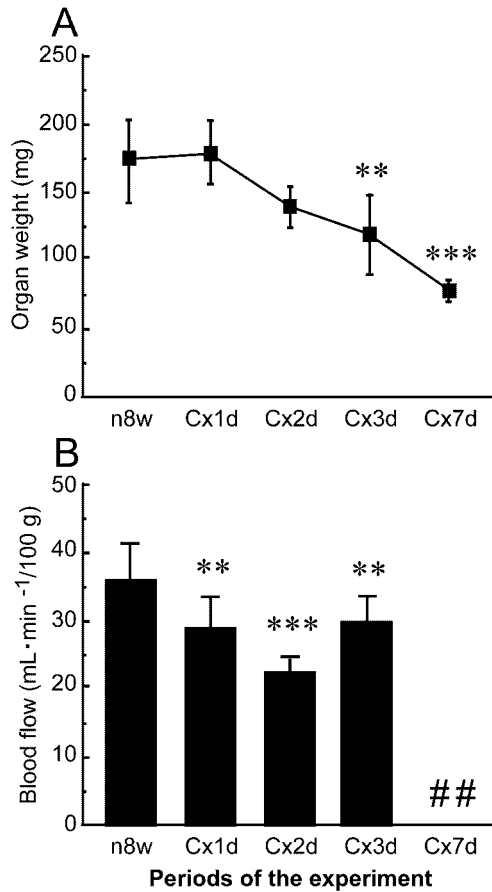


Fig. 2. The changes in organ weight and organ blood flow after castration. A: The organ weight was significantly reduced on day 3 and 7 after castration. B: Organ blood flow was significantly reduced from 36.5 (n8w) to 28.6 on day 1, 21.9 on day 2 and 29.6 on day 3 after castration ($\text{mL} \cdot \text{min}^{-1}/100 \text{ g}$ of organ weight, mean \pm S.D.). On day 7 after castration, the organ was too small to acquire stable measurements (##). n8w, normal rat 8 weeks of age; Cx, castration; ##, no data to be evaluated. ** $p < 0.01$, *** $p < 0.001$.

of luminal area was significantly protected by graded T supplementation ($p < 0.05$ in the T10⁻², and $p < 0.01$ in the T10⁻¹, T10⁰ and T10¹ groups, Fig. 3C).

A shift from type 1 (Fig. 1A) and 2 (Fig. 1B) to types 3 (Fig. 1D) was revealed after castration (Table 1). Regarding the morphological type of the capillaries, type 3 (Fig. 1D) gradually decreased and type 1 (Fig. 1E) capillaries

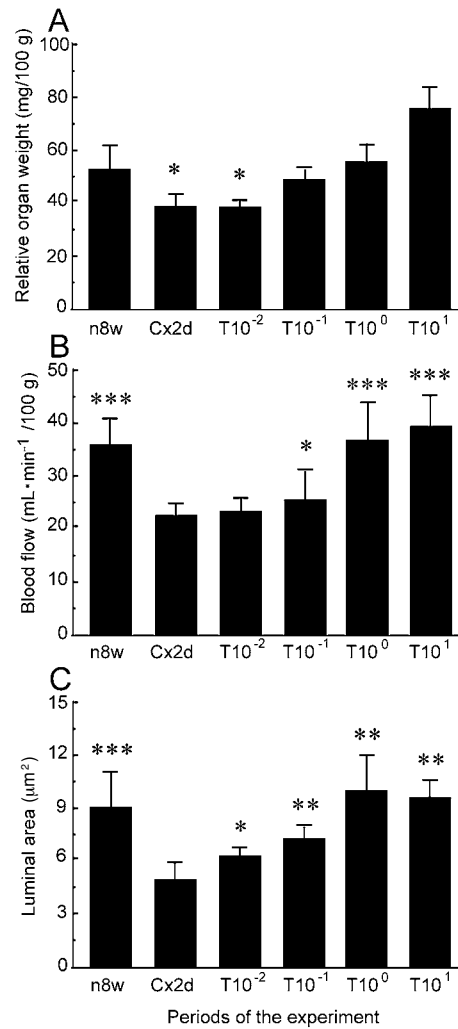


Fig. 3. The changes in the blood flow and luminal area after castration and graded T supplementation. A: Relative organ weight of SV comparing to 100 g of body weight was 52.8 ± 8.8 in the normal group (mean \pm S.D.). It ranged between that in T10⁻¹ group (4.94 ± 4.1) and that in T100 group (56.8 ± 5.4). In this series, T supplementation level that reached to normal biological activity was suggested to range between the T10⁻¹ and T100 group. B: The reduction of the blood flow after castration was significantly protected in the T10⁻¹, T100 and T101 groups (mean \pm S.D.). C: The luminal area of subepithelial capillary was significantly reduced on day 2 after castration and this reduction was significantly protected in the T10⁻², T10⁻¹, T100 and T101 groups (mean \pm S.E.). n8w, normal rat 8 weeks of age; Cx, castration; T10⁻², T10⁻¹, T100 and T101, castration with graded T supplementation (T was adjusted to 10⁻², 10⁻¹, 100 and 101 mg/kg-body weight). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

TABLE 1. *The changes of capillary types with graded T supplementation after castration*

Capillary type	n8w (%)	Cx 2d (%)	T10 ⁻² (%)	T10 ⁻¹ (%)	T10 ⁰ (%)*	T10 ¹ (%)**
Type 1	15 (57.7)	20 (48.8)	20 (44.4)	17 (65.4)	18 (69.2)	32 (91.4)
Type 2	8 (30.8)	5 (12.2)	15 (33.3)	7 (26.9)	7 (26.9)	3 (8.6)
Type 3	3 (11.5)	16 (39.0)	10 (22.2)	2 (7.7)	1 (3.8)	0 (0)
<i>n</i>	26	41	45	26	26	35

n8w, normal rat at 8 weeks of age; Cx, castration; Cx 2d, 2 day after Cx; T10⁻², T10⁻¹, T10⁰ and T10¹, Cx and testosterone supplementation (10⁻², 10⁻¹, 10⁰ and 10¹ mg/kg-body weight); **p*<0.05 vs. Cx2d; ***p*<0.01 vs. Cx 2d.

gradationally increased depending on the T supplementation (Table 1). Ultrastructural and morphological changes in capillaries were significantly protected and resumed in the T10⁰ (*p*<0.05) and T10¹ group (*p*<0.01).

DISCUSSION

Seminal vesicles have an important role in ensuring *in vivo* fertility, and the androgen-dependency of their epithelial cells has been extensively studied (Mata 1995). Their epithelial cells have androgen receptors and castration induces apoptotic changes (Sar et al. 1990; Bentvelsen et al. 1995; Mata 1995). In addition, the development and proliferation of their epithelial cells depends on several growth factors, including the fibroblast growth factor family independent of androgen (Thomson and Cunha 1991). These behaviors are very similar to those of the prostate (Fukabori et al. 1994; Nakano et al. 1999), however, the regulation of blood flow in SV has not been investigated as extensively as in the prostate.

In the rat prostate, microvasculature and ultrastructural features have been investigated and subepithelial capillaries have been detected by scanning electron microscopy with corrosion casts and TEM (Shabsigh et al. 1999b). The vascular luminal area was found to be significantly decreased in size after castration, and was recovered following T supplementation using semi-quantitative procedure by light microscopy with $\times 400$ magnification (Lissbrant et al. 1998). We have extended these investigations and revealed

the androgen-dependent changes in the luminal area and the morphological features of the subepithelial capillaries correlating with the organ blood flow using TEM and laser Doppler flowmetry in androgen ablation and replacement models of rat prostate (Ono et al. 2004). We speculated that these mechanisms showing the basic roles in epithelial-stromal interaction would also occur in SV and designed this study. In addition, we have revealed that castration induces the reductive changes in the subepithelial capillaries and thickening of the extracellular matrix surrounding the capillaries in immature rat prostate and SV using TEM images (Ono et al. 2003). We herein focused on these subepithelial capillaries in adult rat models to extend the studies using TEM and laser Doppler flowmetry.

As a result, in the present study we revealed that the luminal area and the organ blood flow were significantly reduced after castration with a shift of capillary type from type 1 (oval opened) and type 2 (intermediate) to type 3 (collapsed), and these changes after castration were significantly protected by graded T supplementation in SV also. Type 1 capillaries likely maintain good blood flow and type 3 showed interrupted blood flow. Organ blood flow was minimal on day 2 (21.9 ml · min⁻¹/100 g of organ weight) and increased on day 3 (29.6 ml · min⁻¹/100 g of organ weight) after castration (Fig. 2B). This suggests that rapid reduction of the organ blood flow proceeds to the reduction of organ weight by a rapid shifting of capillary type from type 1 or 2 to type 3.

This tendency has been revealed in a rat prostate model (Ono et al. 2004). On the contrary, in T supplementation groups, the organ weight of SV increased with the increase of organ blood flow and with the shift of capillary type from type 2 or 3 to type 1 in a T10¹ group. It has been reported that excessive testosterone induces the proliferation of the epithelial cells and organ enlargement in SV (Mata 1995). This suggests that excessive supplementation of testosterone regulates organ blood flow at a higher than normal level in correlation with the organ enlargement. The results in this study demonstrated that organ blood flow and morphological features especially in subepithelial capillaries depend on androgen. Quantitative and morphological evaluations in estrogen-supplementation models and in stromal capillaries are the subjects for upcoming studies.

For further investigation of stromal-epithelial interaction, blood flow regulatory factors which are directly or indirectly dependent on androgen are very important, because controlling such factors is directly connected with strategies to treat hyperplastic or neoplastic diseases in accessory sex organs including the prostate. Some studies have suggested that nitric oxide (NO) and vascular endothelial growth factor (VEGF) are regulatory factors of the androgen-dependent local blood flow in the rat prostate (Häggström et al. 1998; Hayek et al. 1999; Burchardt et al. 2000). In SV as well, several studies concerning the local blood flow regulation and the changes in VEGF and NO depend on androgen have been reported (Brown et al. 1995; Chamness et al. 1995; Joseph et al. 1997). A fine and end capillary itself has no regulatory mechanism for blood flow, but terminal arterioles or precapillary sphincters that proximate to the fine capillaries do (Harris and Longnecker 1971). Vasoactive intestinal polypeptide (VIP) has also been reported to be an important factor in control of androgen-specific vascular smooth muscle, and whether the detailed functional mechanism depends on androgen in rat SV and prostate remains unclear (Guijarro et al. 1991; Barroso et al. 1999). These factors mediate

the androgen-dependent microcirculatory control in SV. However, it is still unclear what cells mediate to control these factors directly or indirectly via androgen, whether these interactions are regulated via androgen receptor or not, and why this androgen-dependent blood control system is specific to the SV and prostate.

Laser Doppler flowmetry has been fully investigated in its principles and has been widely used in clinical and experimental fields (Stern 1975; John et al. 2002; Ono et al. 2004). The laser Doppler flowmetry system used in the present study was designed to measure the blood flow within a radius of 1 mm around the tip of a laser probe using a 780 nm laser, and to automatically compute the blood flow ($\text{ml} \cdot \text{min}^{-1}/100 \text{ g}$ of organ weight). However, it was of limited use in measuring blood flow in very small organs in SV on day 7 after castration. A probe with a smaller tip or non-contact type probe would be more useful for measuring smaller areas.

Further investigation is required to clarify the regulatory mechanism of the SV microcirculatory system. We used TEM and laser Doppler flowmetry to demonstrate that the changes of the capillaries depended on androgen, and that the reduction in the luminal area of the subepithelial capillaries after castration was morphologically and quantitatively prevented by gradational T supplementation.

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