

# The Changes of Prostatic Microvessel Density in Sprague-Dawleyrats After Castration Under the Effect of Estrogen/ Androgen at Different Concentrations

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## Research Article

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# Abstract

**Background:** Currently, there are relatively few studies on the effects of changes in estrogen and androgen levels on prostatic MVD. This article aimed to study the changes of prostatic MVD in SD rats after castration under the effect of estrogen/androgen at different concentrations.

**Methods:** Male Sprague-Dawley (SD) rats aged 3-4 months were randomly divided into the control group, castration group, and different concentrations of estrogen/ androgen treatment after castration. Dihydrotestosterone (DHT) and estradiol (E) were administered daily by subcutaneous injection for one month. All the rats were sacrificed by cervical dislocation after one month, and the serum DHT and E concentrations of the rats in each group were measured by ELISA assay. Prostate tissues specimens were immunohistochemically stained with monoclonal antibodies against CD-34 and factor VIII for the MVD.

**Results:** Compared with the control group, the MVD decreased significantly in the castration group ( $P < 0.05$ ). When the exogenous E concentration was constant, in general, the MVD of rats in all the groups increased with the increase of exogenous DHT concentration; Among them, compared with the castration group, the MVD increased significantly in the E0.05+DHT0.015 mg/kg group, E0.05+DHT0.05 mg/kg group, E0.05+DHT0.15 mg/kg group, E0.05+DHT0.5 mg/kg group, and E0.05+DHT1.5 mg/kg group ( $P < 0.05$ ). In addition, when the exogenous DHT concentration was constant, the MVD increased with the increase of exogenous E concentration in all the groups; Among them, compared with the control and castration group, the MVD increased significantly in the DHT0.15+E0.015 mg/kg group, DHT0.15+E0.15 mg/kg group, and DHT0.15+E0.5 mg/kg group ( $P < 0.05$ ).

**Conclusions:** Androgens carried an important role in the regulation of prostatic MVD in SD rats, and the decrease of DHT concentration can induce a decrease in prostatic MVD. In contrast, prostatic MVD can be increased with the increase of DHT concentration. In addition, prostatic MVD can be increased gradually with the increase of estrogen concentration.

## Background

Transurethral resection of the prostate (TURP) is currently one of the major treatments for benign prostatic hyperplasia (BPH)<sup>[1]</sup>. Bleeding constitutes one of the complications of TURP, and its cause has attracted increasing attention. Increased bleeding during TURP has been found to be associated with increased prostate volume and increased angiogenesis in prostate tissue<sup>[2]</sup>. Lekås et al.<sup>[3]</sup> found that castration reduced the number and the proliferation rate of endothelial cells in the ventral prostate of adult rats. However, after testosterone supplementation with androgen levels, the number and the proliferation rate of endothelial cells can be restored to normal levels. In addition, testosterone treatment can rapidly normalize ventral prostatic blood flow in adult rats<sup>[4]</sup>. Therefore, these studies suggested that androgen can directly or indirectly regulate the vasculature in rat prostate tissue.

On the other hand, we noted that normal males have a certain concentration of estrogen and in the prostate epithelium and stroma, there exist a large number of estrogen receptors, the activation of which

also profoundly affects the development and progression of prostate diseases<sup>[5]</sup>. Estrogen induces remodeling of the collateral vasculature, and may stimulate the growth of resistance vessels, thereby providing protection during the development of coronary artery occlusion<sup>[6]</sup>. In addition, administration of oestrogen in vitro or in vivo can promote the proliferation and migration of endothelial cells, thereby promoting the formation of new blood vessels<sup>[7]</sup>.

Currently, there are relatively few studies on the effects of changes in estrogen and androgen levels on prostatic MVD. Therefore, this article aimed to study the changes of prostatic MVD in SD rats after castration under the effect of estrogen/androgen at different concentrations, and to provide a new theoretical basis for revealing the influence of sex hormone levels on prostatic MVD.

## Methods

### 1.1 Experimental Animals

52 male SD rats, aged 3 to 4 months, weighing 250g-350g, were provided by the Experimental Animal Center of Guizhou Medical University. License no. SCXK (Guizhou) 2012-0001. The animals were handled in accordance with the "Opinions under the Guidance of Treating Experimental Animals". All the animals (2 rats in each cage) were housed at a room temperature of 20~26°C and relative humidity of 40%~70% during a light cycle from 8:00 to 20:00. During the feeding period, the animals were free to access water and food (soy-free rodent diet). This experiment was approved by the Ethics Committee of Guizhou Provincial People's Hospital (No. 2018025).

### 1.2 Surgery and experimental groups

SD rats were castrated by removing both testis through the scrotal approach under general anesthesia. The rats were divided into 14 groups with 4 rats in each group<sup>[8,9]</sup>, thereinto, the E0.05+DHT0.15 mg/kg group and DHT0.15+E0.05 mg/kg group were the same group. The experimental protocol was shown in Table 1.

### 1.3 Detected the MVD of the prostate tissue in each group of SD rats

The immunohistochemical method was used to detect the expression of CD-34 and factor VIII in the prostate tissue of SD rats in each group. Five areas of each specimen were randomly selected, and the number of CD-34 and factor VIII positive cells in each high-power field of vision were counted to calculate the positive incidence of CD-34 and factor VIII by using the light microscope at a magnification of 400×. Furthermore, the mean score of CD-34 and factor VIII positive cells were calculated in each specimen to obtain the MVD<sup>[10]</sup>.

### 1.4 Statistical Methods

The experimental data were statistically processed by SPSS24.0 software. Nonparametric tests and [one-way analysis of variance](#) were used to analysis the result. A value of  $P<0.05$  was considered statistically significant.

## Results

### 1.1 The changes of serum DHT and E concentrations in each group

As shown in Figure 1, compared with the control group, the serum DHT concentration significantly decreased in the castration group ( $P<0.05$ ). When the concentration of exogenous E was constant, the serum DHT concentration increased with the increase of exogenous DHT concentration. In addition, compared with the castration group, the serum DHT concentration significantly increased in the E0.05+DHT0.05 mg/kg group, E0.05+DHT0.15 mg/kg group, E0.05+DHT0.5 mg/kg group, and E0.05+DHT1.5 mg/kg group ( $P<0.05$ ).

As shown in Figure 2, when the exogenous DHT concentration was constant, and the serum E concentration increased with the increase of exogenous E concentration. Furthermore, compared with the control and castration groups, the serum E concentration increased significantly in the DHT0.15+E0.015 mg/kg group, DHT0.15+E0.05 mg/kg group, DHT0.15+E0.15 mg/kg group and DHT0.15+E0.5 mg/kg group ( $P<0.05$ ).

### 1.2 The expression of CD-34 and factor VIII in each group

The expression of CD-34 and factor VIII in the prostate tissue of SD rats in each group was detected by immunohistochemistry (Figure 3 and 4), and the quantitative analysis showed that the positive rate of CD-34 in the castration group was significantly decreased compared with the control group ( $P<0.05$ ); However, the positive rate of factor VIII increased compared with the control group, but the difference was not statistically significant.

When the exogenous E concentration was constant (Figure 5), except for the E0.05+DHT0.015 mg/kg group, the positive rate of CD-34 gradually increased with the increase of exogenous DHT concentration in the E0.05+DHT0 mg/kg, E0.05+DHT0.05 mg/kg, E0.05+DHT0.15 mg/kg, E0.05+DHT0.5 mg/kg, and E0.05+DHT1.5 mg/kg groups. Among them, the E0.05+DHT1.5 mg/kg group compared with the control group, the difference was statistically significant ( $P<0.05$ ). The difference between the E0.05+DHT0.15 mg/kg group, E0.05+DHT0.5 mg/kg group and E0.05+DHT1.5 mg/kg group were statistically significant ( $P<0.05$ ) compared with the castration group. However, there was no significant linear relationship between Factor VIII positive rate expression and DHT concentration; the positive rate increased significantly in the E0.05+DHT0.015 mg/kg group, E0.05+DHT0.05 mg/kg group, and E0.05+DHT0.15 mg/kg group compared with the control and castration groups ( $P<0.05$ ).

When the exogenous DHT was constant (Figure 6), the positive rate of CD-34 expression in each group gradually increased with the increase of exogenous E concentration; among them, compared with the

castration group, there appeared a significantly higher positive rate in the DHT0.15+E0.015 mg/kg group, DHT0.15+E0.05 mg/kg group, DHT0.15+E0.15 mg/kg group, and DHT0.15+E0.5 mg/kg group, the difference was statistically significant ( $P<0.05$ ). In addition, compared with the control and castration group, the expression of factor VIII positive rate increased significantly in the DHT0.15+E0.005 mg/kg group, DHT0.15+E0.015 mg/kg group, DHT0.15+E0.05 mg/kg group, and DHT0.15+E0.5 mg/kg group ( $P<0.05$ ).

### 1.3 The prostatic MVD in each group

As shown in Figure 7 and 8, compared with the control group, the MVD decreased significantly in the castration group ( $P<0.05$ ). When the exogenous E concentration was constant (Figure 7), in general, the MVD of rats in all the groups increased with the increase of exogenous DHT concentration; Among them, compared with the castration group, the MVD increased significantly in the E0.05+DHT0.015 mg/kg group, E0.05+DHT0.05 mg/kg group, E0.05+DHT0.15 mg/kg group, E0.05+DHT0.5 mg/kg group, and E0.05+DHT1.5 mg/kg group ( $P<0.05$ ). In addition, when the exogenous DHT concentration was constant (Figure 8), the MVD increased with the increase of exogenous E concentration in all the groups; Among them, compared with the control and castration group, the MVD increased significantly in the DHT0.15+E0.015 mg/kg group, DHT0.15+E0.15 mg/kg group, and DHT0.15+E0.5 mg/kg group ( $P<0.05$ ).

## Discussion

Benign prostatic hyperplasia is one of the prevalent diseases among middle-aged and elderly men, and the exact pathogenesis remains unclear. At present, TURP is the gold standard for the surgical treatment of BPH<sup>[11]</sup>, with less painful, less invasive, and faster recovery. However, intraoperative bleeding is still a common complication that can be life-threatening for patients. Deering et al.<sup>[12]</sup> found that the prostate tissue of BPH patients may contain an "angiogenic switch" that can lead to an increase in MVD, and that this angiogenic effect may begin in the early stages of BPH. MVD is currently recognized as the standard for the evaluation of angiogenesis. Immunohistochemical staining is performed using specific vascular endothelial monoclonal antibody against specific vascular endothelial components (cell adhesion molecule CD-34 or factor VIII antigen) to display microvascular components of tissues and calculate MVD values. Therefore, both CD-34 and factor VIII can be used to assess MVD, with the successful application reported by Bettencourt et al.<sup>[13]</sup> and Gettman et al.<sup>[14]</sup>.

Finasteride, as a non-competitive 5 $\alpha$ -reductase inhibitor, inhibits the conversion of testosterone to DHT, and reduces the concentration of DHT in vivos<sup>[15]</sup>. After preoperative administration of finasteride, a reduction in DHT concentration was found to be associated with a significant reduction in perioperative bleeding of TURP<sup>[16,17]</sup>. This may be related to the reduction in DHT concentration, atrophy of the prostate gland, MVD, and lower blood flow after finasteride treatment<sup>[18-20]</sup>. In addition, it has been suggested that androgens can regulate the vascular distribution in the prostate by regulating different growth factors, such as basic fibroblast growth factor, vascular endothelial growth factor, and epidermal growth factor and so on<sup>[18,21-23]</sup>. Therefore, changes in androgen levels are essential for the regulation of prostate MVD.

In this study, rats were induced by castration combined with different concentrations of estrogen/androgen, and the results showed that the prostatic MVD in the castration group was significantly lower than that in the control group due to androgen source blocking. Some studies have demonstrated that testosterone acts as a stimulator of vascular endothelial growth factor, while androgen deprivation could lead to reduced blood flow to the prostate<sup>[24-26]</sup>. Furthermore, androgenic effects are mediated by vasoactive substances produced by dihydrotestosterone-regulated stroma and epithelial cells, which contain androgen receptors<sup>[27]</sup>. In this study, when exogenous estrogen concentration was constant, the prostate MVD of rats in each group increased with the increase of exogenous DHT concentration except for the E0.05+DHT0.015 mg/kg group. Furthermore, we confirmed that in vitro experiments that androgens play an important role in regulating prostate MVD. The decrease of DHT concentration can cause the decrease of prostatic MVD. Conversely, prostatic MVD can be increased with the increase of DHT concentration. Therefore, for patients with a large prostate volume scheduled for TURP, preoperative non-competitive 5 $\alpha$ -reductase inhibitor to reduce DHT concentration in vivo can help reduce the risk of perioperative bleeding.

In our clinical experience, we have found that BPH rarely occurs in young patients with relatively high androgen levels and low estrogen levels, and more often in middle-aged and elderly patients with relatively low androgen levels and high estrogen levels. Devlin et al.<sup>[28]</sup> found that, in the aging male, serum androgen levels are decreased and estrogen levels are relatively increased, which can be the possibly vital reasons for the development of BPH. Therefore, it is important to clarify the effect of changes in estrogen levels on the MVD of prostate tissue using TURP treatment in patients with enlarged prostate. Estrogen plays an important role in angiogenesis in a variety of tissues by increasing coronary blood flow and decreasing coronary resistance and peripheral vascular tension. In this sense, estrogen could induce endothelial cells proliferation and migration, and increase the expression of vascular endothelial growth factor<sup>[29]</sup>. In addition, Zhang et al.<sup>[30]</sup> studied on changes in bone microvascular structure and vascular density after exogenous estrogen supplementation in rats, and found that the changes in estrogen levels could affect the bone changes in MVD, and that the formation of blood vessels in bone increases with increased dose of estrogen. Diedrich et al.<sup>[31]</sup> reported the effect of vulvovaginal atrophy and local estrogen therapy on vaginal microcirculation structure. Using in vivo non-invasive techniques, estrogen therapy was found to have a significant effect on vaginal microcirculation structure, and local estrogen therapy can restore vaginal blood vessels.

At present, there is still a lack of relevant researches on the effect of changes in estrogen levels on MVD of prostate tissue. This is the first systematic study in vitro of the effect of estrogen/androgen changes on prostate MVD. We not only confirmed that androgens play an important role in the regulation of MVD in the prostate of SD rats. In addition, when SD rats were castrated, a certain concentration of exogenous DHT was supplemented to increase estrogen concentration. We first confirmed that the prostatic MVD can be increased with the increase of estrogen concentration. However, it is worth noting that the angiogenic effects of estrogen are caused by several mechanisms. On the one hand, estrogen replacement therapy can increase the expression of vascular endothelial growth factor and its

receptor<sup>[32]</sup>, which regulates endothelial cell proliferation and permeability<sup>[33,34]</sup>. On the other hand, at weeks 3 and 4 of estrogen therapy, estrogen can increase endothelial progenitor cells, directly stimulate their mitosis and migration activity, and inhibit apoptosis of endothelial progenitor cells<sup>[35,36]</sup>. Furthermore, the angiogenic effects of estrogen depend on the form, dose, and duration of administration, as well as on the cellular environment<sup>[37]</sup>. It is suggested that long-term estrogen replacement therapy may increase the expression of vascular endothelial growth factor and endothelial progenitor cells, thereby increasing the synthesis, migration, and lumen formation of prostate microvascular endothelial cells. Without doubt, it is of necessity to be confirmed by more comprehensive and systematic experiments in vivo and in vitro.

In addition, in this study, we also found that the incidence of factor VIII positivity was different from that of positive CD-34. These two endothelial cell antigens, namely factor VIII and CD-34, mark blood vessels of various maturity<sup>[10]</sup>. Anti-CD34, an antibody that is very sensitive to endothelial cell differentiation, stains tumor endothelial cells more strongly than normal endothelial cells<sup>[38,39]</sup>. Da Silva et al.<sup>[40]</sup> found that the MVD detected by anti-CD34 was significantly higher than that of anti-factor VIII in breast cancer tissues. However, there exists a lack of systematic studies on whether there is a difference between CD-34 and factor VIII in detecting MVD in non-tumour tissues, especially in the prostate, which provides a new direction for our research in the next stage.

## **Conclusion**

Androgens carried an important role in the regulation of prostatic MVD in SD rats, and the decrease of DHT concentration can induce a decrease in prostatic MVD. In contrast, prostatic MVD can be increased with the increase of DHT concentration. In addition, when rats were supplemented with a constant dose of exogenous DHT, their prostatic MVD increased gradually with the increase of estrogen concentration.

## **Declarations**

### **Ethics approval and consent to participate**

This experiment was approved by the Ethics Committee of Guizhou Provincial People's Hospital (No. 2018025). The committee approved the requirement for verbal informed consent to be obtained from participants.

### **Consent for publication**

Written informed consent was obtained from all participants..

### **Availability of data and materials**

The datasets analyzed during the current study is available from the corresponding author on reasonable request.

## Competing interests

The authors declare that they have no competing interest.

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## Authors' contributions

BW contributed to the study design, data collection, interpretation and manuscript writing. YB and ZLS contributed to data collection and interpretation. YT and GHJ contributed to data analysis and manuscript writing. All authors read and approved the final manuscript.

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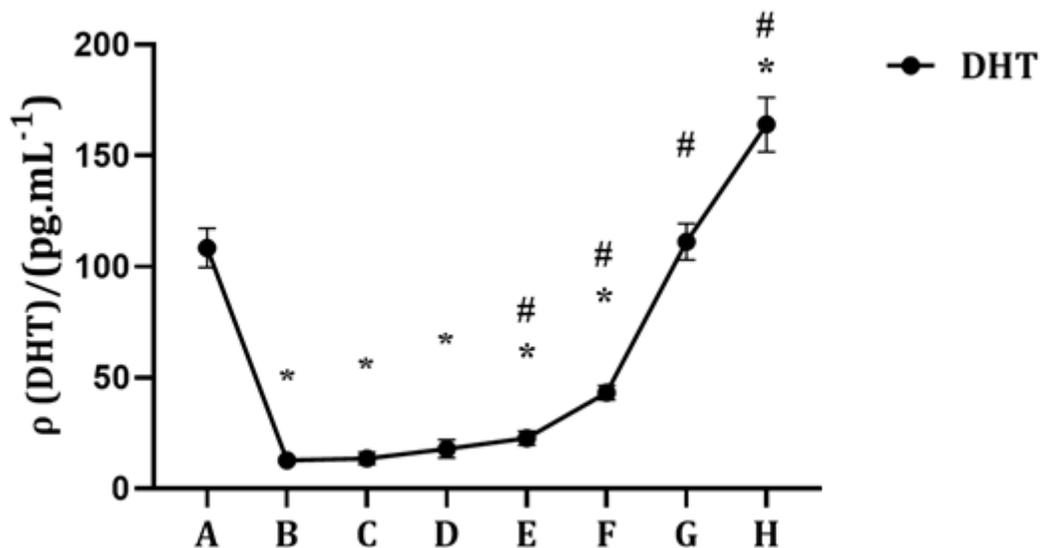
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## Tables

### Table 1 Structure of the Experiment

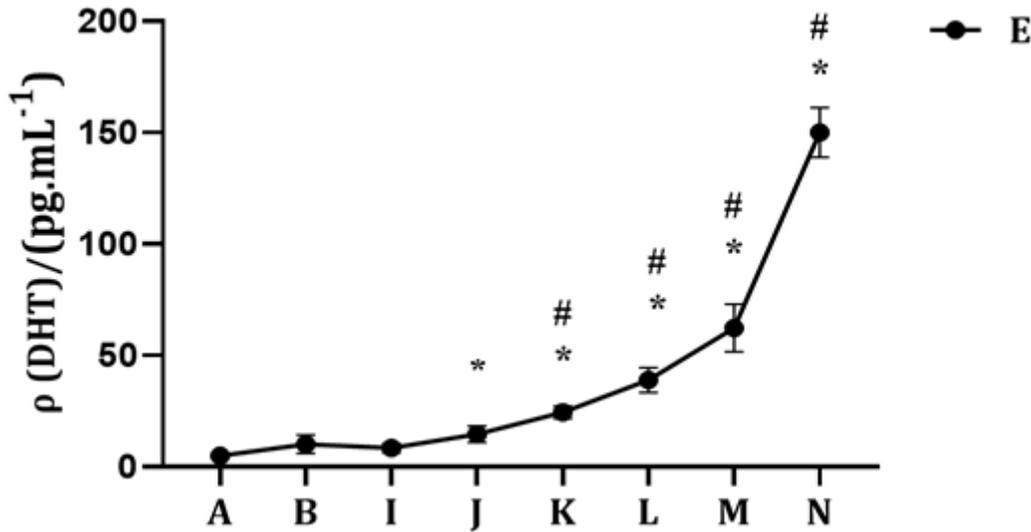
Group	Surgery	Agents and doses	n
Control group	Non-castrated	Corn oil	4
Castration group	Castrated	Corn oil	4
E0.05+DHT			
0	Castrated	E0.05 mg/kg+DHT0 mg/kg	4
0.015	Castrated	E0.05 mg/kg+DHT0.015 mg/kg	4
0.05	Castrated	E0.05 mg/kg+DHT0.05 mg/kg	4
0.15	Castrated	E0.05 mg/kg+DHT0.15 mg/kg	4
0.5	Castrated	E0.05 mg/kg+DHT0.05 mg/kg	4
1.5	Castrated	E0.05 mg/kg+DHT1.5 mg/kg	4
DHT0.15+E			
0	Castrated	DHT0.15 mg/kg+E0 mg/kg	4
0.005	Castrated	DHT0.15 mg/kg+E0.005 mg/kg	4
0.015	Castrated	DHT0.15 mg/kg+E0.015 mg/kg	4
0.05	Castrated	DHT0.15 mg/kg+E0.05 mg/kg	4
0.15	Castrated	DHT0.15 mg/kg+E0.15 mg/kg	4
0.5	Castrated	DHT0.15 mg/kg+E0.5 mg/kg	4

## Figures



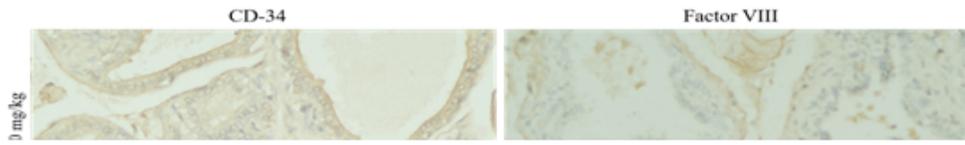
**Figure 1**

The changes of serum DHT concentrations of rats. The data are shown as mean  $\pm$  SD, and the statistical difference was determined using one-way analysis of variance. (n=4). \*P<0.05 compared to control group, #P<0.05 compared to castration group. A: Control group. B: Castration group. I: DHT0.15+E0 mg/kg group. J: DHT0.15+E0.005 mg/kg group. K: DHT0.15+E0.015 mg/kg group. L: DHT0.15+E0.05 mg/kg group. M: DHT0.15+E0.15 mg/kg group. N: DHT0.15+E0.5 mg/kg group.



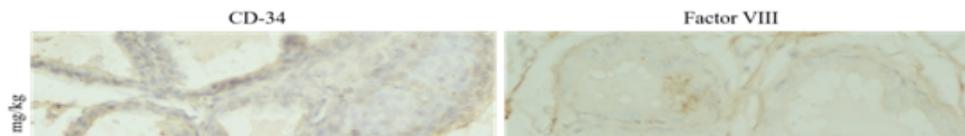
**Figure 2**

The changes of serum E concentrations of rats. The data are shown as mean  $\pm$  SD, and the statistical difference was determined using one-way analysis of variance. (n=4). \*P<0.05 compared to control group, #P<0.05 compared to castration group. A: Control group. B: Castration group. I: DHT0.15+E0 mg/kg group. J: DHT0.15+E0.005 mg/kg group. K: DHT0.15+E0.015 mg/kg group. L: DHT0.15+E0.05 mg/kg group. M: DHT0.15+E0.15 mg/kg group. N: DHT0.15+E0.5 mg/kg group.



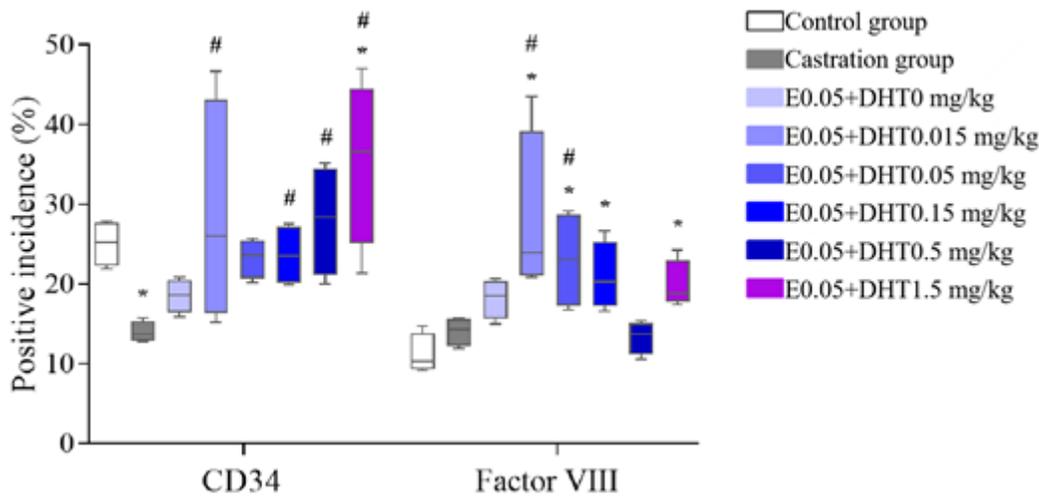
### Figure 3

The expressions of CD-34 and Factor VIII in different DHT concentration groups. IHC ×400



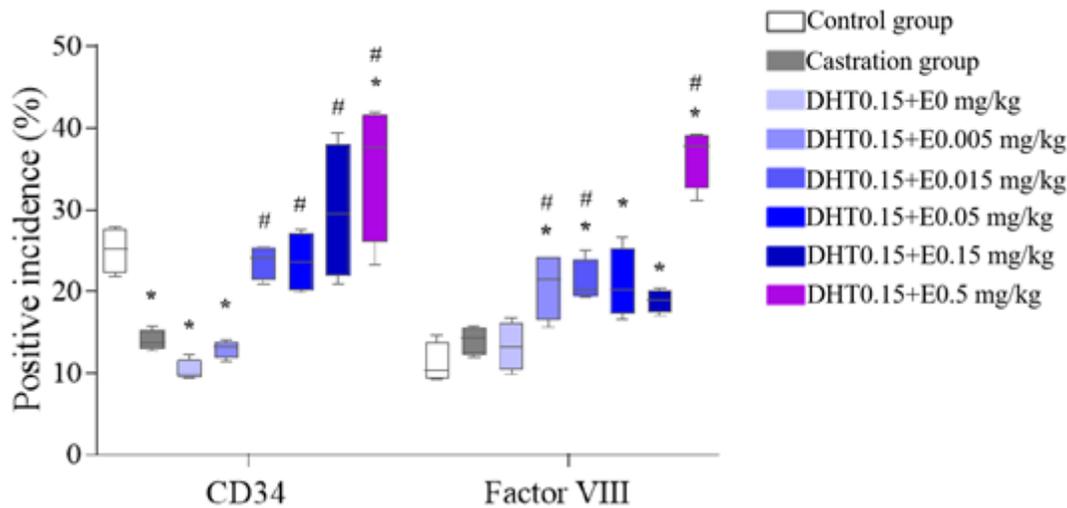
#### Figure 4

The expressions of CD-34 and Factor VIII in different E concentration groups. IHC ×400



**Figure 5**

The positive incidences of CD-34 and Factor VIII in groups treated with different concentrations of DHT. The data are shown as box-and-whisker plots. The plots show the minimum and maximum values (lower and upper horizontal black lines, respectively), the mean value (in black), the H-spread values (mean  $\pm$ 25%, box). Nonparametric tests was used to analysis the data. (n=4). \*P<0.05 compared to control group, #P<0.05 compared to castration group.



**Figure 6**

The positive incidences of CD-34 and Factor VIII in groups treated with different concentrations of E. The data are shown as box-and-whisker plots. The plots show the minimum and maximum values (lower and upper horizontal black lines, respectively), the mean value (in black), the H-spread values (mean  $\pm$ 25%, box). Nonparametric tests was used to analysis the data. (n=4). \*P<0.05 compared to control group, #P<0.05 compared to castration group.

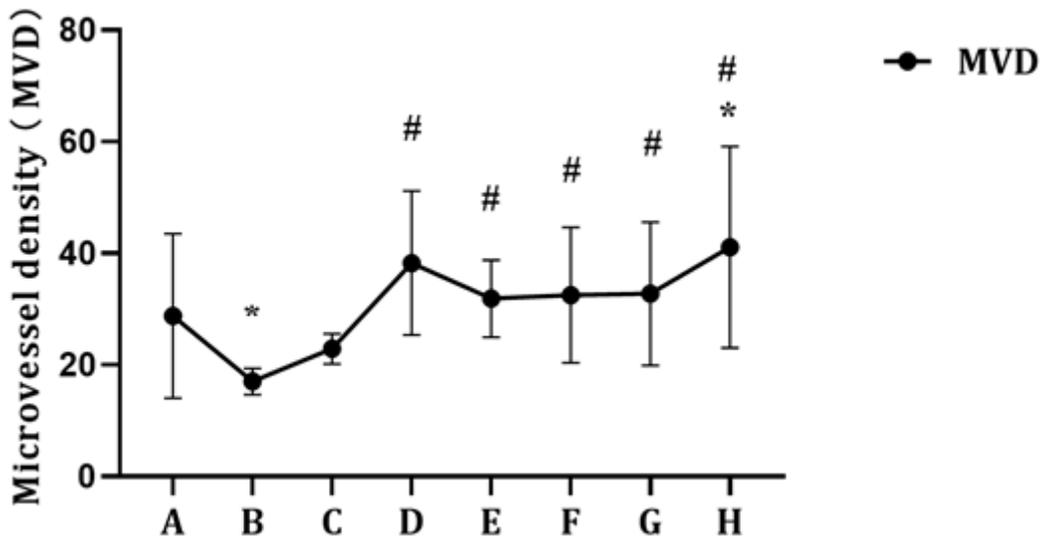


Figure 7

The prostatic microvessel density (MVD) of rats treated with different concentrations of DHT. The data are shown as mean  $\pm$  SD, and the statistical difference was determined using one-way analysis of variance. (n=4). \*P<0.05 compared to control group, #P<0.05 compared to castration group. A: Control group. B: Castration group. C: E0.05+DHT0 mg/kg group. D: E0.05+DHT0.015 mg/kg group. E: E0.05+DHT0.05 mg/kg group. F: E0.05+DHT0.15 mg/kg group. G: E0.05+DHT0.5 mg/kg group. H: E0.05+DHT1.5 mg/kg group.

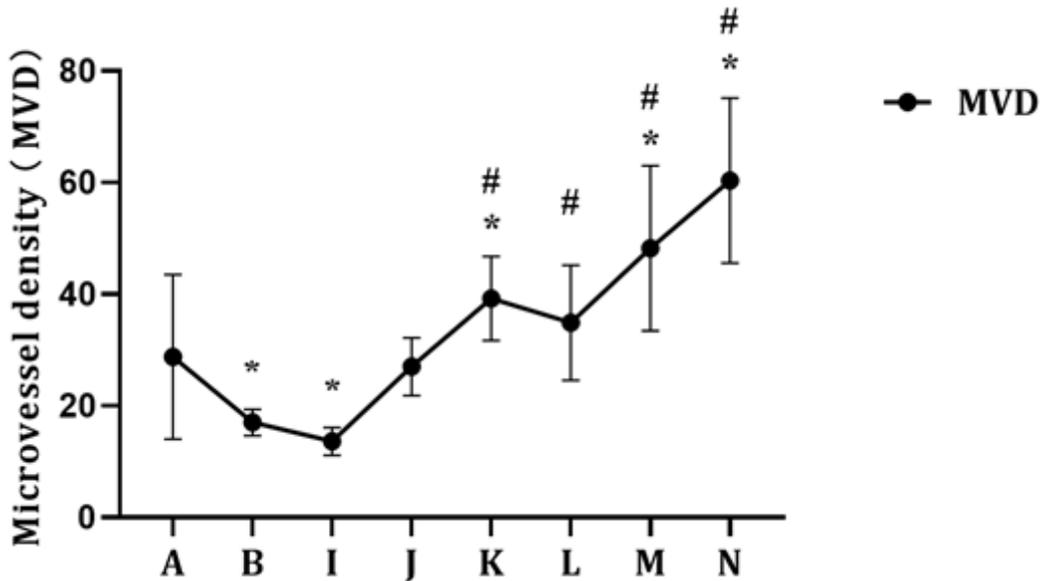


Figure 8

The prostatic microvessel density (MVD) of rats treated with different concentrations of E. The data are shown as mean  $\pm$  SD, and the statistical difference was determined using one-way analysis of variance.

(n=4). \*P<0.05 compared to control group, #P<0.05 compared to castration group. A: Control group. B: Castration group. I: DHT0.15+E0 mg/kg group. J: DHT0.15+E0.005 mg/kg group. K: DHT0.15+E0.015 mg/kg group. L: DHT0.15+E0.05 mg/kg group. M: DHT0.15+E0.15 mg/kg group. N: DHT0.15+E0.5 mg/kg group.