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# THE ROLE OF TESTOSTERONE IN MYOCARDIAL VASCULARIZATION AND CORONARY VASODILATION IN MALE RABBITS DURING SEXUAL MATURATION

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## SUMMARY

It has been reported that coronary vasodilator reserve (CVDR) is reduced at the age of sexual maturity. In this report, we studied the influence of testosterone (T) and / or castration (C) on CVDR, capillary density (CD) and capillary - to - fiber ratio (C/FR) in male rabbits during sexual maturation. Twenty four immature male rabbits of about similar age and weight were divided into four equal groups, treated as follows: 1) Intact + oil (I+O); 2) Castrated + Oil (C+O); 3) Castrated + testosterone (C+T); and 4) Intact + testosterone (I+T). Testosterone was given daily for 2 months (25 ug/Kg body weight) dissolved in 0.1 ml corn oil (i.m.) to groups 3 and 4. Groups 1 and 2 received same volume of solvent. Control (baseline) coronary flow (CCF) and maximum coronary flow (MCF) "induced by reactive hyperemia" were measured in Langendorff - perfused hearts. Histological sections were made to measure CD and C/FR. The results showed that CCF (ml/min/g) was reduced in I+T hearts. MCF of groups C+T and I+T was significantly attenuated when compared with its values in groups I+O and C+O. CD and

C/FR were both lowered in hearts of I+T group in comparison to their values in hearts of the other three groups. Data of this study suggest that, during maturation, testosterone could be one of the factors that reduces CVDR, CD and C/FR in male rabbits.

## INTRODUCTION

A number of important changes in the fiber - capillary relationship take place during the physiological growth of mammalian heart. Morphological studies have revealed a gradual decline in fiber - to - capillary ratio of the developing heart as a result of capillary proliferation. This ratio is highest in the neonate (about 4:1) and reaches almost unity at the age of sexual maturity (Wearn, 1940; Rakusan *et al.*, 1965 and Tomanek and Hovanec, 1981). Despite the increase in capillary proliferation, myocardial capillary density decreases gradually after birth and levels off at the age of sexual maturity as a result of muscle fiber hypertrophy (Wearn, 1941; Rakusan *et al.*, 1967 and Tomanek *et al.*, 1982). More recently, Toma and coworkers (Toma, 1985 and Toma *et al.*, 1985) showed that coronary vasodilator reserve decreases in a similar fashion. It is highest in the neonate and declines gradually during maturation and levels off at the age of sexual maturity. On the other hand steroid sex hormones are known to have a regulatory effect on some myocardial metabolic activities (Schiable *et al.*,1984 and Scheuer *et al.*, 1987). The effect of testosterone on myocardial vascularization has not been studied. Since the morphological and physiological changes in the developing heart take place during sexual maturation, therefore, we designed this study to investigate the effect of testosterone and/or castration on coronary vasodilator

reserve (CVDR), capillary density (CD) and capillary - to - fiber ratio (C/FR) in the male rabbit heart during sexual maturation.

## MATERIALS AND METHODS

### Animals and Experimental Design:

Twenty four immature rabbits (50-60 days old and 1.0-1.4 Kg body weight) were randomly divided into 4 equal groups. Animals of the first group were shamly operated (by incising then suturing of the scrotal skin) and injected with the vehicle (corn oil). This group was named : Intact - sham operated + Oil (I+O). Animals of the second group were castrated and treated with oil, and thus were named: Castrated + Oil (C+O) group. The third group consisted of castrated animals to which testosterone was administered and were reffered to as : Castrated + Testosterone (C+T). The forth group consisted of sham operated animals and received testosterone and were reffered to as : Intact sham operated + Testosterone (I+T).

Testosterone was administered intramuscularily to groups (C+T) and (I+T) at a dose of 25 ug/kg body weight as testosterone anatate (Shering AG Berline/Bergkamen, Germany) dissolved in 0.1 ml corn oil. The same volume of the vehicle was administered (i.m.) to the other two groups. All groups had this treatment daily for 2 months, where the animals reach sexual maturity. All animals had the same housing and feeding managements over the period of experimentation.

### Perfusion of the isolated heart:

At the end of the 60 day treatment, the animals were sacrificed (stunned by a blow to the head) to study

coronary perfusion by a Langendorff preparation described earlier (Toma *et al.*, 1985 and Toma *et al.*, 1988). Following a 30 minute period of equilibration, baseline and maximum coronary flow (CF) were measured by time collection of the coronary effluent from the pulmonary artery and were expressed as ml/min/g of wet heart weight. Maximum coronary flow was obtained as the reactive hyperemic response following a 45 second occlusion period of the aortic cannula.

Preparation of histological sections:

At the end of each experiment, heart weight was measured after trimming away the great blood vessels and fat, and blotting with filter paper. The left and right ventricles were separated at the septal wall, and each ventricle was cut into 3 (upper, middle and lower) pieces, then transferred into Bouin's fixative (Humason, 1967). Serial histological sections (6 microns thick) were prepared from the middle portion of each ventricle and were stained with hematoxylin and eosin for further histological examination.

Determination of capillary density and capillary-to-fiber ratio:

Histological sections were examined under the high power lense (x100) of a light microscope for counting CD. The number of capillaries in 20 microscopic fields was counted which represents a surface area of about 1 mm (Kayer and Banchero, 1985). Total CD (cap/mm$^2$) of the ventricles and of the right and left ventricles were measured separately.

The number of capillaries and myofibriles in 3 microscopic fields was counted from each ventricular portion to calculate the C/FR (Bloor and Leon, 1970). The

capillaries were diagnosed by the single layer of endothelial cells lining the capillary vessels.

Statistical analysis:

Results are expressed as Mean±SEM. We used one - way between - group analysis of variance and least significant difference tests for multiple comparisons between mean data (Steel and Torrie, 1980) to compare values for baseline CF, maximum CF, heart weight, body weight, heart weight-to-body weight ratio, CD and C/FR in the 4 groups. Differences between mean data were considered significant at $P < 0.05$.

## RESULTS

Relationship between heart weight and body weight:

The data pertaining to the heart weight and body weight for each of the four groups are summarized in table 1. None of the parameters showed significant differences among the means of the four groups. However, the heart weight in (C+O) group was lower than the other groups, but it did not reach statistical significance.

Effect of testosterone and castration on coronary flow:

Table 2 illustrates the effects of testosterone and castration on baseline CF and maximum CF (ml/min/g wet heart weight) and total maximum CF (ml/min; i.e. coronary flow not corrected per cardiac mass which is considered as an index of vascular cross-sectional area) in the four groups of hearts. Testosterone has significantly ($P<0.05$) reduced the baseline CF in (I+T) group vs. the other three groups. Furthermore, maximum CF

Table 1 : Effect of Testosterone (T) and/or castration (C) on heart weight (HW), body weight (BW) and heart weight-to-body weight ratio. Data are prested as Mean±SEM.

| Parameters | Groups | | | |
|---|---|---|---|---|
| | I+O | C+O | C+T | I+T |
| Heart weight (g) | 4.14 ±0.1 | 3.87 ±0.08 | 4.45 ±0.1 | 4.21 ±0.07 |
| Body weight (g) | 1738 ±39.3 | 1705 ±61.9 | 1801 ±65.8 | 1695 ±28.9 |
| HW/BW ratio ($X10^{-3}$) | 2.38 ±0.1 | 2.28 ±0.1 | 2.47 ±0.1 | 2.48 ±0.07 |

Table 2 : Data of this table show levels (mean±SEM) of baseline coronary flow, maximum coronary flow and total maximum coronary flow in the four treated groups. * $P<0.05$ vs. the corresponding parameters.

| Parameters | Groups | | | |
|---|---|---|---|---|
| | I+O | C+O | C+T | I+T |
| Baseline CF (ml/min/g) | 5.3 ±0.3 | 5.5 ±0.2 | 5.1 ±0.3 | 3.8 * ±0.2 |
| Maximum CF (ml/min/g) | 8.4 ±0.2 | 9.0 ±0.8 | 6.8 * ±0.4 | 6.8 * ±0.2 * |
| Total maximum CF(ml/min) | 34.5 ±2.0 | 34.8 ±2.7 | 27.2 * ±0.8 | 28.5 * ±0.9 |

values were similar in the (C+T) and (I+T) groups, but were both significantly ($P<0.05$) lower than their corresponding values in the (I+O) and (C+O) groups. In addition, total maximum CF was also reduced by testosterone in (C+T) and (I+T) groups in a similar fashion. These results point out to the possibility that testosterone reduces the vasodilator capacity of the coronary vessels.

**Effect of castration and testosterone on capillary density:**

A. Total myocardial capillary density:

The total cardiac CD (capillary/$mm^2$) of each of the four groups is shown in figure 1. Exogenous testosterone (group I+T) caused a significant ($P<0.05$) reduction in total cardiac CD. The values of CD were : 2922±21; 3092±39; 3111±27 and 3134±23 cap/$mm^2$ for groups I+T; I+O; C+O and C+T, respectively. These results suggest that testosterone might have an inhibitory effect on myocardial capillary proliferation.

B. Capillary density of the left and right ventricles:

To test whether castration and/or testosterone preferentially affect capillary growth of one of the ventricles, we measured CD of the right and left ventricles separately. Figure 2 shows that indeed, additional testosterone (I+T) caused a significant ($P<0.05$) reduction in CD of the right but not of the left ventricle, where CD (cap/$mm^2$) of the right ventricle was : 2877±50; 2957±80; 3033±50 and 3095±25 for the groups I+T; I+O; C+O and C+T, respectively. Whereas in the left ventricle, the CD was : 2968±24 in I+T group which was

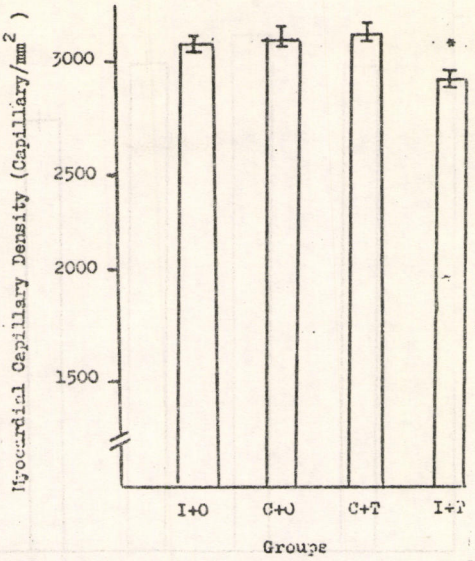


Figure 1: Effects of testosterone and/or castration on capillary density (Mean±SEM). * P<0.05 vs. groups I+O. C+O and C+T.

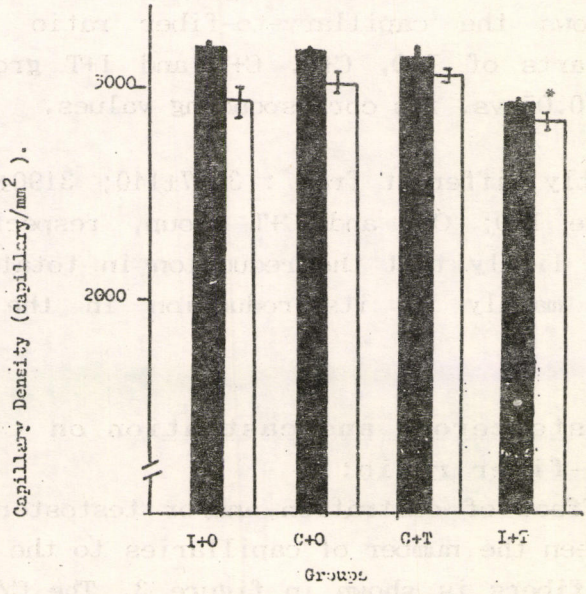


Figure 2: Illustrates the effect of castration and testosterone on capillary density of the Right and left ventricles. (Mean±SEM). * P<0.05 vs. hearts of I+O, C+O, and C+T groups.

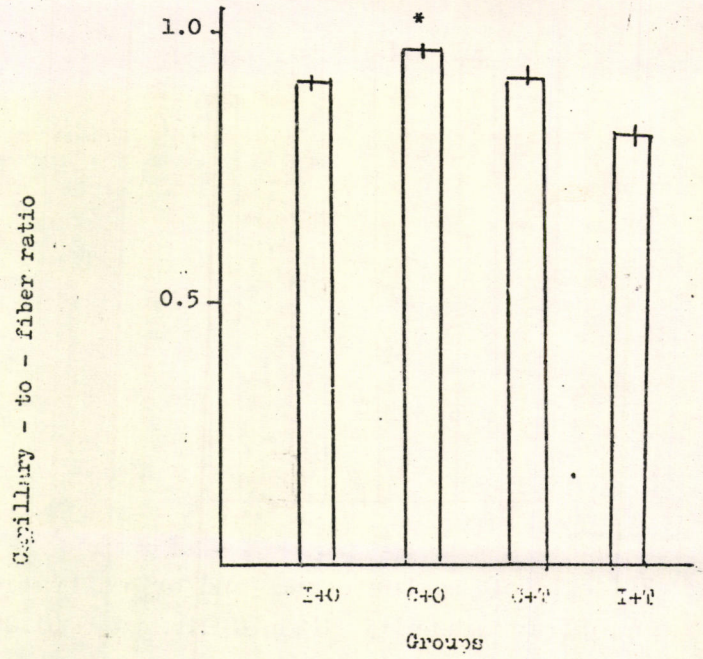


Figure 3: Shows the capillary-to-fiber ratio in the hearts of I+O, C+O, C+T and I+T groups. * P<0.05 vs. its corresponding values.

not significantly different from : 3227±140; 3190±32 and 3175±70 in the I+O; C+O and C+T group, respectively. Thus, it seems likely that the reduction in total CD is brought about mainly by its reduction in the right ventricle.

**Effect of testosterone and castration on capillary-to-fiber ratio:**

The effect of castration and/or testosterone on the ratio between the number of capillaries to the number of myocardial fibers is shown in figure 3. The C/FR was significantly (P<0.05) lower in the I+T group (0.82±0.02) vs 0.92±0.02; 0.93±0.02 and 0.98±0.02 for the I+O; C+T and C+O groups, respectively.

## DISCUSSION

The role of testosterone in myocardial vascularization during sexual maturation is unknown. We investigated the effect of testosterone and/or castration on cardiac vascularity (represented by CD and CVDR) in immature male rabbits. Laboratory male rabbits reach sexual maturity between 120 to 150 days of age (Hafez, 1970). Therefore, we have chosen animals of 60 day-old and treated them for another 60 days to reach sexual maturity. The dose of testosterone (25 ug/Kg body weight) was used according to its equivalent level in the plasma of male rabbits which does not inhibit the release of leutinizing hormone (Berger *et al.*, 1982). The use of Langendorff preparation in studying myocardial vascularization and coronary physiology has well been established by many investigators (Bunger *et al.*, 1972; Toma *et al.*, 1985 and Toma *et al.*, 1988). Furthermore, it has similar properties of the in vivo blood-perfused hearts (Wangler *et al.*, 1982).

### Effect of castration and testosterone on baseline and maximum coronary flow:

This study showed that exogenous testosterone reduced the baseline as well as the maximum coronary flow. Thus it seems likely that the decreased dilating capacity to reactive hyperemia reported in this study and the decreased CVDR reported by Toma *et al.* (1985) and Toma *et al.* (1988) might be related somehow to testicular testosterone during sexual maturity.

There are several possible explanation for these findings:

1) The decreased CVDR in C+T and I+T groups could not be due to an increase in growth of myocardial mass in respect to vascular growth, since we could not detect

a significant anabolic effect of testosterone on heart weight of these 2 groups (table 1).

2) Another possibility is that testosterone has increased coronary stiffness in the hearts of C+T and I+T groups. This is because testosterone increases the collagen-to-elastin ration in the aortic wall (Wilinsky, 1972). However, sexual maturation did not dramatically affect the coronary vasoelasticity during autoregulation of flow (Toma *et al.*, 1985). Yet, we can not rule out this possibility without further investigations.

3) Finally, it has been shown that testosterone decreases, and estrogen increases the release of $PGI_2$ from cultured coronary endothelial cells (Batres and Dupont, 1984). Since $PGI_2$ is a potent coronary vasodilator, it seems likely that testosterone might have reduced the release of $PGI_2$, and thus lowered the coronary vasodilating capacity reported in this study. However, further studies are needed to support this hypothesis.

Effect of testosterone on capillary density:

The results of this investigation showed that exogenous testosterone (group I+T) causes a significant reduction in cardiac CD, whereas the CD was almost similar in the hearts of the other 3 groups. These results suggest that the decrease in myocardial CD reported by Wearn (1941) and others during sexual maturation, might be related to testosterone release during this period.

Again, an increase in cardiac fiber growth, relative to capillary growth, can not be an explanation for the decreased CD, since heart weight was not altered

in this group (table 1). Malinow *et al.*, (1962) showed that testosterone has an inhibitory effect on some mitochondrial oxidative enzymes of the aortic wall, such as cytochrome oxidase and succinate dehydrogenase. Furthermore, Hudlicka (1984) proposed that local hypoxia is a potent stimulus for capillary proliferation. Thus, it seems likely that testosterone administration had inhibited the production of local hypoxia and hence lowered capillary proliferation and CD reported in this study.

Effect of testosterone on capillary-to-fiber ratio:

The direct effect of testosterone and castration on the myocardium has reveiled variable effects on C/FR. The results of this study showed that castration (group C+O) caused a significant increase in the C/FR in comparison to the other 3 groups. This increase in C/FR could be accounted for by the absence of the anabolic effect of testosterone on the growing heart.

In conclusion, the results of this study suggest that testosterone might have a depressive effect on myocardial vascularization, which is manifested by the decrease in coronary vasodilator reserve, decreased capillary density and decreased capillary-to-fiber ratio.

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## دور هورمون التستوستيرون في المد الوعائي القلبي والاحتياطي التوسعي التاجي في ذكور الارانب خلال مرحلة النضوج الجنسي

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### الخلاصـــــة

ان المد الوعائي في قلوب اللبائن يتمثل بالمعايير التالية: كثافة الشعيرات الدموية، نسبة الشعيرات الدموية الى الالياف العضلية القلبية والاحتياطي التوسعي للشرايين التاجية. لقد

اثبتت الدراسات التركيبية-الوظيفية السابقة بان المد الوعائي في القلب يقل تدريجيا خلال مراحل النمو المختلفة حيث يكون في اعلى مستوياته عند حديثي الولادة ويقل تدريجيا الى حين مرحلة البلوغ الجنسي، حيث يثبت تقريبا عند حد معين في المراحل التي تلي مرحلة البلوغ الجنسي. لذلك صمم هذا البحث لدراسة دور هورمون التستوستيرون في المد الوعائي القلبي في ذكور الارانب خلال مرحلة البلوغ الجنسي. استخدمنا في هذا البحث ٢٤ ارنبا ذكرا متقاربة الاوزان وبعمر ٥٠-٦٠ يوما. وزعت الارانب الى اربعة مجاميع متساوية كما يلي: المجموعة الاولى : شملت الحيوانات المخصية والمحقونة بزيت الذرة (C+O) المجموعة الثانية : تضمنت الحيوانات المخصية والمحقونة بالتستوستيرون (C+T) حيوانات المجموعة الثالثة اجريت لها عملية خصاء كاذبة وحقنت بزيت الذرة (I+O) اما حيوانات المجموعة الاخيرة فقد اجريت لها عملية خصاء كاذبة ايضا وتم حقنها بهورمون التستوستيرون (I+T) تم حقن الهورمون الذكري في العضل بجرعة (٢٥) مايكروغرام/كيلوغرام من وزن الجسم مذابا بـ (٠ر١) مل من زيت الذرة في مجموعتين C+T، I+T. اما المجموعتين الاخريين فقد تم حقن حيواناتهما بمقدار (٠ر١) مل من زيت الذرة في العضل. جرى الحقن بهذه المواد يوميا ولمدة شهرين، حيث يتم النضوج الجنسي خلال هذه المدة. في نهاية مدة التجربة تمت التضحية بالحيوانات واستؤصلت قلوبها لغرض اروائها بطريقة لانكندورف للقلوب المعزولة لغرض قياس الجريان التاجي القراري والاقصى المستحدث بواسطة فرط الدم التفاعلي حيث

ان الحريان التاجي الاقصى يمثل الاحتياطي التوسعي للاوعية الدموية. بعد ذلك تم تحضير شرائح نسيجية لغرض حساب كثافة الاوعية الشعرية الدموية ونسبة عدد الاوعية الشعرية الدموية الى عدد الالياف العضلية القلبية. اظهرت النتائج بان الحريان التاجي القراري قد انخفض بشكل معنوي في قلوب الارانب السليمة والمحقونة بالتستوستيرون (I+T) مقارنة مع المجاميع الاخرى. فضلا عن ذلك كان الحريان التاجي الاقصى للمجموعتين اللتين حقنتا بالتستوستيرون اقل بشكل ملحوظ عنه في المجموعيتين الاخريين. كما اظهرت هذه الدراسة ايضا بان كثافة الاوعية الشعرية الدموية ونسبتها الى الالياف العضلية كانت في مجموعة I+T اقل مما هي عليه في المجاميع الاخرى. ان نتائج هذه الدراسة تشير الى ان التستوستيرون قد يكون احد اسباب انخفاض المد الوعائي القلبي في ذكور اللبائن اثناء مرحلة النضوج الجنسي.