Functional arterio-venous anastomoses between the testicular artery and the pampiniform plexus in the spermatic cord of rams


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Summary. Blood flow to the testis, haemoglobin oxygen saturation and testosterone concentration in arterial and venous testicular blood vessels were studied in Texel rams in the breeding and non-breeding season.

Blood flow in the proximal and distal testicular artery was measured electromagnetically. The mean flow in the proximal testicular artery was 18.5 ml/min and in the distal testicular artery 7.5 ml/min, and there was no detectable seasonal influence.

Haemoglobin oxygen saturation and testosterone concentration were measured in the saphenous artery and vein, the distal testicular artery and vein, and in the proximal testicular vein. The haemoglobin oxygen saturation in the proximal testicular vein was significantly higher than in the distal testicular vein in both seasons. The mean testosterone concentration was significantly lower in the proximal testicular vein than in the distal testicular vein in both seasons. Based on haemoglobin oxygen saturation and testosterone data, it was calculated that between 28 and 46% of the testicular arterial blood was bypassing the testis and was directly flowing through arterio-venous anastomoses towards the pampiniform plexus in the spermatic cord of conscious rams. In anaesthetized rams 55 and 64% of the blood was flowing directly from the testicular artery to the pampiniform plexus based on blood flow data.

Transfer of testosterone and oxygen by passive diffusion from the testicular artery to the pampiniform plexus and vice versa in the spermatic cord was not detected.

Introduction

The single, very long and tightly coiled testicular artery is intimately associated with the pampiniform plexus (testicular veins) in the spermatic cord of most eutherian mammals. To this vascular complex several functions have been assigned, such as temperature regulation of the testicular blood (Waites & Moule, 1961), reduction of the testicular arterial pulse (Waites & Moule, 1960) and transfer of steroids from the pampiniform plexus to the testicular artery. Transfer of steroids is considered to take place by passive diffusion down a concentration gradient in rats (Free & Jaffe, 1975). However, the blood of rats does not contain testosterone binding globulin. In the ram, contradictory evidence has been reported on testosterone transfer (Jacks & Setchell, 1973; Cooper & Waites, 1974).

Anatomical evidence has been presented for direct arterio-venous anastomoses (shunts) between the testicular artery and the pampiniform plexus in the spermatic cord of the bull, ram and boar (Wensing & Dijkstra, 1981; Wensing, Dijkstra & Frankenhuys, 1981; Hees, Leiser, Kohler & Wrobel, 1984).
This study was designed (a) to identify a possible shunting of blood from the testicular artery to the pampiniform plexus in the spermatic cord of the ram, and (b) to investigate possible differences in shunting between the breeding (high testicular activity) and the non-breeding (low testicular activity) season.

Materials and Methods

Mature Texel rams in the breeding (November/December) and non-breeding (April/May) season were used.

Surgery was performed in all animals under general anaesthesia. The rams were premedicated with acepromazine (0·1 mg/kg i.m.; Vetranquil: Clin-nidy, Rijswijk, The Netherlands) and atropine sulphate (0·01 mg/kg i.m.). Anaesthesia was induced 20 min later with thiopentone sodium (10 mg/kg i.v.; Nesdonal: Rhône-Poulenc, Paris, France). Anaesthesia was maintained with pentobarbitone sodium (2·5 mg/kg i.v.; Nembutal: EVA, Paris, France), administered approximately every 30 min. The animals were ventilated throughout the experiments by means of a positive pressure respirator (Bennett BK-4) using a mixture of oxygen and nitrous oxide (1:1, v/v). Heart rate was recorded from the electrocardiogram which was derived from limb leads. Mean heart rates between 80 and 120 beats/min were accepted as physiological. Haemoglobin concentrations in venous saphenous blood samples were measured (Vitatron LCP/Hb, Vitatron Scientific Dieren, The Netherlands). Body temperature was monitored with a rectal probe and testicular temperature with a testicular probe connected to an electrothermometer (Ellab TE3: Ellab Instruments, Copenhagen, Denmark). Body temperature was kept at ~39°C and testicular temperature at 33–34°C.

Blood flow was measured in 8 rams 47–60 kg in weight in the breeding season and 8 rams 60–74 kg in weight in the non-breeding season. Continuous pulsatile blood flow signals were measured with a sine wave electromagnetic blood flow meter (Skalar-Transflow 600; Skalar Instruments, Delft, The Netherlands). After laparotomy, flow sensors were mounted proximally on the left and right testicular arteries between their origin from the aorta and the internal inguinal ring. Flow sensors with an internal diameter of 1·75, 2·0 and 2·25 mm were used. Thereafter incisions were made on the left and right side of the scrotum. Flow sensors with an internal diameter of 1·5, 1·75 and 2·0 mm were mounted distally on the left and right testicular arteries just before the ramification of the arteries on the surface of the testes (Noordhuizen-Stassen, Beijer, Charbon & Wensing, 1983). The cranial and caudal epididymal blood vessels in the left and right spermatic cords were ligated with a polyfilament nylon thread (Synthacord: PW Beyvers K.G., Berlin).

Values for the degree of acidity (pH), oxygen tension (Po2), haemoglobin oxygen saturation (satO2) and testosterone concentrations in various blood vessels were obtained in 7 rams in the breeding and 7 rams in the non-breeding season. Testes and spermatic cords were exposed by means of a scrotal incision. Cranial and caudal epididymal blood vessels in the left and right spermatic cords were ligated. Modified intravenous canulae (20 gauge, i.d. 0·6 mm, o.d. 1 mm; Abbott Laboratories Ltd, Sligo, Ireland), connected to polyvinyl catheters (i.d. 1 mm, o.d. 1·8 mm; Norton Plastics and Synthetics Division, Cleveland, Ohio, U.S.A.) were inserted in a branch of the testicular artery on the testis, with only the tip located in the main artery to avoid disturbance of the blood supply to the testis, and in a distal testicular vein on the testis. Proximal testicular veins (spermatic veins) were canulated with polyvinyl catheters which tapered to the tip (i.d. 0·5 mm, o.d. 1 mm). The catheters were inserted just proximal to the pampiniform plexus to avoid sampling of blood other than that of the testicular outflow (Text-fig. 1). Subsequently, polyvinyl catheters were placed into the saphenous artery (i.d. 1 mm, o.d. 1·8 mm) and vein (tapered to i.d. 0·5 mm, o.d. 1 mm at the tip). Blood values obtained from the saphenous artery were considered to be representative for the proximal testicular artery (internal spermatic artery; Jacks & Setchell, 1973).
All catheters were filled with heparinized saline and sealed with metal plugs. They were led subcutaneously and exteriorized through a small incision in the skin on the back. The rams were allowed at least 24 h to recover from surgery before blood sampling was performed. Blood samples of all catheterized blood vessels were taken simultaneously and in duplicate. Animals from which collection of one or more samples of the different blood vessels failed were excluded from presentation and calculations. During sampling the animal stood quietly in a cage.

The pH and \( P_O_2 \) values in the various arterial and venous blood samples were measured with a blood–gas analyser (ABL2: Radiometer, Copenhagen, Denmark). The percentages of haemoglobin oxygen saturation (satO\(_2\)) were derived from the oxygen dissociation curve of the ram (Bartels & Harms, 1959) after correction for the Bohr effect (Severinghaus, 1974). The \( P_O_2 \) values measured were corrected for temperature differences (Severinghaus, 1974).

Plasma (0·05 ml) prepared from the arterial and venous blood samples was assayed for testosterone by a radioimmunoassay procedure (Verjans, Cooke, de Jong, de Jong & van der Molen, 1973). The within-assay coefficient of variation was 8·9% and the inter-assay coefficient of variation was 9·6%. Sensitivity was 5 pg/tube.

**Histology**

After the experiments 5 rams were killed. To visualize the shunting of fluid (e.g. blood) from the testicular artery to the pampiniform plexus in the spermatic cord the testicular blood vessels of 5 testes were perfused with luke-warm water, and then the distal testicular artery on the surface of the testes was ligated. Thereafter, a canula was inserted in the proximal testicular artery, and India-ink coloured gelatine was infused. Samples were obtained from the median part of the spermatic cords of these testes, which were subsequently fixed in Bouin's solution, embedded in paraffin wax, sectioned at 5 \( \mu \)m, and stained with haematoxylin and eosin.

The other 5 spermatic cords were fixed in Bouin's solution and embedded in paraffin wax. Sections (5 \( \mu \)m) of the lower part were stained with haematoxylin and eosin. The diffusion distance between the testicular artery and the pampiniform plexus was determined by measuring the inner wall distance between the testicular artery and the pampiniform plexus.

**Latex casts**

Five rams were killed after the experiments to calculate the diffusion surface between the testicular artery and the pampiniform plexus in the spermatic cord. Therefore, the testicular artery in the spermatic cord was perfused with luke-warm water and subsequently filled with latex. After corrosion of the tissue, the total length and diameter of the latex cast of the testicular artery in the spermatic cord were measured, so that the diffusion surface could be calculated.

**Data handling and analyses**

To study the amount of oxygen diffusion and arterio-venous blood flow (shunting) from the testicular artery to the pampiniform plexus in the spermatic cord, the following equations were used.

Diffusion in the spermatic cord was estimated using a modification of the first law of Fick (Brown, 1974):

\[
\frac{dm}{t} = K \times \frac{S \times \triangle P_O_2}{D}
\]

where diffusion was defined as the amount of oxygen (\( dm: \) in \( \mu \)mol \( O_2 \)) which passed per minute (t) through the testicular arterial wall (\( S: \) diffusion surface) at a certain oxygen tension gradient.
Text-fig. 1. Schematic drawing of the experimental approach to the testis, indicating catheterization sites of the testis and the spermatic cord. 1, proximal testicular vein; 2, distal testicular vein; 3, distal testicular artery; 4, pampiniform plexus; 5, spermatic cord; 6, testis; 7, epididymis.

$(\Delta P_{O_2})$ between the testicular artery and the pampiniform plexus (D: diffusion distance). The diffusion constant ($K$) for muscle:

$$1.4 \times 10^{-5} \frac{cm^3 O_2}{cm \cdot min \cdot atm}$$

was used in the calculations (Thews & Niesel, 1959).

The percentage of blood ($a - vS$) flowing directly from the testicular artery to the pampiniform plexus, without passing the testicular capillary bed, was calculated for each ram using the formula:

$$a - vS = \frac{(TVP - TVD) satO_2}{\frac{1}{2}(SA + TAD) satO_2 - TVD satO_2} \times 100\%$$

where $(TVP - TVD) satO_2$ is the difference in haemoglobin oxygen saturation between the testicular veins distal and proximal to the pampiniform plexus (Text-fig. 1), $\frac{1}{2}(SA + TAD) satO_2$ is the mean haemoglobin oxygen saturation of saphenous and distal testicular artery, and $TVD satO_2$ is the haemoglobin oxygen saturation of the testicular outflow.

The oxygen flux, the amount of oxygen flowing through arterio-venous anastomoses from the testicular artery to the pampiniform plexus, in the spermatic cord was calculated for each ram as follows:

$$O_2 flux = Q_s \times \frac{\frac{1}{2}(SA + TAD) satO_2}{100} \times (Hb) \times 1.34, \text{ in } \mu mol O_2/min,$$
where 1.34 is the haemoglobin oxygen binding capacity and \( Q_s \) is the amount of blood flow through the arterio-venous anastomoses between the testicular artery and the pampiniform plexus and is defined as:

\[
Q_s = \frac{a - vS}{100} \times Q
\]

where \( Q \) is the blood flow measured in the proximal testicular artery.

The results are expressed as mean \( \pm \) s.e.m. In Tables 1 and 2, for each animal an average of two values of each measurement was taken. The results were analysed for statistical significance with the paired Student's \( t \) test and the Sign test. Differences were considered to be statistically significant if \( P < 0.05 \).

### Results

The results are summarized in Tables 1 and 2. The mean haemoglobin concentration in the venous saphenous blood was 6.3 ± 0.4 (\( n = 14 \)) mmol/l.

#### Blood flow

A nearly total reduction of the pulse amplitude of the blood flow in the distal testicular artery was encountered (Text-fig. 2). Blood flow (ml/min and ml/min 100 g\(^{-1}\)) measured in the proximal testicular artery was more than twice that in the distal testicular artery (Table 1).

#### Oxygen saturation

More oxygen was extracted from the testicular arterial blood in the breeding season than in the non-breeding season (Table 2). In both seasons there was a significantly higher oxygen content in the proximal testicular vein than in the distal testicular vein.

<table>
<thead>
<tr>
<th>Table 1. Testicular weight and blood flow in the proximal and distal testicular arteries measured in anaesthetized rams in the breeding and non-breeding seasons</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Non-breeding season</strong></td>
</tr>
<tr>
<td>No. of rams</td>
</tr>
<tr>
<td>Testicular weight (g)</td>
</tr>
<tr>
<td>Blood flow</td>
</tr>
<tr>
<td>Proximal testicular artery*</td>
</tr>
<tr>
<td>ml/min</td>
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<tr>
<td>ml/min 100 g(^{-1})</td>
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<tr>
<td>Distal testicular artery*</td>
</tr>
<tr>
<td>ml/min</td>
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<tr>
<td>ml/min 100 g(^{-1})</td>
</tr>
</tbody>
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Values are mean \( \pm \) s.e.m.

* No difference could be measured between left proximal and distal testicular artery and right proximal and distal testicular artery. Therefore, left and right testicular blood flows were averaged.
Table 2. Haemoglobin oxygen saturations, oxygen tensions and testosterone concentrations in the saphenous artery and vein, the distal testicular artery and the proximal and distal testicular vein measured in conscious rams in the breeding and non-breeding seasons

<table>
<thead>
<tr>
<th></th>
<th>Non-breeding season</th>
<th>Breeding season</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Oxygen saturation (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of rams</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>Saphenous artery</td>
<td>95.7 ± 0.4</td>
<td>95.7 ± 0.3</td>
</tr>
<tr>
<td>Distal testicular artery</td>
<td>95.3 ± 0.3</td>
<td>94.7 ± 0.5</td>
</tr>
<tr>
<td>Distal testicular vein</td>
<td>50.4 ± 3.5</td>
<td>33 ± 1.7*</td>
</tr>
<tr>
<td>Proximal testicular vein</td>
<td>65.1 ± 2.1</td>
<td>50.1 ± 2.1*</td>
</tr>
<tr>
<td>Saphenous vein</td>
<td>72.5 ± 1.1</td>
<td>68.4 ± 4.4</td>
</tr>
<tr>
<td>Difference in oxygen saturation between:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distal testicular artery and vein</td>
<td>44.9 ± 3.6</td>
<td>60.8 ± 1.7</td>
</tr>
<tr>
<td>Proximal and distal testicular vein</td>
<td>14.7 ± 2.1†</td>
<td>17.1 ± 2.0†</td>
</tr>
</tbody>
</table>

| **Oxygen tension (mmHg)**     |                     |                 |
| No. of rams                   | 5                   | 7               |
| Saphenous artery              | 100 ± 4             | 98 ± 3          |
| Distal testicular artery      | 82 ± 3              | 78 ± 3          |
| Distal testicular vein        | 27 ± 1.3            | 21 ± 0.6*       |
| Proximal testicular vein      | 42 ± 1.3            | 34 ± 1*         |
| Saphenous vein                | 47 ± 0.9            | 45 ± 3.3        |

| **Testosterone concentration (nmol/l)** | | |
| No. of rams | | |
| Saphenous artery | 1.8 ± 0.4 | 1.9 ± 0.2 |
| Distal testicular artery | 3.3 ± 1.1 | 2.2 ± 0.3 |
| Distal testicular vein | 136 ± 31 | 116 ± 11 |
| Proximal testicular vein | 78 ± 20 | 80 ± 6.5 |
| Difference in testosterone concentration between: | | |
| Distal testicular artery and vein | 132 ± 30 | 114 ± 15 |
| Proximal and distal testicular vein | 58 ± 12† | 37 ± 8.9† |

Values are mean ± s.e.m.
* Significantly different from value in non-breeding season, P < 0.05.
† Significantly different from value for distal testicular artery and vein, P < 0.05.

**Testosterone concentrations**

In both seasons a considerable release of testosterone was measured (Table 2). In all rams a higher concentration of testosterone was measured in the distal than in the proximal testicular vein.

**Arterio-venous shunting and diffusion in the spermatic cord**

Approximately 64% and 55% of the blood flow in the proximal testicular artery in the spermatic cord bypassed the testis in the non-breeding and breeding seasons, respectively (Table 1).
In both seasons 30.1 ± 3.7% (n = 10) of the amount of oxygen in the testicular artery was flowing to the pampiniform plexus in the spermatic cord (Table 2) and created the increase of oxygen in the testicular venous blood passing distally to proximally (Table 2). This represented an amount of oxygen flux from the testicular artery to vein in the spermatic cord of 43.5–46.1 µmol O₂/min. There was an oxygen diffusion from the testicular artery to the pampiniform plexus of 23 × 10⁻² µmol O₂/min. This calculation was based on an average length and diameter of the testicular artery of 181 ± 11 cm and of 0.19 ± 0.01 cm, respectively, and on a diffusion surface and distance of 85 ± 5 cm² and 0.018 ± 0.008 cm, respectively in both seasons.

From the testosterone values it could be calculated that 45 ± 2.7% and 30 ± 3.8% of the testicular arterial blood bypassed the testis in the non-breeding and breeding seasons respectively and created the dilution of testosterone in the testicular vein as it passed proximally in the spermatic cord (Table 2).

**Histology**

India-ink coloured gelatine was visible in the testicular vein and the testicular artery in sections of the spermatic cord (Text-fig. 3). Because the distal testicular artery was ligated on the surface of the testis, no gelatine could have passed through the capillary net of the testis, and therefore must have passed through arterio-venous anastomoses in the spermatic cord.

**Text-fig. 3.** A section of the spermatic cord showing the appearance of India-ink coloured gelatine (1) in the testicular artery (2) and in the pampiniform plexus (3). × 25.

**Discussion**

Until now, only capillary blood flow measurements of the testis have been presented (Setchell, Waites & Thorburn, 1966; Courrot & Joffre, 1977). Courrot & Joffre (1977) suggested that blood flow to the testis in various sheep breeds might be influenced by the season. They found a 50% increase in testicular capillary blood flow in the breeding season in Ile-de-France rams. However, after measurement of blood flow in the proximal as well as in the distal testicular artery, it was indicated that blood might bypass the testis in the spermatic cord, particularly in the non-breeding season (60%) in Clun Forest rams (Fleet, Laurie, Noordhuizen-Stassen, Setchell & Wensing, 1982). Transfer of steroids in the spermatic cord has been examined in various species based on measurements made in peripheral blood samples and in venous and arterial testicular blood samples taken on the surface of the testis (Einer-Jensen & Waites, 1977). However, we compared
testosterone concentrations and haemoglobin oxygen saturations in both the arterial and venous testicular blood in the proximal and distal parts of the spermatic cord. To examine diffusion and/or shunting from the testicular artery to the pampiniform plexus and vice versa in the spermatic cord, testicular arterial blood flow in the proximal as well as the distal part of the spermatic cord was measured. The epididymal blood vessels were ligated in all rams to avoid admixture from the testicular outflow with that of the epididymis. From several rams used in this experiment and in a pilot study, corrosion casts (Technovit 7001: Kulzer & Co., GmbH, Bad Hamburg, Germany) of the blood vessels in the spermatic cord and the testis were made to check the effectiveness of this ligation.

It was surprising that, although more oxygen was extracted from the testicular arterial blood in the breeding than in the non-breeding season, indicating a higher metabolic rate of the testes in the breeding season, no difference in release of testosterone between the two seasons could be detected (Table 2).

In this study the blood flow measured in the distal testicular artery was only about 40% of the blood flow in the proximal testicular artery in both seasons, indicating that about 60% of the blood was bypassing the testis in the spermatic cord and not contributing to the capillary blood flow of the testis. No seasonal changes in distal and proximal testicular arterial blood flow could be observed in the Texel rams used.

A significant increase of haemoglobin oxygen saturation was measured in the testicular vein from the surface of the testis as it passed proximally in the spermatic cord. It was calculated that only 0.5% of this increase could be explained by passive diffusion from the testicular artery to the pampiniform plexus, but this is already an overestimation because optimal data for diffusion distance, diffusion surface and coefficient were used to calculate the amount of diffusion in the spermatic cord (Hees et al., 1984). About 30% of the testicular arterial blood bypassed the testis through non-metabolic channels to create the rise in oxygen saturation from the distal to the proximal testicular vein in both seasons.

Testosterone concentrations showed a significant decrease from the distal to proximal testicular vein. We calculated that about 28% of the testicular arterial blood passing directly from the artery to vein was responsible for this dilution in the breeding season, well comparable with the oxygen saturation data. In the non-breeding season an admixture of about 46% of the testicular arterial blood in the pampiniform plexus was calculated. Our data did not prove unequivocally that any testosterone transfer was due to diffusion, because no significant difference in testosterone concentrations between the saphenous and distal testicular artery was detected. The amount of blood bypassing the testis in the spermatic cord was greater when measured with the electromagnetic blood flow technique than with the other two techniques used. The influence of anaesthesia and the dorsal recumbent position during blood flow measurements might be responsible for this difference.

It is possible that the arterio-venous anastomoses between the testicular artery and the pampiniform plexus can be preferentially opened or closed. Considering the blood flow data of different authors, in some breeds (e.g. Clun Forest and Ile-de-France) the blood flow in the distal testicular artery increased considerably in the breeding season, but blood flow in the proximal testicular artery in the same animals was not influenced by the breeding season (Courtot & Joffre, 1977; Fleet et al., 1982). However, the Texel rams used in this study and Australian Merinos and Southdown rams showed no difference of blood flow in the distal testicular artery between the two seasons. The Texel rams showed no difference of blood flow in the proximal testicular artery either.

Considering the possible effect of biogenic substances, Free & Duc Kien (1973) demonstrated that the major site of action of serotonin on testicular blood vessels was in the spermatic cord and not in the resistance vessels of the testis. They stated that venous–arterial interaction involving serotonin may be more important as an example of a more general shunting mechanism. The blood vessels of the testis, like those of the head, but in contrast to those of the viscera and the extremities, are far more sensitive to serotonin than to noradrenaline (Free & Duc Kien, 1973; Saxena, 1978;
Noordhuizen et al., 1983). It is therefore possible that a drop in serotonin concentration in the blood during pathophysiological conditions (e.g. migraine headache, abdominal testis) can lead to dilatation of large arteries and a closure of arterioles. The increased blood flow in the large arteries would then preferentially pass through the dilated arterio-venous anastomoses, thus bypassing the high resistance in the capillary bed (Saxena, 1978). Other biogenic substances (e.g. prostaglandin F-2α) could also affect the regulation of capillary and direct arterio-venous blood flow.

Further studies are needed to establish whether arterio-venous shunting of blood in the spermatic cord provides an additional mechanism involved in blood flow and/or temperature regulation of the testis.

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References


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