Testicular morphology and androgen profiles following testicular ischaemia in rams

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Arteriosclerotic changes were induced in the internal spermatic artery of rams to determine whether there is a link between this condition and some pathological conditions of the testes, similar to those that cause infertility in men. Eight weeks after the induction of testicular ischaemia, blood plasma was collected simultaneously from the jugular and spermatic veins after an LH injection (10 µg) and assayed for testosterone. The rams were then castrated and sections of the testis, ductuli efferentes and spermatic cord were examined quantitatively and qualitatively. Vascular disturbance decreased the percentage of normal spermatogenic epithelium (P < 0.01) and the diameter of the seminiferous tubules (P < 0.001). These effects were accompanied by an increase in the percentage of the interstitial region within the testis (P < 0.05). Macrophages, lymphocytes and other inflammatory cells became numerous in the interstitium as damage to the seminiferous epithelium progressed. The most striking feature of the ischaemic testis was the focal damage of the spermatogenic epithelium, that is, sections of the same testis exhibited both normal and germ cell-depleted seminiferous tubules. Concentrations of testosterone in peripheral plasma were not significantly altered by either unilateral or bilateral testicular ischaemia; however, the concentration of testosterone was higher in the experimental spermatic vein than in the contralateral spermatic vein (P < 0.05) as was the ratio of LH:testosterone (P < 0.05). Unilateral vascular disturbance of the testis did not cause damage in the contralateral testis. The ductuli efferentes of these rams also showed structural changes as a result of vascular disturbance. The tubules of the most proximal regions were reduced in calibre compared with the control tissue (P < 0.005) and pigment-laden macrophages had invaded the lumina, the epithelium of the ducts and the interstitium. These morphological and endocrine profiles that were induced in rams are typically seen among fertile men. These similarities suggest that vascular disturbance may be implicated in the formation of some gonadal pathologies that cause infertility in men.

Introduction

Primary testicular disease is a common cause of infertility and men presenting with this condition constitute 23% of the infertile male population (Jequier and Holmes, 1993). Spermatogenic arrest can result in either azoospermia, which is often associated with germinal aplasia (Sertoli cell only), or tubular atrophy, whereas oligospermia generally results from maturation arrest of germ cells. For reasons as yet unknown, this latter condition often occurs at foci within the testis and leaves some seminiferous tubules completely undamaged. Patients with primary testicular disease show abnormal patterns of LH secretion (Jequier and Holmes, 1993), but it has not been established whether this is due to an alteration in the function of the Leydig cells or to factors that affect FSH and LH.

The caput epididymidis (which in humans mostly comprises ductuli efferentes) is also a common site of disease and is obstructed in 21% of men aged less than 50 years and 39% of older men (Ball and Mitchinson, 1984). Upper epididymal obstruction is characterized by the accumulation of lipofuscin pigment (Mitchinson et al., 1975; Ball and Mitchinson, 1984), a well-established product of oxidative damage in some tissues (Roubal, 1970). The testis and epididymis may well be susceptible to oxidative damage, as the blood vessels supplying these tissues often show sclerotic changes. Such vascular anomalies have been observed in a variety of mammals (Jensen et al., 1962; El-Etreby, 1969; Pearson and Slinger, 1982), including both young and old humans (Suoranta, 1971; Oshima et al., 1984; Brehmer-Andersson et al., 1985; Womack and Ansell, 1993).
and, in many cases, when there is no evidence of systemic arteriosclerosis.

On the basis of these observations, we developed the general hypothesis that vascular disturbance may be implicated in the formation of testicular pathology that is a cause of infertility in men. This was tested by surgically inducing testicular ischaemia in rams and observing the morphological and endocrine changes that ensued within the period of approximately one spermatogenic cycle. The ram was used as an experimental model for the human because of the similarities between the species in size and the anatomical relationship of the testis and ductuli efferentes to the ductus epididymis (Hemeida et al., 1978).

Materials and Methods

Twenty-one Polled Merino rams, 14–20 months of age, were used in this study. They were obtained from the CSIRO farm (‘Yalanbee’) and maintained on pasture at the University of Western Australia. Sixteen rams formed the experimental group and five acted as controls.

Surgery

The rams were not allowed feed and water for 24 h before surgery. Anaesthesia was induced with sodium thiomyal (10 mg kg\(^{-1}\) body mass) i.v. and maintained by 3% halothane in oxygen. Under sterile conditions, the spermatic cord was exteriorized and the spermatic artery gently isolated from the pampiniform plexus for approximately 1.5 cm. A piece of silk ligature (n = 11) or polyethylene tubing (n = 11) was placed around the artery to cause various degrees of arterial occlusion ranging from very minor to major ischaemic effects, respectively. Unilateral arterial occlusion was performed on 10 of the 16 experimental rams; in these animals the contralateral testis was sham-operated. Bilateral arterial occlusion was performed on the remaining six rams. A total of 22 spermatic arteries were therefore subjected to partial arterial occlusion. After surgery, the rams were given an injection of penicillin–streptomycin i.m. They were left for 56 days after surgery, slightly longer than the 49 day course of the entire spermatogenic process in rams.

After 56 days, the rams were anaesthetized as outlined above. A cannula was placed in one vein of the pampiniform plexus and passed up for 12–15 cm to a point where the spermatic veins unite to form a single vein. Blood plasma was sampled simultaneously from the jugular and spermatic veins every 15 min for 3.5 h after administration of a single injection of ovine LH (NIADDK-oLH-25) i.v. The plasma was separated and stored at −20°C until it was assayed for testosterone. Cannulation of the jugular vein, experimental spermatic vein and contralateral spermatic vein was successful in 11 of the 16 experimental rams. Problems arose in the remaining five rams when extensive fibrosis of the region, due to surgical trauma, hindered correct placement of the cannula within the single spermatic vein.

The rams were bilaterally castrated and the testis, ductuli efferentes and spermatic cord were perfused via the spermatic artery with Karnovsky’s fixative (2% paraformaldehyde, 2.5% glutaraldehyde in 0.1 mol phosphate buffer 1\(^{-}\)). The testis was dissected and 1 cm × 1 cm × 0.5 cm pieces from each of the quadrants were removed. The ductuli efferentes were dissected and four pieces, approximately 1 cm × 1 cm × 0.5 cm, were removed. Care was taken to ensure that sampling was consistent owing to the lack of uniformity of tubule diameter in this region. The tissue was post-fixed in Karnovsky’s fixative, dehydrated by a series of alcohols (50%, 70%, 90% and 100%) and 100% acetone, infiltrated by a series of Epon–Araldite: acetone solutions, and finally embedded in pure Epon–Araldite.

Morphometric analysis

Blocks were trimmed and 2 μm sections were cut using a JB4 ultratome with a glass knife. Sections were stained with methylene blue azur II basic fuchsin (BDH, Melbourne) and Schmorl’s stain (BDH) for the identification of lipofuscin, and mounted with a glass coverslip. For stereological analysis of the testis (volume density of normal spermatogenic epithelium and interstitial region) each section was viewed at ×25 magnification through a Wild Heitz microscope fitted with an eyepiece graticule containing a multipurpose test system M42 (Weibel, 1979). Tissue variables were measured by viewing four randomly chosen areas per section of testis. Each testis was represented by four tissue blocks taken from each of the quadrants of the testis, so that a total of 16 areas were measured from individual testes. The cross-sectional diameter of the seminiferous tubules, the height of the epithelium and the diameter of the lumen of the seminiferous tubule were measured by fitting a graticule of a calibrated linear scale in the ×10 eyepiece of a Wild Leitz microscope with a ×40 objective lens. Only those tubules that were circular, or near circular, in profile were measured. The cross-sectional diameter of the ductuli efferentes and height of the duct epithelium were estimated in the same manner.

All linear measurements of the testis and ductuli efferentes were made by viewing three randomly chosen tubules per section of tissue, of which four equally distant points around each tubule were measured. Each testis or epididymal region was represented by four tissue blocks so that a total of 60 measurements were made for each variable. A testicular biopsy score count was also performed on approximately 100 seminiferous tubules of each testis. The criteria for scoring is outlined by Johnsen (1970) and ranges from score 10, which denotes a full complement of all germ cells within the seminiferous tubules, down to a score of 1, which represents no cells within the tubules.

The luminal area of each cross-section of spermatogenic artery was measured using an MD-20 Image Analyzing System and a digitizing pad attached to a Wild Leitz microscope. Measurements were made at the point of partial occlusion (occlusion), in the region either side of the occlusion point (non-occlusion) and in the contralateral artery.

Radioimmunoassay

Plasma LH was measured in duplicate in 100 μl aliquots of all samples using a double-antibody radioimmunoassay described by Martin et al. (1980). The preparation CNRS-M3 (biopurity
1.8 IU NIH-LH-S1 mg⁻¹) used for both iodination and reference was kindly supplied by M. Jutisz (College de France, Paris). The limit of detection of the assay, calculated by subtracting two standard deviations from the mean counts bound in nine replicates of the zero standard, was 0.125 µg l⁻¹. Intra-assay variation was estimated in each assay using at least five replicates of three pooled plasma samples containing 0.30 (12.60%), 1.26 (6.5%) or 4.94 (11.7%) µg l⁻¹. All samples were measured in one assay to avoid interassay variation.

Plasma testosterone was measured in duplicate in 25 µl aliquots of all samples using a non-extraction radioimmunoassay developed by M. A. Blackberry (Faculty of Agriculture, University of Western Australia). Spermatic vein samples were diluted from a range of 1:5 to 1:50 as testosterone concentration was much higher in these samples than in the jugular vein samples. Standards made by serial dilution of a stock containing 34.67 nmol 4-androsten-17β-ol-3-one l⁻¹ (Sigma, Sydney) to 0.22, 0.43, 0.87, 1.73, 3.47, 6.94, 13.87, 27.74 nmol l⁻¹ in wether plasma. The tracer (1,2,6,7[3H]testosterone; Amersham (Sydney); specific activity 90 Ci mmol⁻¹) was diluted in gelatin-phosphate buffer. The antiseraum (R3) was raised against testosterone-3-carboxymethyl-oxime-human serum albumen using an antigen donated by R. I. Cox (CSIRO, Division of Animal Production, Prospect, NSW) and was diluted to 1:1000 in gelatin-phosphate buffer and saturated with BSA before storage at −20°C. It was used at a working dilution of 1:70 000 (final dilution 1:280 000) and crossreacted primarily with dihydrotestosterone (70%) and androstenedione (3.7%). Crossreactions with progesterone, oestradiol, oestron and oestradiol were all less than 0.05%. Assays contained duplicate 25 µl aliquots of up to 300 unknown samples and six replicates each of three quality control samples (pooled plasma). All incubations were done at 4°C overnight. Bound and free testosterone were separated using a double-antibody technique. After centrifugation (2000 g for 30 min), the supernatant was aspirated and the precipitate was dissolved in 0.5 ml HCl (0.05 mol l⁻¹) before radioactivity was measured. The limit of detection was 0.59 nmol l⁻¹. Included in each assay were six replicates of three pooled plasma samples containing 2.36, 7.35 and 14.46 nmol l⁻¹. They were used to estimate the coefficients of variation within assay (10.80 ± 1.85, 3.30 ± 1.55, 2.19 ± 1.40%) and between assays (16.40, 6.30, 6.80%).

The concentrations of LH and testosterone in the jugular vein, experimental spermatic vein and contralateral spermatic vein were calculated as the data under the curve collected at intervals of 15 min from 0–180 min. The concentration of testosterone was also calculated as a ratio of the LH concentration for that testis to maximize precision of this measure of testicular responsiveness.

Statistical analysis

The testicular and epididymal parameters of the contralateral (n = 10) and control group (n = 5) were compared using an unpaired Student's t test. As there was no significant difference between these groups for all variables measured, the data were pooled and compared with the experimental group using a Student's t test. The occlusion, non-occlusion and contralateral arteries were compared by a one-way ANOVA. A mean value ± SEM was calculated for each group.

Results

Spermatic artery

Analysis of the spermatic artery revealed that the tunica intima and media had undergone hyperplasia during the 8 week post-operative period resulting in a narrowed arterial lumen compared with the contralateral and control arteries (Fig. 1). The mean cross-sectional area of the lumen in the occlusion artery was 0.57 ± 0.09 mm² which was significantly smaller than the non-occlusion artery (2.72 ± 0.42 mm²; P < 0.05) and the contralateral artery (2.78 ± 0.36 mm²; P < 0.05). There was no significant difference between the contralateral and control arteries for this measurement. Within the experimental group there was a range in the degree of arterial occlusion from severe luminal reduction to little luminal reduction, and this
was reflected by a range in the degree of testicular and epididymal damage caused by the ensuing reduction in blood flow. However, it was not possible to correlate the degree of arterial occlusion with either the morphology of the testicular and ductuli efferentes or the venous testosterone concentration.

Tests

There was no significant difference between the contralateral group (n = 10) and the control group (n = 5) for any of the testicular measurements; data from these groups were therefore pooled and they were termed the control group (Table 1).

Partial occlusion of the internal spermatic artery led to a reduction in the diameter of the seminiferous tubules compared with the control group (P < 0.01). This difference was due to reductions in the height of the seminiferous tubule epithelium (P < 0.05) and the diameter of the seminiferous tubule lumen (P < 0.05) in the experimental group. The percentage of the testis occupied by normal spermatogenic epithelium (containing a full complement of germ cells) was lower in the experimental group than in the control group (P < 0.01). Conversely, the percentage of testis occupied by interstitial regions was higher in the experimental group than in the control group (P < 0.05). The mean testicular biopsy score count in the experimental group indicated a depletion of germ cells within the seminiferous tubules, and was significantly lower than in the control group (P < 0.01), which generally showed a full complement of germ cells. Qualitative assessment of the ischaemic testes revealed focal damage of the seminiferous tubules. Within the same testes, some seminiferous tubules showed germinal aplasia, in which Sertoli cells were the only type of cells present, or maturational arrest and therefore an absence of more mature germ cells (spermatocytes and spermatids), while other tubules showed a full complement of spermatogenic cells.

Of the 22 experimental testes, a number revealed little or no disruption of spermatogenesis despite vascular disturbance (n = 8; Fig. 2a, b). The testes started to exhibit adverse changes, particularly within the interstitium, when the testicular biopsy score count fell below 8.4. Lymphocytes, plasma cells, large epithelioid macrophages and neutrophils infiltrated the interstitium, at first in low numbers, but these populations escalated as the testicular biopsy score count declined to 4.7 (n = 7). Within this range of scores, the Sertoli cells became vacuolated and the heterogeneity of the spermatogenic epithelium was greatest (Fig. 2c). Below a testicular biopsy score count of 4.7, tissue damage was generally homogeneous (n = 7). At the stage of total seminiferous tubule atrophy (Fig. 2d), the interstitial region showed a proliferate capillary network that enhanced the massive infiltration of lymphocytes, neutrophils and epithelioid macrophages into the region (Fig. 3a). Many of the macrophages contained a pigment that was identified as lipofuscin using specific histochemical staining (Schmorl's stain). Leydig cells were present within the interstitium yet they occasionally contained an abundance of lipid droplets in their cytoplasm which is quite atypical of these cells in rams. This was in marked contrast to the histology of the interstitial region of the normal testes within the experimental group which contained characteristic Leydig cells (Fig. 3b).

Ductuli efferentes

As with the testis, there was no significant difference between the contralateral and control groups for all the epididymal measurements; these data were therefore pooled and they were termed the control group. The diameter of the ductuli efferentes of the experimental group was smaller than that of the control group (P < 0.005) (Table 2). This was due to a significant decrease in the lumen diameter of the ducts within the experimental group compared with the control group (P < 0.005). The height of the epithelium in the ductuli efferentes did not change between the experimental and control groups.

There was a range of ischaemic damage in the epididymides of the experimental group, as was described in the testis, and changes in the dimensions of the tubules between the testis and ductuli efferentes seemed to occur concomitantly. This was demonstrated by the positive correlation between the diameter of the seminiferous tubules within the experimental testes and the diameter of the ductuli efferentes in the corresponding epididymides (r = 0.61; P < 0.01).

Qualitative assessment of the proximal caput epididymidis revealed that partial occlusion of the spermatic artery caused changes in morphology that were very similar to those already described in the ram ductuli efferentes following ligation of the superior epididymal artery (Markey, 1993). These changes included a decrease in tubule calibre and the invasion of pigment-laden macrophages into the tubule lumen, epithelium and interstitial region.

Table 1. Testicular characteristics in ischaemic (n = 22) and control (n = 15) groups of rams

<table>
<thead>
<tr>
<th>Variable</th>
<th>Experimental</th>
<th>Control</th>
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<tr>
<td>Seminiferous tubule diameter (µm)</td>
<td>171.21 ± 4.75 **</td>
<td>190.70 ± 4.94</td>
</tr>
<tr>
<td>Seminiferous epithelium height (µm)</td>
<td>50.11 ± 2.53 *</td>
<td>58.30 ± 2.05</td>
</tr>
<tr>
<td>Lumen diameter (µm)</td>
<td>54.57 ± 6.94 *</td>
<td>73.49 ± 2.59</td>
</tr>
<tr>
<td>Spermatogenic epithelium (%)</td>
<td>41.20 ± 7.04 **</td>
<td>68.55 ± 1.37</td>
</tr>
<tr>
<td>Interstitial region (%)</td>
<td>20.86 ± 1.98 *</td>
<td>14.15 ± 0.85</td>
</tr>
<tr>
<td>Testicular biopsy score count</td>
<td>5.81 ± 0.72 **</td>
<td>8.72 ± 0.08</td>
</tr>
</tbody>
</table>

Values given are the group means ± SEM derived from a single value calculated for each ram. Mean values are significantly different: *P < 0.05, **P < 0.01 (unpaired t test).
Production of testosterone

The concentration of LH did not vary significantly between the jugular vein, experimental spermatic vein and contralateral spermatic vein indicating that both the experimental and contralateral testes received a similar LH stimulus. There was no significant difference between the experimental and control groups in the concentration of testosterone in the jugular vein, nor was there a significant difference between the experimental and control groups in the LH:testosterone ratio. The concentration of testosterone was higher in the experimental...
spermatic vein than in the contralateral spermatic vein \( (P < 0.05) \) (Table 3). This difference was reflected in the LH:Testosterone ratio between the experimental spermatic vein and contralateral spermatic vein \( (P < 0.05) \).

**Discussion**

Partial occlusion of the internal spermatic artery in rams caused hyperplasia of the arterial wall and a consequent narrowing of the lumen characteristic of arteriosclerosis. These surgically induced changes in the artery caused a range of changes in morphology in both the testes and ductuli efferentes of rams. The ischaemic testis exhibited focal maturational arrest of germ cells, a phenomenon that is quite typical of some human pathologies (Hargreave and Jequier, 1978; Pryor et al., 1978). These results demonstrated that vascular disturbance may be implicated in the onset of testicular pathologies that cause male infertility.

The reason for this selective damage of tubules is unknown. The link between the testicular artery and branches of the inferior epididymal artery are unlikely to provide an additional blood supply to isolated seminiferous tubules, so this concept does not reasonably explain the random, focal damage in the ischaemic testis. A similar pattern has been noted following administration of hCG (Kerr and Sharpe, 1989) and the withdrawal of testosterone (Russell and Clermont, 1977; Bartlett et al., 1986) with stages III and IX-XI, and VII and VIII of the spermatogenic cycle, respectively, being disrupted. Since, in the study reported here, testicular ischaemia was induced for 56 days and the spermatogenic cycle of the ram is 12 days, all seminiferous tubules would have presented all stages of spermatogenesis and would therefore be expected to succumb to damage if such a mechanism was responsible.

Macrophages and lymphocytes have been identified in the interstitial region of the testis (Miller et al., 1983; Pollanen and Niemi, 1987). Under normal conditions, these cells are not observed in the seminiferous tubules but were present in that region of the ischaemic testis in the study reported here. These macrophages were laden with lipofuscin pigment, suggesting their role in phagocytosis of cellular debris that accumulated as a result of ischaemic damage. Testicular macrophages are known to generate high concentrations of reactive oxygen species (Wei et al., 1988) and the large stores of polyunsaturated fatty acids, which to a great degree constitute spermatозoa, act as a substrate for reactive oxygen species. Free radicals are powerful oxidizing agents and the combination of these with high concentrations of polyunsaturated fatty acids in ischaemic testes provides an optimum environment for lipid peroxidation, tissue damage and formation of lipofuscin. The particular sensitivity of the testes to such degenerative processes has been confirmed by Reichel (1968) and Miquel et al. (1978).

The breakdown of the blood-testis barrier is a most likely consequence of ischaemic damage to testes in the study reported here. The presentation of sperm antigen and the ensuing autoimmune response in the testis is a significant cause of infertility in men after testicular ischaemia (Bandhauer, 1982) and this can often result in damage to the contralateral testis. This phenomenon, termed sympathetic orchiopathia, has been shown to occur after both unilateral testicular torsion (Lipschultz et al., 1976; Heindel et al., 1990) and unilateral testicular occlusion of the artery (Harrison et al., 1981; Kearney and Lewis-Jones, 1985). This phenomenon was not seen in the study reported here, a finding that concurs with a number of other studies (Turner, 1987; Bergh et al., 1988; Stern et al., 1990).

The changes in morphology induced in the ductuli efferentes of rams by partial occlusion of the spermatic artery are very similar to those that are characteristic of obstruction of the
Table 3. Effects of testicular ischaemia on the concentration of testosterone in plasma samples from the experimental spermatic vein (n = 22) and contralateral spermatic vein (n = 10) of rams

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Experimental</th>
<th>Contralateral</th>
</tr>
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<tbody>
<tr>
<td>LH:testosterone ratio</td>
<td>20.56 ± 4.37*</td>
<td>7.80 ± 2.52</td>
</tr>
<tr>
<td>Testosterone concentration (nmol l⁻¹)</td>
<td>2696.00 ± 159.15*</td>
<td>1108.88 ± 448.34</td>
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</table>

Values are the group means ± SEM derived from a single value calculated for each ram. Mean values were calculated as the data under the curve collected at 15 min intervals from 0–180 min. Mean values are significantly different: *P < 0.05 (unpaired t test).

higher epididymis in men (Mitchinson et al., 1975). These features have been described in rams after ligation of the superior epididymal artery (Markey, 1993), the major supply of the proximal caput epididymidis. The ductuli efferentes are narrower and contain lipofuscin pigment within their epithelial compartment. Numerous macrophages reside between the epithelial cells of the tubules, in the interstitial region and also within the lumina of the tubules. These cells appear to be actively involved in phagocytosis of spermatozoa and contain vast amounts of lipofuscin pigment, perhaps as a result of the over-accumulation of digested debris.

One objective of this study was to correlate the degree of arterial occlusion induced by surgery and the ensuing damage to gonadal morphology and testosterone concentration. However, such relationships could not be made. Differences in artery length and diameter between rams may have prevented similar changes in blood flow resulting from the same degree of arterial occlusion. Furthermore, arterial diameter must have varied throughout the post-surgery period, as intimal hyperplasia is a progressive condition. Non-invasive, periodic measurement of blood flow would therefore have been the only definitive measurement but, in the study reported here, this was not technically feasible. None the less, quantitation of the spermatic artery confirmed that arterial damage was induced in the experimental group causing a reduction of blood flow to the testis and epididymis. As this oxygen-dependent organ functions in a state of near anoxia, such a decrease in blood flow had profound effects on tissue morphology. The results of this study indicate that there may be a threshold of arterial damage that must occur before testicular and epididymal damage ensues. This contention was supported by the observation that a group of testes and their accompanying epididymides appeared normal when arteriosclerotic changes had clearly been induced in the internal spermatic artery. However, it would seem that such changes were insufficient to alter blood flow, and further, disrupt the morphology of the testes and epididymides.

Systemic concentrations of testosterone remained unchanged after ischaemia of the ram testis, in spite of severe damage to the morphology of the seminiferous tubules. This situation is very often seen in men exhibiting spermatogenic arrest, but in such cases, normal testosterone concentrations are associated with high concentrations of LH and FSH (Jequier and Holmes, 1993). These findings further demonstrate the similarities between ischaemia of the testes, induced in the study reported here, and some human pathological conditions.

The concentration of testosterone was found to be greater in the experimental spermatic vein than in the contralateral spermatic vein. As systemic concentrations of testosterone were not affected by testicular ischaemia, it would seem that this result was due to a reduction in blood flow through the experimental spermatic vein. Indeed, this confirms that the blood supply to the testis and epididymis was reduced by surgical occlusion of the spermatic artery. Similar results were observed after ligation of the efferent duct, and heating and irradiation of the testes (Setchell and Galil, 1983), and were attributed to a decrease in blood flow caused by a reduced requirement of the degenerated spermatogenic epithelium. It has even been suggested that a moderate reduction in blood flow after heating of the testes is compensated for by an increase in the local concentration of testosterone, so that total testosterone secretion is not affected (Galil and Setchell, 1987).

Cryptorchid testes (exposed to high temperatures) show an increased density of Leydig cell mitochondria and a subsequent increase in testosterone production after hCG stimulation (Kerr et al., 1988). As measurements of blood flow were not conducted in the present study it cannot be ascertained whether such a phenomenon occurred. However, some Leydig cells within the severely damaged testes appeared to contain more lipid droplets than normal, and perhaps these cells were increasing steroidogenic activity to accommodate for the reduced blood flow. Furthermore, there is strong evidence that Leydig cells are stimulated by interstitial macrophages (Yee and Hutson, 1985a, b, c) with which they are often in close association (Bergh, 1985; Niemi et al., 1986) and the invasion of numerous macrophages, seen in the present study as a result of testicular ischaemia, may increase steroidogenesis at the local level.

Primary testicular disease and higher epididymal obstruction are common causes of either oligospermia or azoospermia in men. The present study has produced changes in morphology and endocrine changes in the ram testis and ductuli efferentes that are very similar to those seen in humans. These changes were induced by partially occluding the internal spermatic artery and causing arteriosclerosis, which is shown to be quite prevalent in the vessels of the male gonads. This work therefore strengthens the hypothesis that vascular disturbance may be implicated in the formation of some testicular and epididymal pathologies that cause male infertility.

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