

AN ULTRASONOGRAPHIC AND HISTOPATHOLOGICAL STUDY OF THE TESTIS AND EPIDIDYMIS FOLLOWING EXPERIMENTALLY INDUCED UNILATERAL ISCHEMIA IN MALE GOATS AND RAMS.

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ABSTRACT

A study was conducted to explore the use of ultrasound imaging in assessing the progressive development of testicular and epididymal lesions in male goats and rams. For this purpose, ultrasound imaging of the testis and related structures was carried out fortnightly for 20 weeks following unilateral ligation of the testicular artery and veins in four goats and three rams. Following this, these animals were killed, their testes and epididymides removed and examined for gross and histopathological lesions. Ultrasonographically, within 24 hours, the ligated testis showed a reduced echogenicity and was mottled in appearance. There was a thin layer of anechoic fluid between the testicular tunics which later contained a network of hyperechogenic fibrin. The tunica albuginea was hyperechogenic, probably due to fibrosis. The testis was atrophied and showed a diffused coagulative necrosis postmortem. The epididymal tail in the beginning was reduced in echogenicity and had lost its heterogenous texture. Later, it showed increased echogenicity due to marked fibrosis, and was atrophied, except in one goat in which it appeared enlarged and showed chronic epididymitis with cystic dilatation of the tubules histologically. Ultrasound imaging appears to be a valuable diagnostic tool for assessing the progressive development of testicular and epididymal lesions.

INTRODUCTION

Diagnostic ultrasound is a non-invasive and innocuous technique which permits tissue interfaces to be detected and their shape and size described. The ultrasonographic appearance of the testis and related structures in normal rats (Ikinger *et al.*, 1983), boars (Cartee *et al.*, 1986), bulls (Pechman and Eilts, 1987; Eilts and Pechman, 1988; Cartee *et al.*, 1989), dogs (Pugh *et al.*, 1990) and goats and rams (Ahmad *et al.*, 1991) has been described. Ahmad *et al.* (1993) and Ahmad and Noakes (1995a) reported the ultrasonographic appearance of infertile goats with testicular degeneration. Ahmad and Noakes (1995b) described the echotexture of testes and epididymides after inducing lesions through the injection of a mild irritant chemical into these organs in rams and male goats. The same workers in a subsequent study (Ahmad and Noakes, 1995c) described ultrasonographic changes in the testis and the epididymis of rams and goats following unilateral vasectomy.

By inducing lesions experimentally and unilaterally, it should be possible to study progressive changes and at the same time to compare ultrasonically with the adjacent normal control organs. One method of inducing lesions in the testis and epididymis is to

restrict the blood supply from the testicular artery by its ligation. This has been shown to induce a predictable, ischemic necrosis of the ram testes that simulated testicular reaction to traumatic injury (Vrzgulova, 1981). In a later study, Eilts *et al.* (1989) reported ultrasonographic and histological changes upto 30 days following unilateral ligation of testicular artery in male goats. However, the ultrasonographic and histological appearance of the epididymis following this treatment was not described.

In the study described here, ultrasound imaging was used to assess the progressive development of testicular and epididymal lesions following unilateral ligation of the artery and veins of the pampiniform plexus in four male goats and three rams. Attempts were also made to correlate ultrasonographic observations with gross and histological findings postmortem.

MATERIALS AND METHODS

Four mature male goats and three rams with clinically normal external genitalia were used. They were housed in a partitioned loose box and were fed good quality hay and water *ad libitum* with 0.4 kg of concentrate ration per animal per day.

Treatments

The testicular artery and veins of the pampiniform plexus in experimental animals were unilaterally ligated, while the normal organs on the contralateral side served as controls for ultrasonographic comparisons. Ligation of the blood vessels was performed under general anaesthesia, with the animal in dorsal recumbency, using strict surgical asepsis. A 5-6 cm incision was made through the skin and subcutaneous fascia on the cranial aspect of the neck of the scrotum over the spermatic cord. After isolation of the spermatic cord and incising the vaginal tunic, the spermatic artery and surrounding veins of the pampiniform plexus were ligated at two points, about 4 cm apart, before they were returned, together with other structures of the spermatic cord, into the vaginal sac. The skin incision was then closed with 4-5 mattress sutures.

Post treatment monitoring

Experimental animals were monitored for 20 weeks post-treatment. Ultrasonographic imaging was carried out before surgery and on postoperative days (POD) 1, 8, 15 and then at approximately 14 day intervals, using a B-mode, real time scanner fitted with a 7.5 MHz linear array transducer designed primarily for intra-rectal use. Before imaging, the hair or fleece of the scrotum was closely clipped and a water soluble coupling gel was applied to the scrotum to ensure good contact between its surface and the transducer, by excluding air. Transverse images of paired testes and epididymides and longitudinal images of individual testis and epididymis were made. The sonographic images were copied with a video graphic printer.

Twenty weeks after treatment, the experimental animals were killed and scrotal contents were removed immediately. Each testis and the epididymis was sectioned longitudinally and examined for the presence of gross lesions. About 2-4 mm thick tissue samples were taken from the upper, middle and lower parts of each testis and from the three segments of the epididymis and preserved in 10 per cent formal saline solution until they were processed and stained with hematoxylin and eosin.

RESULTS

Ultrasonographic findings

The ultrasonographic findings at various stages of the study were comparable in all experimental animals except one goat (B 4). On POD 1, the ligated testis and the epididymal tail were enlarged and firm in all animals. The ultrasonographic appearance of these

organs differed from those on the non-ligated side (Fig. 1A, B and C). The ligated testis appeared to be larger and less echogenic than the contralateral normal testis. The testicular parenchyma was not homogeneous, but was mottled in appearance (Fig. 1A). The mediastinum testis could not always be identified. A hydrocele was evident as an anechoic line between the testicular tunics (Fig. 1B). The epididymal tail was enlarged, reduced in echogenicity and had lost its heterogeneous texture (Fig. 1C). A small amount of fluid was also seen around the epididymal tail.

On POD 8, the gross and ultrasonographic appearance of the ligated testis changed little, except that the thickness of anechoic fluid around it increased. The echogenicity of the epididymal tail was increased. A thick layer of anechoic fluid could also be identified around the pampiniform plexus, the latter was represented by hyperechogenic tissue (Fig. 1D). The tunica albuginea was thickened and then mediastinum testis was visible.

On POD 15, a thick layer of anechoic fluid was still seen around the testis, the epididymal tail and the pampiniform plexus (Fig. 1E, F and G). Hyperechoic threads, presumably representing fibrin, were seen floating within the anechoic fluid.

On POD 30, the ligated testis appeared to be smaller than the non-ligated testis. A true hydrocele was not observed. Instead, a thick network of hyperechogenic fibrin was seen within the anechoic fluid around the testis and the epididymal tail (Fig. 1H and K). Aspiration of fluid at this stage confirmed the presence of fibrin threads within the straw-coloured fluid.

In images taken POD 15-30 and later, linear hyperechoic spots, presumably representing the mediastinum testis, were identified in longitudinal images, while in the transverse images, 2-3 hyperechoic spots located in the middle of the organ, were seen. From postoperative weeks 6 to the end of the study (Fig. 1L, M and N), the ligated testis was atrophied and slightly less echogenic than the non ligated testis, with a thick echogenic tunica albuginea. The testicular parenchyma was homogeneous and the mottled appearance was lacking. The epididymal tail was also atrophied but more echogenic than the normal control. However, in one goat (B 4), an increase in the size of the epididymal body and the tail was noted six weeks after operation, which otherwise were hyperechogenic. The epididymal body appeared as a hyperechogenic mass between the paired testes on transverse images (Fig. 1M) in this goat. Relatively hyperechogenic tissue was seen between the scrotal skin and the testis in images taken 8 weeks after ligation and later (Fig. 1N).

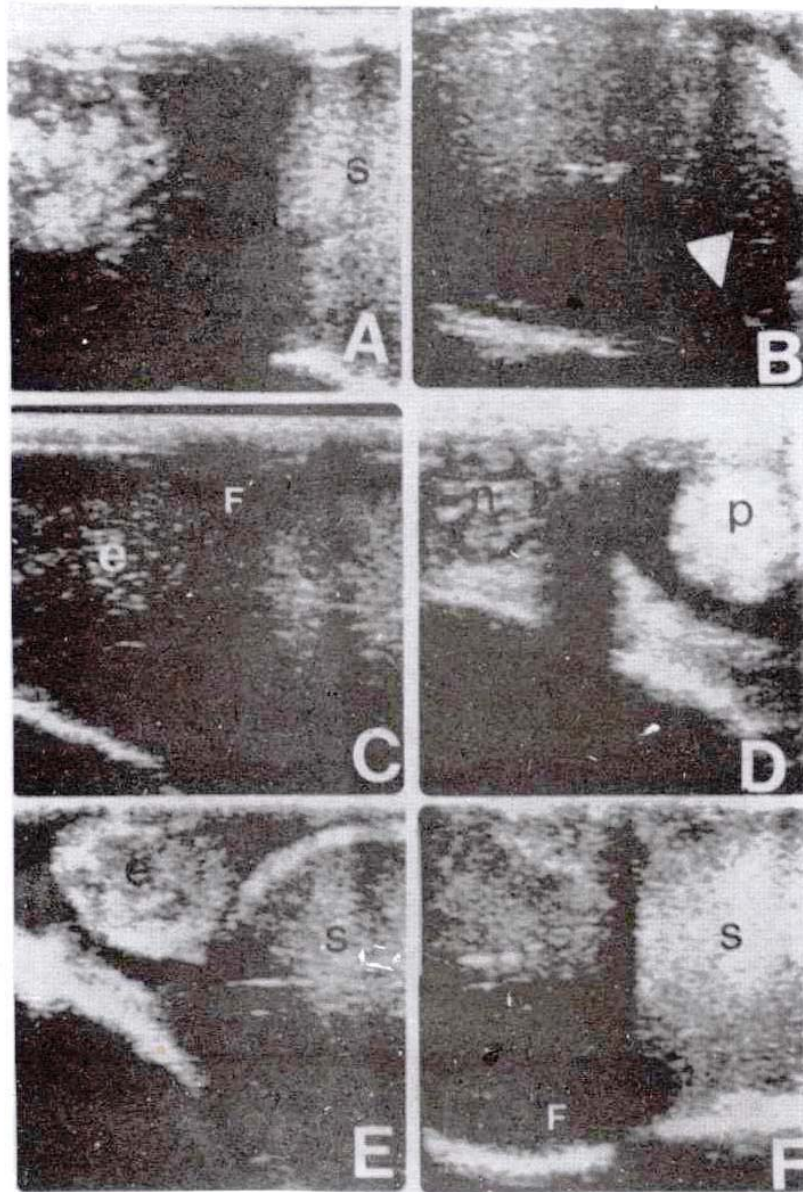


Fig. 1 (A-F): Ultrasound images of testes and related structures of goats and rams at different time intervals after ligation of testicular blood vessels. A). Day 1 The ligated (left) testis is less echogenic than the normal right testis (S), and has a mottled appearance. B). Day1 Hydrocele is visible (arrow) between the testicular tunics. C). Day 1 Epididymal tail (e) surrounded by anechoic fluid (F) testis (S) is also visible. D). Day 8 the right (ligated) pampiniform plexus (P) is represented by hyperechogenic tissue, and is surrounded by anechoic fluid. The left pampiniform plexus (n) is normal. E). Day 15 The ligated epididymal tail (e) is hyperechogenic and is surrounded by anechoic fluid. Tunica albuginea of the ligated testis (s) is thickened. F). Day 15 The ligated (left) testis is surrounded by anechoic fluid (f), normal testis (s) is also shown.

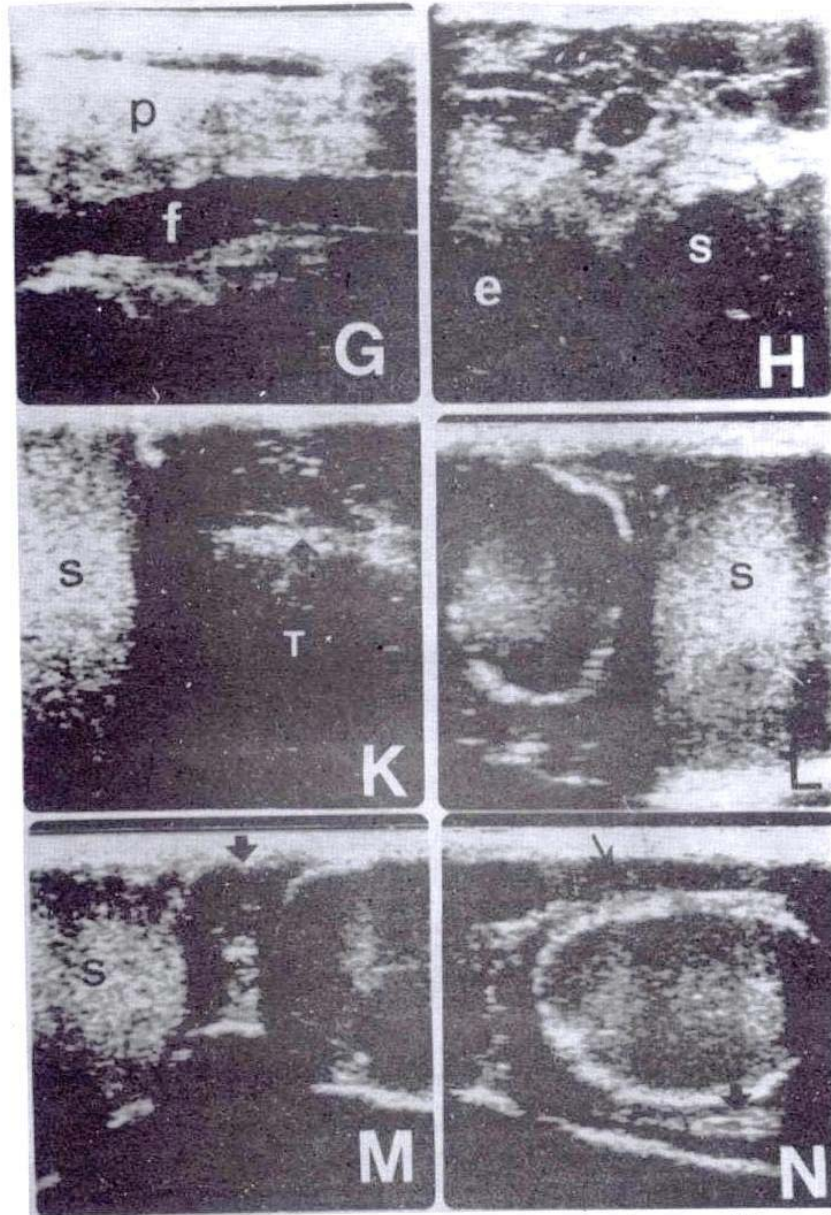


Fig. 1 (G-N): Ultrasound images of testes and related structures of goats and rams at different time intervals after ligation of testicular blood vessels. G). Day 15 Thick anechoic fluid (f) is present around the pampiniform plexus (p). H). Day 30 Hyperrechogetic fibrin threads are seen within the fluid around the testis (s) and the epididymal tail (e). K). Day 30 Fibrin network (arrow) is seen around the right testis (T). Left testis (s) appears normal. L). Week 12. Normal right testis (s) and the ligated left testis are shown. M). Week 16. An hyperechoic mass (arrow), probably the epididymal body, is seen between the normal testis (s) and the atrophied right testis in the goat B 4. N). Week 16. Atrophied ligated testis with thickened capsule (thin arrow) and echogenic material (thick arrow) between testicular capsule and the skin.

Postmortem findings

The gross and histological appearance of the ligated testis and related structures in all experimental animals, except one goat (B 4) were comparable. On the ligated side, the testis was atrophied and grey white in appearance, there was no network of blood vessels on the surface (Fig. 2); there were diffuse but loose adhesions. Sectioning of the ligated testis showed that the organ was surrounded by a thickened fibrous capsule, followed by a thin, grey brown layer and then the parenchyma, which was yellowish brown in color at the periphery and pinkish in the middle. It was dry and necrosed, and did not protrude from the cut surface. Histologically, the ligated testis showed diffuse coagulative necrosis with mineralization at the edge of the necrotic focus. A fibrous tissue reaction was present at the periphery.

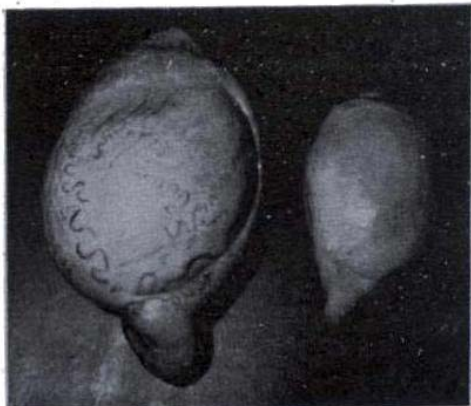


Fig. 2: Paired testes and epididymides of a goat in which testicular blood vessels on the right side were ligated. Testis and three segments of the epididymis on the right side are atrophied.

The three segments of the epididymis were atrophied, hard, dry and whitish in appearance; the tail showed a few small yellowish areas. Histologically, in the three segments of the epididymis, the normal tubules were not seen, the specimen was composed of a small number of thin and empty ducts, lymphatics and vessels within an extensive fibrous tissue mass; macrophages laden with hemosiderin were also identified.

In the goat B 4, the ligated testis was atrophied and was similar in gross and histological appearance as for other animals. The epididymal head was also atrophied; the epididymal body and the tail were enlarged and were of hard consistency (Fig. 3), but were dry due to the absence of secretions. Prominent blood vessels were seen around the epididymal tail as well as alongside the epididymal body. Histologically,

the epididymal tail showed chronic epididymitis with cystic dilatation of the tubules. There was evidence of fibrosis with patchy areas of necrosis and scattered areas of inflammation (neutrophilic).

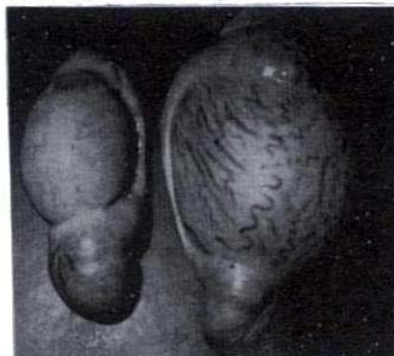


Fig. 3: The ligated (left) testis and epididymal head of goat B 4 are atrophied while epididymal body and the tail are enlarged.

DISCUSSION

The time sequence and the ultrasonographic appearance of the degenerating testicular parenchyma in goats and rams are similar to those reported upto 30 days after unilateral ligation of the testicular artery in goats (Eilts *et al.*, 1989). Experimentally induced 720° torsion of the canine testis resulted in decreased echogenicity of the testicular parenchyma within 15-60 minutes (Hricak *et al.*, 1983). The ultrasonographic appearance of the testis later than 30 days after ligation of the blood vessels has not been reported. Similarly, no reports describing changes in the echotexture of the epididymis after such treatment could be traced in the existing literature.

Oedema of the interstitium observed immediately after the ligation of the testicular artery in rams (Vrzgulova, 1979) and the distended veins filled with fibrin clots in goats (Eilts *et al.*, 1989) probably accounted for the decreased echogenicity and the mottled appearance of the testis on POD 1. By POD 3, histologically, the ligated testis had increasing amounts of tubular necrosis that progressed to total autolysis by POD 5 (Eilts *et al.*, 1989). The thickened appearance of the testicular tunics may partly be attributed to the ease of their identification caused by the hypoechoic testicular parenchyma, and partly to congested and thrombosed capsular veins (Eilts *et al.*, 1989). During the later stages, however, its thick hyperechoic appearance was due to the fibrosis which was seen at necropsy.

The decreased echogenicity and increased size of the epididymal tail on POD 1 may also have been due

to oedema and venous congestion. Later, the epididymal tail increased in echogenicity, probably due to fibrosis which was identified at the histological examination at the end of the study. The hyperechoic fibrin network, observed around the ligated testis and the epididymal tail on POD 30 in goats and rams has not been reported previously.

The ligated testis in all the animals showed a remarkable atrophy and diffuse coagulative necrosis associated with the ischaemia. Such changes were expected if the blood supply to this organ was completely removed, it also implies that there was no collateral blood supply to this organ. However, the results are complicated in this study because the method used also involved occlusion of the venous drainage of the organ.

All the three segments of the epididymal tail had atrophied in all the animals except on goat, in which the epididymal body and the tail appeared enlarged and prominent blood vessels were seen on their surface. The deferential artery supplying the vas deferens could have been responsible for maintaining adequate blood supply to the epididymal body and the tail in this animal.

Results of this study indicate that the decreased echogenicity of testicular tissue may represent the seminiferous tubular necrosis, while increased echogenicity of the epididymis may be associated with fibrosis. Focal hyperechoic areas within the testicular parenchyma have been shown to represent areas of mineralization (Ahmad *et al.*, 1993; Ahmad and Noakes, 1995a). An association between ultrasonographic, and gross and histological findings suggests that this technique may be a valuable diagnostic tool for assessing the intra-scrotal lesions in domestic animals.

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