



Ultrasonographic and Gross Pathological Studies on Testes and Epididymides of Rams and Bucks with Potential Lesions

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ABSTRACT

Ultrasonographic imaging of 30 organs of adult rams and bucks with spontaneous lesions of testes and epididymides and 10 normal organs was carried out, using a B-mode ultrasound scanner. Each organ was incised and the nature of lesions and their contents was observed. Ultrasonically, testes of normal rams and bucks appeared homogeneous and moderately echogenic. Mediastinum testis was seen as a centrally located hyperechoic line in longitudinal images and by an almost circular hyperechoic area in the middle of the organ in transverse images. The epididymal head and body were less echogenic than the testis and were homogeneous; the epididymal tail was heterogeneous, but less echogenic than the testis. In organs with lesions, various lesions included sperm granulomata in the epididymis, testicular degeneration with or without mineralization, testicular abscesses and chronic epididymitis. In early stages, sperm granuloma appeared as an anechoic mass with ill-defined border. In advanced stages, it exhibited a mixture of anechoic and hyperechoic areas, encircled by a thick hyperechoic border. In early cases of testicular degeneration, testicular parenchyma showed reduced echogenicity. However, in advanced cases, hyperechoic areas were scattered in affected parenchyma. Testicular abscesses showed anechoic cavities with some hyperechoic areas. In chronic epididymitis, echogenicity of epididymal tail was increased, probably due to fibrosis of organ. It was concluded that diagnostic ultrasound may be used to confirm the presence, and to some extent nature, of lesions in the testis or the epididymis.

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Authors' Contribution

SM designed the study. MA and RA helped in data analysis. NA, MAR, MSA and MMA helped in manuscript writing and contributed in experimental work.

Key words

Echotexture, Gross pathology, Testis, Epididymis, Ovine.

INTRODUCTION

Small ruminants, including sheep and goats, play a significant role in the economy of Pakistan. The present sheep and goat population in the country is estimated to be 29.8 and 70.3 million heads, respectively (GoP, 2015-16). Besides providing 906 thousand liters of milk per year, sheep and goats also provide wool, hair, mohair and skins, which are an important source of foreign exchange for the country. These animals are also the major source of mutton in country, which is preferred over beef due to its taste and low fat content. However, during the last decade, due to draught and starvation in various parts of the country, especially in Balochistan, Cholistan and Tharparkar, drastic

decreases in sheep and goat populations have been noticed. Extensive export of these animals to Afghanistan and Gulf states has further increased the economic significance of small ruminants in the country. The population of sheep and goats in Pakistan can be increased through better health coverage and improved conception rates. Artificial insemination is the best available tool for the rapid genetic improvement in livestock within shortest possible time. However, for the success of this technique, it is necessary that males are fertile and free from any lesions of reproductive organs.

Diagnostic methods of assessing the health of testes and epididymides in domestic animals mainly include manual palpation, measurement of scrotal circumference, testicular diameter and evaluation of semen. Thermography, tonometry and biopsy have also been tried as diagnostic aids, but their results were not quite encouraging in veterinary practice. The findings of clinical

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examination and semen evaluation are usually not enough to specify reasons for reduced fertility (Jedraszczyk, 2003). Diagnostic B- Mode and Color Doppler ultrasound techniques are a non-invasive, painless, simple and quick method to screen out the animals with abnormal testes and poor conception rates (Samir *et al.*, 2015). Moreover, B-Mode ultrasonographic examination of the genital organs of a male can be a more economical and useful measure to complement clinical findings and could be of great value in diagnosing subclinical changes in these organs (Cardilli *et al.*, 2009).

In theriogenology, the use of diagnostic ultrasound is mostly used for monitoring physiological changes in, or diagnosing the pathological lesions of, female reproductive organs. However, it seems to be an effective technique to confirm the presence or absence of intra-testicular masses, since a moderately homogeneous echogenic pattern of normal testis serves as standard for the detection of small focal lesions of this organ. Testes and epididymides seem to be particularly suited for this type of examination due to their superficial location in the scrotum. It is a useful tool to monitor developmental changes in the testes and accessory glands (Derar *et al.*, 2012). Moreover, color doppler and B-mode ultrasound examination of testes have no adverse effects on semen quality or sperm production (Kastelic and Brito, 2012). There are relatively few reports on the use of the diagnostic ultrasound in assessing the lesions of male reproductive organs in domestic animals. These include: induced testicular lesions in goat bucks (Ahmad and Noakes, 1995a; Ali *et al.*, 2011), spontaneous testicular lesions in rams and male goats (Ahmad *et al.*, 2000; Ahmad and Noakes, 1995c; Gouletsou *et al.*, 2003), bulls (Abu-Seida, 2012), spermatic granuloma in the ram (Karaca *et al.*, 1999) non-palpable Sertoli cell tumors in infertile dogs (England, 1995) and segmental aplasia in bulls (Williams *et al.*, 2010). Some authors also tried to correlate breeding soundness evaluation (BSE) with trans-scrotal ultrasonography (Chapwanya *et al.*, 2008; Yimer *et al.*, 2011).

In Pakistan, a study was conducted on the ultrasonographic appearance of testes and epididymides of infertile cattle and buffalo bulls (Ali *et al.*, 2011). However, these researchers could not correlate ultrasonographic findings with gross pathological observations. The present study describes ultrasonographic and gross pathological picture of testes and epididymides of rams and bucks with ill-defined lesions of these organs but previously only in normal ram testes (Ahmadi *et al.*, 2013).

MATERIALS AND METHODS

Collection and physical examination of organs

A total of 30 male reproductive organs (testes and

epididymides) with spontaneous lesions were collected immediately after the slaughter from adult rams and bucks, slaughtered at a local abattoir. Another five reproductive organs from normal adult rams and bucks each were included as control. No pre-slaughter information about the age and reproductive functions of these animals was available. Immediately after collection, these organs were wrapped in the plastic sheets and taken to the laboratory within 1-2 h after slaughter. In the laboratory, each organ was properly cleaned and the extraneous tissue, if any, was removed. Each organ was examined for the nature of testicular or epididymal lesions. The consistency and location of each lesion was recorded.

Ultrasonographic examination

After physical examination, each testis was examined ultrasonographically, using a B-mode, real time ultrasound scanner (Micrus, SSD 500, Aloka, Japan), fitted with a 5.0 MHz convex-array transducer, as described earlier by Ali *et al.* (2011). Before imaging, testicular capsule was removed and ultrasound coupling gel was applied on each organ. Each organ was imaged in the longitudinal, as well as transverse plane. Similarly, three segments of epididymis *i.e.* the head, body and the tail were also scanned to determine nature of lesions. Ultrasound images were frozen and printed on a glossy paper, using a thermal video graphic printer (Echo Copier SS2-307E, Aloka, Japan).

Pathological examination

After ultrasound examination, each organ was dissected to grossly confirm the nature of pathological lesions. For this purpose, the organ was incised and nature of each lesion and its contents, if any, was recorded. Contents of sperm granulomata and abscesses were also cultured on the nutrient agar to monitor any bacterial growth. About 2-4 mm thick tissue samples from the diseased site were taken from selected samples, preserved in 10% buffered formal saline and processed for histopathological examination through Haematoxylin and Eosin stain, using standard procedure.

RESULTS AND DISCUSSION

Echotexture of normal testes and epididymides

On ultrasonographic examination, testes of normal rams and bucks appeared quite homogeneous and moderately echogenic. Mediastinum testis is a fibrous cord of connective tissue which is an extension of tunica albuginea. It runs through length of organ and gives rise to radiating sheets of connective tissue which divide testicular parenchyma into lobes and lobules (Ahmad *et al.*, 1991). In ultrasonographs, the mediastinum testis was represented by a centrally located hyperechoic line in

longitudinal images (Fig. 1A) and by an almost circular hyperechoic area located in the middle of the organ in transverse images. The testicular tunic was seen as a hyperechoic line encircling the testicular parenchyma.

Similar type of echotexture of normal testes as observed in the present study has been described previously in rabbits (Aksoy *et al.*, 2009), bulls (Derar *et al.*, 2012), rams and male goats (Ahmad *et al.*, 1991), and camels (Pasha *et al.*, 2011). A thin layer of non-echogenic fluid was (Ahmad *et al.*, 1991) identified between the parietal and visceral layers of testicular tunics in bucks and rams (Ahmad *et al.*, 1991). No such fluid could be identified in organs of rams and male goats used in the present study. This could have been due to the fact that testicular capsule, which contains this fluid, was removed from these organs before ultrasound examination.

The epididymal head was less echogenic than the testicular tissue and was homogeneous. However, the epididymal tail was heterogeneous, but less echogenic than the testis. The epididymal body appeared as a

homogeneous hypoechoic structure with echogenic margins. The vascular pampiniform plexus was seen as comprising numerous convoluted hypoechoic tubular structures. A similar ultrasonographic appearance of the normal epididymis has also been described in the bulls (Derar *et al.*, 2012), bucks and rams (Ahmad *et al.*, 1991; Gouletsou *et al.*, 2003). Tail of the epididymis was relatively more heterogeneous ultrasonically than testis, most probably due to its convoluted tubular structure.

Echotexture of testes and epididymides with lesions

In this study, a total of 30 organs with spontaneous lesions were examined. Among these, 13 showed sperm granulomata in epididymal head, while one had sperm granulomata in both epididymal head and tail. Moreover, two organs had mild testicular degeneration and four organs showed testicular degeneration with mineralization. Testicular abscesses were seen in four organs, three had problem with epididymal tail (epididymitis) and three had miscellaneous lesions.

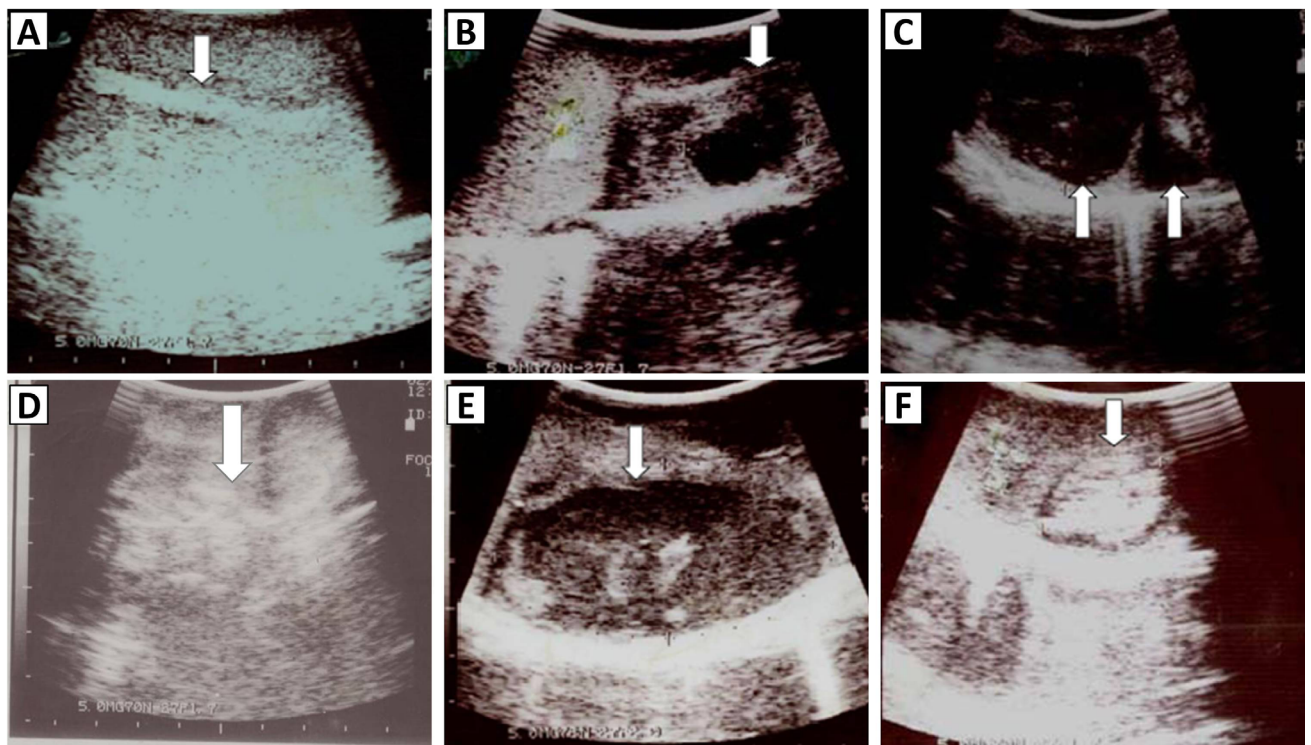


Fig. 1. Longitudinal ultrasonograph of A, normal testis showing homogeneous and moderately echogenic parenchyma with hyperechoic mediastinum testis (arrow); B, showing sperm granuloma (arrow) at advance stage, having hyperechoic and anechoic areas; C, transverse ultrasonograph of the epididymal tail showing two sperm granulomata (arrows). Tissue below the lesion is hyperechoic, which is an ultrasound artifact known as enhanced through transmission; D, testis showing many hyperechoic areas scattered throughout the parenchyma (testicular mineralization); E, testicular abscess (arrow), showing hypoechoic and hyperechoic areas, with enhanced through transmission underneath the lesion (bright area); F, testis, showing a hyperechoic area surrounded by a hypoechoic line (arrow).

Sperm granulomata

Sperm granulomata are usually seen in the head or the tail of the epididymis. They are formed when spermatozoa escape from their normal ducts due to extravasation or rupture of the epididymal tubules. In the beginning, it appears thin walled inflammatory reaction around thick creamy semi-fluid mass consisting of tail-less spermatozoa with a few phagocytes in its wall. Later, it develops a thick grayish red wall with putty-like contents consisting of spermatozoa, phagocytes, giant cells and amorphous debris (Karaca *et al.*, 1999).

Sperm granuloma is considered to be a significant lesion of the epididymis. In this study, 13 samples showed granulomata in the epididymal head, while in one sample granuloma was found both in the head and tail of the epididymis. Thus, in this study approximately, sperm granulomata were seen approximately, in 50% of the organs examined. This reflects high incidence of this problem in rams and bucks, and may be a major cause of infertility in males of these two species.

The gross and microscopic appearance of epididymal sperm granuloma has been described in ram (Ahmad and Noakes, 1995b; Karaca *et al.*, 1999) and dog (Yamasaki and Sawaki, 1991). It has been stated by researchers (Ahmad *et al.*, 2000) that during the early stages, sperm granulomata appeared as anechoic mass with through transmission indicative of fluid filled cavity with an indistinct wall. With time, the cavity changed from anechoic to hypoechoic mass, presumably due to fibrosis of the lining tissue, as seen in histological sections postmortem, or due to deposition of ceroid material from phagocytosis and disintegration of the trapped spermatozoa (Ahmad *et al.*, 2000; Ahmad and Noakes, 1995b). Similarly, in the present study, most of the sperm granulomata appeared as anechoic masses with indistinct wall (Fig. 1B). They seemed to be in their early stages of development. However, a few sperm granulomata showed hyperechoic heterogeneous appearance with distinct border (Fig. 1C). These seemed to be at advance stages. Some samples showed multiple sperm granulomata (up to three) in the same organ. When epididymides with granulomata were incised for gross examination, thick, semi-solid creamy-white material was seen in the granuloma. In-vitro culture of contents of sperm granulomata did not show any bacterial growth, excluding possibility of the abscess.

Testicular degeneration

Testicular degeneration is an important cause of infertility in male animals. In this study, testicular degeneration showed second highest frequency after sperm granuloma; Six samples showed testicular degeneration of varying degree; severe degeneration with mineralization

was seen in four samples, while two samples had mild degeneration. The ultrasonographic images of organs with severe testicular degeneration showed remarkable changes in their echogenicity; the testicular parenchyma was quite heterogeneous and showed dense hyperechoic areas scattered throughout the parenchyma (Fig. 1D). These appeared to represent the mineralized foci (Ahmad *et al.*, 1993). In two organs suspected for mild testicular degeneration, hyperechoic areas in the testicular parenchyma were not seen. Instead, the testes showed reduce echogenicity. Histological examination of these organs revealed sloughing of epithelium and vacuolization of seminiferous tubules without mineralization. A study was done on induced degeneration/necrosis of testicular tissues in rams and male goats by injecting a mild irritant solution locally into the organ (Ahmad and Noakes, 1995a). They observed reduced echogenicity of the testes within 24 h after injection as same done by other researchers in buck kids (Samir *et al.*, 2015). In a subsequent study, an ischemia was induced in testis and reduced echogenicity was observed in testis after 24 h (Ali *et al.*, 2011).

Ultrasonically image of testes were obtained after slaughter from bulls of unknown breeding history by scientists working on bovines (Jedraszczyk, 2003). They found hyperechoic areas in the testes; these areas corresponded to foci of grey-white fibrous looking tissue seen on gross examination (Fig. 1D). However, in the absence of any available information about semen quality and histological appearance of the testes, these workers could not ascertain the true nature of these hyperechoic areas. On the other hand, results of the present study and those of Ahmad *et al.* (1993) indicate that these hyperechoic areas were representing foci of mineralization observed on gross or histo-pathological examination. Thus, diagnostic ultrasonography seems to be useful in advanced cases of testicular degeneration with mineralization when semen collection and its evaluation is a problem.

Testicular abscesses

Testicular abscesses usually arise from the extension of a pyogenic epididymitis or proliferation of pyogenic organisms when there is traumatic injury on testis. Ultrasonographically, an increased number of internal echoes have been reported with interspersed areas of anechogenicity assumed to indicate areas of edema in the organ (Ülker *et al.*, 2009). In the present study, four testicular samples were enlarged and swollen. In these samples, some areas were soft in consistency and others were hard. Ultrasound imaging showed increased number of internal echoes with interspersed areas of lucency thought to arise from areas of edema, and some bright echogenic spots were seen in the middle (Fig. 1E).

When testes were opened for gross examination, thick creamy material oozed out, indicating abscess with fibrous lining. Bacterial growth was seen when contents of these lesions were cultured on nutrient agar. Abundance of leukocytic infiltration and exudates in seminiferous tubules were seen histologically, which confirmed the lesion to be an abscess. On ultrasound scan, an abscess appeared quite heterogeneous and was represented by mixture of anechoic and hyperechoic areas. A similar picture was seen for sperm granuloma in advanced stages. Thus, it does not seem possible to differentiate between these two lesions ultrasonically. Aspiration of contents of these lesions and their examination/culture in the laboratory is necessary for differential diagnosis between these two lesion types.

Epididymitis

Epididymitis commonly causes enlargement of the epididymis, accompanied by tenderness, erythema and pain. Ultrasonically, epididymitis appears as enlargement of the organ, together with a reduction in the echogenicity; the structure may appear slightly heterogeneous with diffuse areas of anechogenicity, probably due to edema. In this study, three samples showed increased echogenicity of the epididymal tail. Testis appeared normal ultrasonically. Grossly, the epididymal tail was enlarged, hard in consistency and appeared fibrosed and seemed to be a case of chronic epididymitis. However, histological examination of the organ could not be made. Increased echogenicity might have been due to fibrosis of the organ. A similar study performed with humans where fibrosis and tumor like lesions were confirmed which ultrasonically appeared as hypo and hyper echoic images, respectively (Shtricker *et al.*, 2015).

Miscellaneous lesions

In the present study, three organs showed miscellaneous lesions, as histo-pathological examination of these lesions to confirm their nature could not be performed. The first organ showed many hyperechoic bright areas in the testis upon ultrasound examination. On sectioning, gross examination of testis indicated that whitish (sperm clot like) material was spread over the whole parenchyma of the testis. When this material was observed under light microscope, sperm clots were identified. This was assumed to be due to traumatic injury of the testis that might have damaged the seminiferous tubules, and sperm might have come out from the damaged seminiferous tubules. No significant abnormality was found in the testis.

For the second organ, testis was found normal on physical palpation. When ultrasound was performed, a hyperechoic mass surrounded by a hypoechoic border was seen in the testicular parenchyma; the remaining testicular

parenchyma did not show any significant abnormality. On sectioning, the testis showed a hard, fibrosed mass, the exact nature of which remained unclear (Fig. 1F).

In one study, it is revealed that fibrotic lesions are common in testis of bulls raised under intensive rearing conditions (Barth *et al.*, 2008). Physical examination of the third organ reflected no abnormality in the testis or epididymis. Ultrasonically, echogenicity of testis along with epididymis was increased. Grossly, dark pinkish parenchyma was observed in the longitudinal section, which could have been due to blood hemorrhaging. Increased echogenicity was thought to be due to blood fibrinogen, which might have occurred due to injury and similar lesion has been reported through color doppler ultrasonography in Shiba goats (Samir *et al.*, 2015). Testicular echogenicity has also been shown to increase due to increased age (Cardilli *et al.*, 2012). However, there was no information about the age of animals from which the subject organ was collected. Histological examination of this organ could have confirmed the nature of such lesions as similar study in prepubescent lamb testes (Giffin *et al.*, 2014).

CONCLUSIONS

Results of the present study show that diagnostic ultrasound may be used to confirm nature of certain lesions in the testis or the epididymis. However, ultrasound scanning supplemented with histo-pathological examination seems necessary for the confirmation of the nature of different lesions of male reproductive organs.

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Statement of conflict of interest

Authors declare that they have no conflict of interest.

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