

Research Article**Studies in Testosterone Hormone Level in Ram Affected with Fascioliasis
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Abstract: Out of the 830 examined livers of ram, of different ages and sexes were slaughtered at abattoirs of Cairo, Giza provinces during the period from January 2014 to October 2014, the incidences of Fascioliasis was recorded in 117 (14.1 %). Highly significant decreases in serum cholesterol, High density lipoprotein HDL and Low density Lipoprotein LDL ($p < 0.01$) were recorded in our study in chronic fascioliasis. While in acute fascioliasis the serum cholesterol, HDL and LDL levels had significantly decreased ($p < 0.05$) in all. Testosterone concentration was found to be significantly decreased ($p > 0.05$) in rams, suffered from chronic fascioliasis. On the other hand, the results showed that there were no significant differences in testosterone concentration in acute fascioliasis. Lesions in acute fascioliasis were severe. Congestion, hemorrhagic migrating tracts formed from degenerated hepatocytes, erythrocytes and eosinophil's beside old parasitic tracts represented by central necrosis surrounded with eosinophil's, macrophages and lymphocytes together with connective tissue capsule. TEM of liver showed eosinophil cells with its characteristic granules. Chronic fascioliasis was characterized by presence of liver flukes in the lumen of the bile ducts. TEM of liver had shown prominent degeneration of the cell organelles. The testes in chronic fascioliasis displayed thickening of tunica albuginea, in addition to leydig cells were found isolated or in small clusters. TEM, showed necrotic leydig cells having deformed mitochondria, reduced smooth endoplasmic reticulum and few lipid droplets.**Keywords:** Ram, Testosterone, Fascioliasis.

INTRODUCTION

Rams have been used in Egypt as the main source of meat and wool. It's necessary to fulfill the gap between the increased population and their demands from animal protein. Fascioliasis caused by the giant liver fluke, *Fasciola gigantica* is an important animal parasite that inhabits the liver and bile ducts of ruminants and occasionally man [1]. Liver is the largest gland in the body, characterized by multiple functions categorized broadly as synthetic, catabolic, detoxifying, secretory and excretory in addition to its role in the metabolism of diverse pharmacologic agents, the liver is also responsible for inactivation or modification of several hormones; therefore [2].

Liver fluke infestation (*Fasciola gigantica*) is the most common cause of liver damage [3]. During migration of young flukes there is direct trauma with necrosis, hemorrhages, and subsequent healing by granulation tissues, which results in cirrhosis of the liver and fibrosis of the bile ducts causing its dilatation, thickening [4] which resulting in great economic losses either directly through condemnation of affected liver at

slaughter houses [5] or indirectly by effect on animal production [6, 7] and reproduction [8, 9].

Chronic liver disease may be accompanied by signs of apparent hormonal imbalance. Abnormalities in testosterone metabolism are believed to be involved in the development of testicular atrophy frequently seen in rams with chronic liver disease.

Lipoproteins transfer various lipids such as cholesterol from the tissue of origin to target sites, where the lipid complex is delivered via lipoprotein receptor-mediated uptake. In addition, the supply of the Steroids required for cellular activities, including membrane formation, steroid hormone secretion, and the post-translational modification of proteins is regulated by lipoproteins [10]. Lipoprotein-derived cholesterol is a major source of substrate for steroidogenic tissues, including the Leydig cells of some species [11-14]. Testosterone, the primary male hormone, is responsible for male characteristics plasma, testosterone levels are related to the serum lipid profile in normal and infertile animals [15]. Sheep infected with *Fasciola*

sp. exhibit substantial changes in Serum lipoprotein [16].

The present study aimed to evaluate the effect of hepatic dysfunction on serum cholesterol, lipoproteins and testosterone levels in rams.

- Investigate the effect of fascioliasis on ram liver and testis from the
- Histological and ultra-structural point of view.

MATERIALS AND METHODS

Animals and sampling

During the period extended from January 2014 to October 2014 January, livers and of 117 rams out of 830 examined once, of different ages and breed were collected from abattoirs of Cairo, Giza provinces, after Postmortem examination of liver by palpation and many incisions through each liver and bile ducts. Then careful visual examination and identification of adult or larval parasitic infestation was carried out according to Soulsby EJL [17] and choose positive cases of fasciola infestation. The gross picture was recorded.

Testes were obtained from the positive infested rams with fasciola.

Each testicle, left and right, after procurement was numbered, put in separate polythene bag brought to the laboratory as soon as possible and the testes were placed in their natural position on the table top. The extraneous material including the fat and fascia were removed from each testicle before gross examination.

Fecal sample

Samples were collected in clean plastic bags from the rectum of all animals under investigations. Fecal samples were examined directly after examination by direct smear, sedimentation and floatation technique [18].

Blood sampling and analysis

Blood samples (15 ml) were taken from the jugular vein and collected in vacuainers (Becton Dickinson Co., USA). Serum was separated by centrifugation at 2500 rpm for 20 minutes and stored at -20°C until analysis. The sera were analyzed for total cholesterol levels using enzymatic kits (Pars AZ moon.). Lipoproteins were isolated from sera by a combination of precipitation and ultracentrifugation. The precipitation method was used for measuring high-density lipoprotein (HDL) particles. In this method, after addition of sodium phosphotungstate- magnesium to the serum, the non-HDL lipoproteins that sedimented by centrifugation (10000 g for 5 minutes) were aggregated. Then, the residual cholesterol was

RESULTS

Animals positive to the fasciola infested was recorded in 117 rams, 14.1 %. Most cases showed

enzymatically measured [19, 20]. Low density lipoprotein (LDL) was calculated as the difference between cholesterol measured in the precipitate and in the HDL fraction, In addition, serum testosterone level was measured by radioimmunoassay method that employed a diagnostic kit (Immunotech, SA, France, PI-1119).

Histopathological examination

Specimens from affected livers and testis were immediately taken and immersed in 10 % formalin. After proper fixation, the specimens were trimmed, washed, dehydrated in ascending grades of ethyl alcohol, then cleared in xylol and embedded in paraffin. Thin sections about 4-6 microns in thickness were prepared and stained with haematoxylin and eosin stain for general microscopic examination [21].

Transmission electron microscopy (TEM)

For ultra-structural evaluation by transmission electron microscopy as described previously by (22), freshly excised liver and testes were cut into small blocks (1×1 mm³), fixed directly in cold 4F1G (i.e. 4% formalin + 1% glutaraldehyde adjusted at pH 2.2) for 24 hours, then were post fixed in 1% osmium tetroxide in 0.1M phosphate buffer (pH 7.3), dehydrated in an ethanolic series culminating in 100% acetone, and infiltrated with epoxide resin. After polymerization overnight at 60°C, semithin sections (0.5 µm) were stained with 1% toluidine blue in 1% sodium borate and examined with light microscope. Areas of seminiferous tubules in testis were selected and the blocks trimmed accordingly.

Ultrathin sections (80-90 nm) were cut, mounted on 200 mesh copper grids, and stained with uranyl acetate and lead citrate. The stained grids were examined and photographed by JEOL. JEM-1400-EX-ELECTRON MICROSCOPE at the Central Laboratory of Faculty of Science, Ain Shams University. The photographs were printed on KODA BROMIDE F5s GLOSSY Black and White-Schwarzweib- Kodak.

Statistical analysis

Results were presented as mean ± standard error of mean (SEM) for serum lipid profile, and the levels of serum testosterone. The data were statistically analyzed using the SPSS statistical software package, version 10.0.1 (SPSS Inc., Chicago, IL, USA. Prior to analysis, the Kolmogorov-Smirnov test was used to check normal distribution of the data. Then, to evaluate the correlation of the serum lipid profile and testosterone secretion, Pearson's partial correlation coefficient analyses were conducted. A p value <0.05 was considered statistically significant.

emaciation. The consistency of feces mostly was pasty and some cases showed normal pellets.

Cholesterol, Lipoproteins and testosterone level

Our results as illustrated in table (1) revealed that highly significant decreases in serum cholesterol, High density lipoprotein HDL and Low density lipoprotein LDL ($p < 0.01$) for all. Whereas, in acute fascioliasis the serum cholesterol, HDL and LDL levels

decreased significantly ($p < 0.05$) in all. In addition, testosterone concentration significantly decreased ($p > 0.05$) in rams that suffered from chronic fascioliasis. On other hand showed that there was no significant differences testosterone concentration in acute fascioliasis.

Table 1: Correlation coefficients between serum lipid profile and serum testosterone concentration in Acute and Chronic fascioliasis rams

	Control	Acute Fascioliasis	Chronic Fascioliasis
Cholesterol (mg/dl)	63.75±8.56	48.76± 6.82*	33.28±6.38**
LDL	11.99±2.27	7.82 ± 1.88*	5.07.0±7.51**
HDL(mg/dl)	43±8.72	23.07 ± 5.51*	19.81±3.62**
Testosterone concentration (ng/ml)	2.69 ± 0.64	1.91 ± 1.05	0.65 ± 0.07**

Data expressed as mean ±SD, *: significant ($p < 0.05$), **:highly significant ($p < 0.01$)

Histopathological investigation

Acute fascioliasis were recorded in 86 cases (10, 36 %), Chronic fascioliasis showed in 31(3.1%).

Macroscopic examination revealed that the majority of the affected livers were enlarged; firm, congested and oozing blood freely from the cut surface with the presence multiple reddish or yellowish foci on the surface.

Microscopically, severe congestion of central veins, hepatic sinusoids and portal blood vessels was seen. Hemorrhagic migrating tracts formed from

degenerated hepatocytes, erythrocytes and infiltrated with eosinophils, macrophages and lymphocytes were noticed (Fig. 1A). Moderate reaction in the portal triad was manifested by formation of numerous newly formed bile ducts, leukocytic cell infiltration and fibroblastic reaction (Fig.1B). Hemosiderin pigments were noticed in migrating tract in ram. Moreover, severe eosinophilic cellular infiltration in the portal areas was seen in some cases. TEM of the liver inacute fascioliasis showed eosinophil cells with its characteristic granules, mature collagenous connective tissue. Presence of cells in stage of pyknosis and karyorrhexis (Fig. 1C).

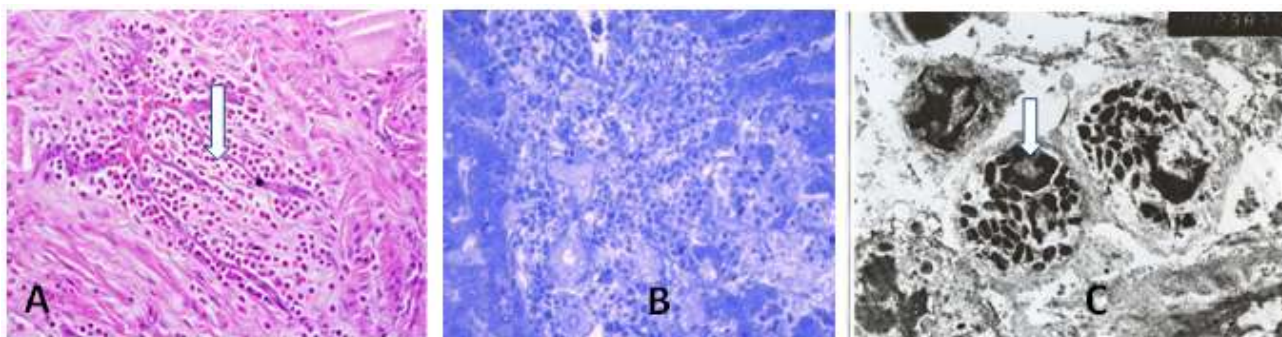


Fig.1 A: Liver of rams showing severe eosinophilic cellular infiltration in the portal area (arrow) (H&E stain x300). B: a light micrograph of tuldine blue stained section from liver demonstrate leukocytes infiltration. The hepatic cells showing moderate cytopathic changes, most of the nuclei of the hepatic cells showing pyknosis. Narrowing of the sinusoids (T.B. stain x 40). C: TEM of ram liver from the acute fascioliasis showing eosinophil cells (arrow) with its characteristic granules (6700 X 2).

Grossly, the Testes showed nearly normal size. Microscopically, the testis of the rams consist of lobules, each lobule contain one or more Seminiferous tubules was irregular or round -shaped, arranged in testicular lobules. The seminiferous multilayer of germinal epithelium lined tubule (Fig. 2A). The testis is enclosed in a thick fibrous capsule, the tunica albuginea. The seminiferous tubules are ensheathed by basal lamina formed of myoid cells. Each tubule Possesses epithelial cells involved of sertoli cells and the germ cells of various stages, covering the complete process of spermatogenesis. Sertoli cells exhibit typical

irregular nuclei and well-defined cytoplasm. Spermatogonia are oval, rest upon the basal lamina of the seminiferous tubule. The interstitium between seminiferous tubules contain distinct Leydig cells and blood vessels. Cells of interstitial tissues of the testes were conspicuous and in large groups (Fig. 2B). TEM of testis showed Normal Leydig cells possess large spherical nuclei with distinct nucleoli, Chromatin and coarse clumps of peripheral heterochromatin, in addition to the cytoplasm containing cisternae of smooth endoplasmic Reticulum, mitochondria and lipid droplets. Monocytes are found in the interstitial tissue.

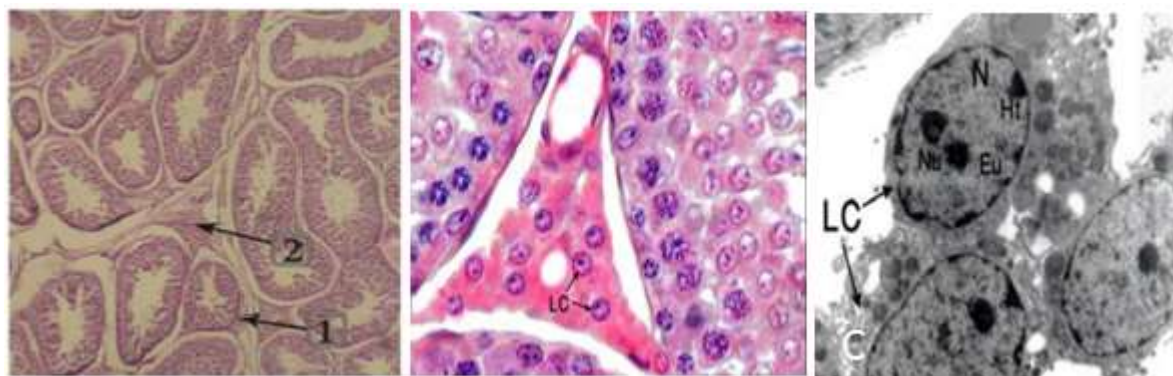


Fig. 2A: Showing normal Seminiferous tubules (1) and interstitial tissues (2) ram testis (H&E, x125); **B:** Normal architecture of interstitial tissue of testis embodying clusters of Leydig cells (Lc) located in between three seminiferous tubules being surrounded by basal lamina containing myoid cells having spermatogonia, and Sertoli cells. (H&E, x1320). **C:** TEM of Interstitial tissue of testis revealing Normal fine structure of Leydig cells (LC), characterized by cytoplasm contain mitochondria, Smooth endoplasmic reticulum and lipid droplets, in addition to oval to rounded nuclei (N) containing one or two nucleoli (Nu), heterochromatin (Ht) and euchromatin (Eu). A monocyte cell is also noticed with its eccentric nucleus (N). (x4000)

Macroscopically, the livers were mostly small in size and firm in consistency with corrugated capsule. Mature liver flukes were occasionally observed within the lumen of the thickened bile ducts. Black minute granules (hematoporphyrin pigment) of gritty sensation were found in the bile duct. Meanwhile, some normal sized liver showed areas of cirrhosis and thickened bile duct wall.

Microscopically, hepatic cirrhosis characterized by severe connective tissue proliferation infiltrated with mononuclear leucocytes with the presence of hepatocellular atrophy and prominent fibroblastic proliferation with formation of large amount of collagenous fibers were seen.

The fibroblastic proliferation and fibrosis mainly begin from the area of the portal triad and

extend into and interlobular areas forming pseudolobulation (Fig. 3A).

Adult fasciola worms with desquamated epithelium were occasionally found inside the ductal lumen (Fig. 3B). Portal tracts showed lymphocytic infiltration in addition to hyalinization and mononuclear leucocytic infiltration in the wall of the blood vessels. Bile ducts revealed severe hyperplasia and desquamation of their epithelial cell lining, TEM of the liver in chronic fascioliasis showing: prominent degeneration of the cell organelles, disintegration of the cisternae of the mitochondria (M). Some of cells contain lipofuscin pigment (arrow). Collagenous connective tissue was found in between atrophied hepatic cells (Fig. 3C).

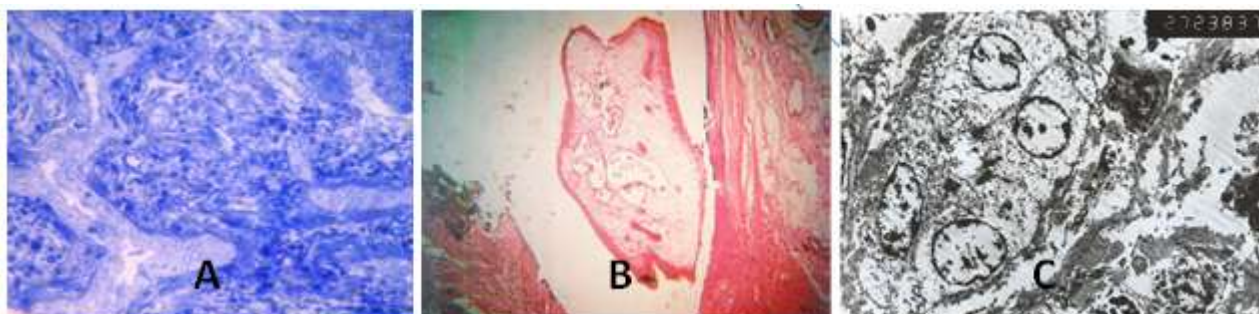


Fig 3: **A:** a light micrograph of tulidine blue stained section of liver showing large branching bile ducts having a narrow lumen, and newly formed bile ducts without formation of lumen. Presence of collagenous fibers with presence of eosinophilic cells occupying wide area in the stroma of the liver (T.b. stain mag. 40x); **B:** Liver of ram affected with chronic fascioliasis showing cross section of fasciola worm in the bile duct lumen, biliary hyperplasia and leucocytic infiltration ductal wall (H&E stain x300); **C:** TEM of liver showing newly formed bile ductules. The epithelial cells contain free ribosomes and poor in other cell organelle. The connective tissue reaction contains collagenous connective tissue eosinophilic cells, lymphocytes and mast cells. 2700 X 2.

Grossly, the testis was small, and was slightly firm. Microscopically, reduced seminiferous tubules in diameter, thickened basement membrane, with hyaline changes were seen. There was increase in per tubular connective tissue and interstitial cells appeared to be increased in number. The spermatocytes were vacuolated and fusion of spermatids produce

intratubular giant cells., Leydig cells were found isolated or in small clusters and lower in number compared to the testis in acute fascioliasis (Fig. 4A).TEM of testis showed necrotic Leydig cells having deformed mitochondria, reduced smooth endoplasmic reticulum and few lipid droplets. Blood capillary lined with endothelial cells is also seen (Fig. 4B)

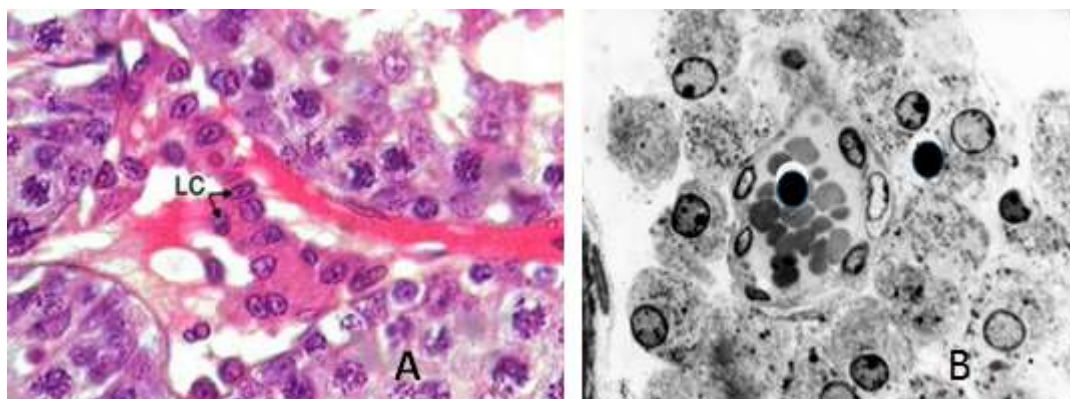


Fig. 4: A Destructed interstitial tissue of testis in between four seminiferous tubules revealing necrotic Leydig cells (LC), vacuolation and infiltrated tissue. The seminiferous tubules showing vacuolated spermatogonia , necrotic primary spermatocytes. Beside, deformed sertoli cells are clearly seen. (H&E, x1320); **B:** Interstitial tissues with Leydig cells (Lc) are commonly located close to blood vessels (bv). Some of the Leydig cells are damaged and disintegrated (1 000 x).

DISCUSSION

The prevalence percentage of fascioliasis in rams in this study, was 14.1 % in it is evident that rams was more susceptible to infested with fasciola probably due to grazing on pasture contaminated by snails beside their higher susceptibility and low immunity [17]. Highly significant decreases in serum cholesterol, High density lipoprotein HDL and Low density Lipoprotein LDL ($p < 0.01$) were recorded in our study in chronic fascioliasis. While in acute fascioliasis the serum cholesterol, HDL and LDL levels significantly decreased ($p < 0.05$) in all. These results were in complete agreement with those obtained by [16]. Who stated that Lipoproteins are synthesized in the liver; the presence of hepatic dysfunction may results in disturbance in their serum levels. The decrease in serum cholesterol ($p < 0.01$) levels may be attributed to decrease synthesis by the liver and sever damage of liver in chronic disease. It was reported that the majority of serum cholesterol in sheep is circulated as HDL [3]. HDL cholesterol in the present study was the major lipoprotein in serum, the decrease in its level may be attributed to decrease serum cholesterol level and to decrease HDL synthesis by the liver. In addition, Serum LDL was significantly decreased and may be attributed to the higher decrease in cholesterol concentration in serum of rams [38].

The findings demonstrated that testosterone concentration significant decrease ($p > 0.05$); in rams suffered from chronic fascioliasis. In other hand showed that there was no significant differences testosterone Concentration in acute fascioliasis. Among serum lipids

levels had a positive correlation with serum testosterone concentration. These results were in complete agreement with those other studies [23, 24].

Regarding the gross lesions of acute fascioliasis among the affected livers, the majority of livers were enlarged, firm, congested and oozed blood freely on cutting. Multiple pinpoint reddish foci or streaks along with grayish- white or yellowish raised foci on the hepatic surface were noticed. These foci on the surface represented the points of entrance of the immature parasite into the liver structure. These lesions were in complete agreement with previous studies [25-27]. Our microscopical findings revealed recent hemorrhagic tracts and old migrating tract. These lesions agree with those mentioned others [28-30]. In the same time our results disagree with Metwally AMM *et al.* [27] who obtained multinucleated giant cells around the old parasitic tract and Kaya G *et al.* [32] who obtained granuloma encircled the fasciola eggs and formed from polymorph nuclear cells, mononuclear cells, giant cells and outermost fibrous layer. In these studies, hepatocytes in the vicinity of the parasitic tract were suffering vacuolar and hydropic degeneration and the bile ducts showed hyperplasia and desquamation of its epithelial cell lining. Moreover, congestion and dilatation of the hepatic blood vessels and hepatic sinusoids were seen. These findings were in agreement with those previously found others [28, 29]. Hemosiderin pigments were detected in the old parasitic tract in liver and these results were similar to that obtained by Ameria FMA [31]. In our investigation, the livers suffering from chronic fascioliasis had corrugated

thick capsule. The cut surface revealed thickened wall bile duct with the presence of mature fasciola worms together with mucoid exudate within the ductal luminae. These results were in a compliance with those mentioned others [25, 26, 33].

Our study showed that, chronic fascioliasis was manifested microscopically by hyperplasia and desquamation of the epithelial lining of the bile ducts. Fasciola worms intermingled with necrotic debris were also noticed inside the bile duct lumen. These results were similar to those obtained in previous studies [29, 33, 34]. Adenomatous growths of the ductal epithelium in the lumen in the form of villous like projections were also observed in our results. Similar findings were mentioned by others [27, 29]. Perilobular and pericellular hepatic cirrhosis accompanied with chronic fascioliasis had been revealed in our work. These results were in a complete agreement with those mentioned prior studies [28, 29, 31, 35]. Vasculitis characterized by thickening and hyalinization of the hepatic blood vessel wall, which infiltrated with leucocytes was observed in our work. These results completely agree with some studies [26, 34] and partially agree with others [35, 36].

The testis is considered the most important organ in the male reproductive system. It is characterized by two main functions, synthesis of steroid hormones and production of spermatozoa [37].

Testicular hypoplasia were reported in our study association with chronic fascioliasis in the present study. Grossly, the hypo plastic testes were smaller and were slightly firm. Histologically, examination revealed seminiferous tubules of reduced diameter, thickened basement membranes and increased per tubular connective tissues these results partially agree with by Monfared AL [38].

There is clear evidence that Leydig cells produce androgenic steroid hormones, principally testosterone, necessary for the germ cells to proliferate and differentiate. It has been reported that high concentrations of testosterone are required for normal spermatogenesis.

In the present study, ultrastructure revealed degeneration of Leydig cells may lead to inhibition of steroid genesis. The steroid genic capacity of Leydig cells in the goat's testis and its change during development of cellular organelles including the mitochondria and smooth endoplasmic reticulum responsible for steroid biosynthesis has been mentioned previously [24, 39]. It was concluded that, the present results seem to indicate that fascioliasis is greatly responsible for hepatic damage and demands immediate attention for taking control and eradication measures. Testicular hypoplasia accompanied chronic fascioliasis. Serum cholesterol and lipoprotein levels decreased in

fascioliasis and positively correlate with testosterone concentration.

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