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PATHOLOGY

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Hepatitis in Growth Promoter Treated Cows

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With 6 figures and 1 table

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Summary

Adult female beef cattle found positive for stanozolol in the urine were investigated for liver pathology. In all the animals toxic hepatitis was found, including cholestasis, periportal fibrosis and inflammation, focal necrosis and blood filled lacunae. As no clinical data of the cows were available, apart from the history of illegal stanozolol abuse, it is not possible to attribute all changes to the illegal hormone treatment. Moreover, the cows have probably been treated with a cocktail, and apart from stanozolol more anabolic steroids may have been used. Management factors, viral and bacterial infections, former caesarean sections and especially feeding regime may also be responsible for the lesions described. Striking similarities with data from hepatotoxicity found in human body builders using similar agents, however, suggest a major role of stanozolol as causative agent.

Introduction

The illegal administration of growth promoting agents is a recurring problem in meat industry. In the Netherlands, routine control is performed by the General Inspection Service for Lifestock and Meat (RVV) and suspected farms are controlled by the General Inspection Service (AID). Apart from this public control system, the sector has its own private control system, which works closely together with the public services.

Samples taken by the General Inspection Service are chemically analysed by RIKILT. The Section of Histopathology, Department of Safety, Health and Food, is sometimes involved in the examination of the animals and screening the tissues for injection sites and specific hormonal effects.

In 2000, many cows were found positive for the illegal growth promoter stanozolol in the urine, and consequently the General Inspection Service confiscated the animals. At slaughter the animals were examined for clinical symptoms and the carcasses were inspected for pathological lesions and injection sites. All carcasses were condemned.

Stanozolol is a 17-α alkylated androgenic steroid, therapeutically used to treat muscle wasting, in some anaemia, hereditary angioedema and in treatment of hypogonadal status (Catlin, 1995; Sheffer et al., 1987). But the illegal use of stanozolol, as Winstrol®, is widespread in sport doping by athletes and bodybuilders (Blue and Lombardo, 1999).

These 17-α alkylated androgens are well-known hepatotoxic agents, which can induce abnormal liver function tests, coagulopathies, liver hyperplasia, cholestasis, lipidosis, adenomas and liver failure (Soe et al., 1992; Blue and Lombardo, 1999). Hepatotoxicity because of anabolic steroids is described in humans (Ishak, 1979; Soe et al., 1992), dogs (Heywood et al., 1977; Yoshida et al., 2001), cats (Harkin et al., 2000), rats (Boada et al., 1999) and mice (Lesna and Taylor, 1986), but not in cattle.

This study describes liver pathology in 37 cows from several herds found positive for stanozolol in the urine.

Materials and Methods

The livers of 37 adult female meat-type cows were examined at slaughter and sampled along with the genital tract (data not shown). The cows originated from six different herds, the only factor they had in common was the presence of stanozolol residues in the urine. The pluriparous adult meat-type cows were in good general condition. The caudate lobe of the liver was sampled in all the animals and fixed in 4% buffered formaldehyde. Routine histology was performed on HE-stained paraffin sections. Moreover, extra stainings were performed using Gomori's Silver Impregnation for reticulin, Periodic Acid Schiffs for glycogen, Fouchet for bile, Rhodamine for copper and Perls Prussian Blue for iron.

Results

At slaughter all cows seemed in good condition. Macroscopically, the livers showed few effects, some were swollen, some were dark, and some more firm. Microscopically, the livers showed a pattern of alterations consisting of mild to severe fibrosis, mostly periportal but sometimes joining adjacent portal tracts. Sinusoidal leucocytosis was present in most livers (Fig. 1). Lymphocytic proliferation was present in most of the portal tract, but also more polymorphonuclear leucocyte infiltrates were present and in some cases plasma cells were seen.

The hepatocytes showed centrilobular hydropic degeneration and at the limiting plate the cells were smaller and more

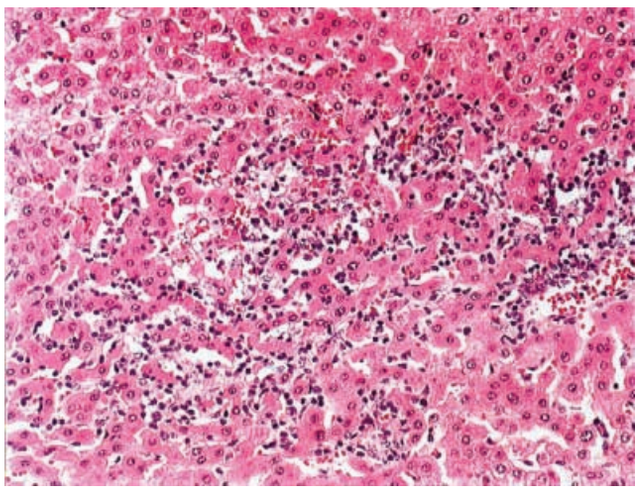

Fig. 1. Sinusoidal leucocytosis (HE, 250×).

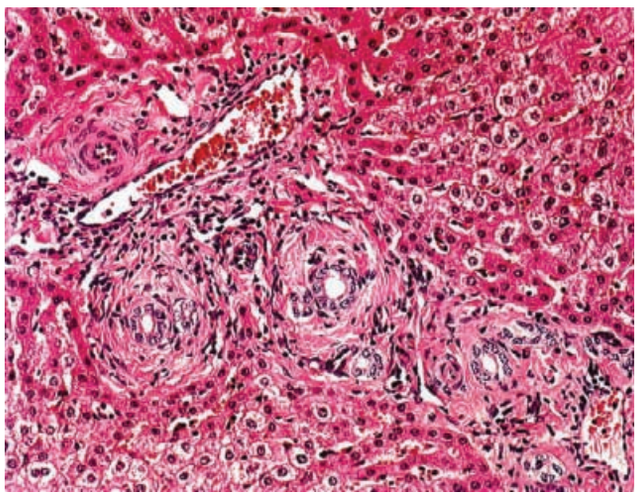

Fig. 4. Sclerotizing cholangitis (HE, 250×).

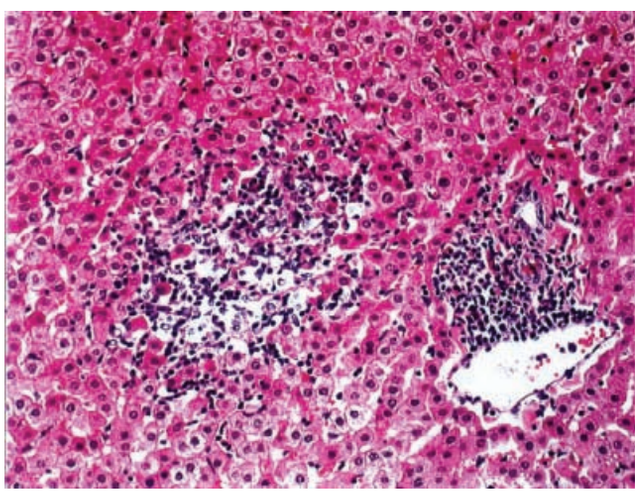

Fig. 2. Focal necrosis with infiltration of inflammatory cells (HE, 250×).

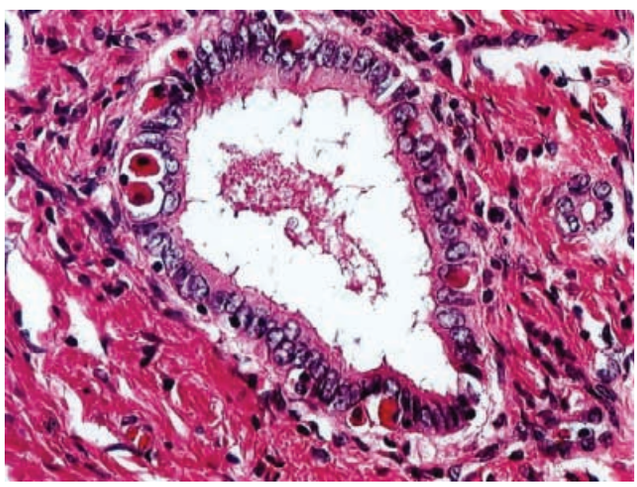

Fig. 5. Apoptosis of the bile duct epithelium (HE, 400×).

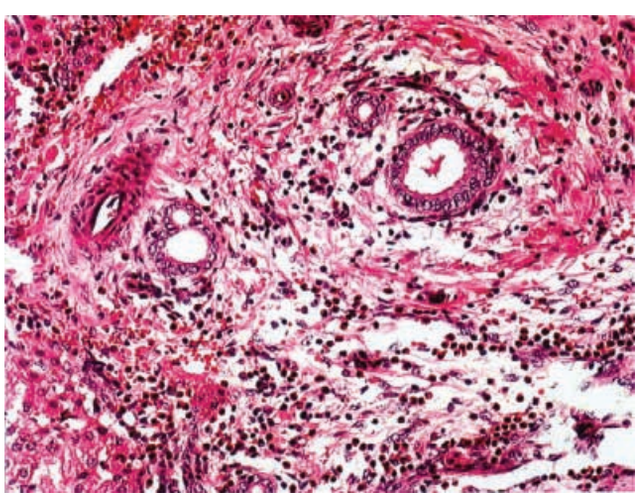

Fig. 3. Bile duct proliferation and infiltration of leucocytes (HE, 250×).

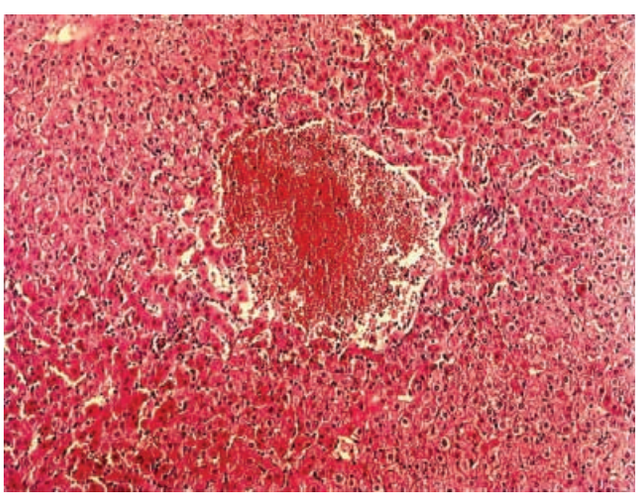

Fig. 6. Blood filled spaces in an area with widened spaces of Disse (HE, 100×).

Table 1. Liver changes, location, degree and number of animals affected ($n = 37$)

Finding	Location	Mild	Moderate	Severe
Inflammation (lymphocytes and pmn's)	Zone 1: 33; Zone 2: 17; Zone 3: 1	18	19	
Necrosis	Zone 1: 2; Zone 2: 32; Zone 3: –	22	8	1
Fibrosis	Zone 1: 37; Zone 2: 1; Zone 3: 14	18	12	4
Bile duct proliferation	Zone 1	28	6	
Hyperplasia	Zone 1: 19; Zone 2: 1; Zone 3: –	21	2	
Iron		7	5	5
Fatty change		10		
Blood filled spaces		9		
Wide sinusses		16	1	

eosinophilic. Midzonal necrotic foci were present in most animals (Fig. 2) as well as single-cell necrosis. Necrotic foci were surrounded by inflammatory cells, mostly lymphocytes but also plasma cells and some polymorphonuclear leucocytes. The portal tracts were enlarged and bile duct proliferation (Fig. 3) was present in most animals. Pericholangiofibrosis was present in many animals (Fig. 4). The bile duct epithelium showed marked apoptosis (Fig. 5) in some animals. Intrahepatic bile plugs were found in some livers.

Other striking features were wide sinusses and blood filled spaces (Fig. 6) without endothelial lining that were present in some livers. Iron staining was positive in most animals, some of them showing extensive staining throughout the liver. Lobular disarray was seen in many animals as well as nodular regenerative hyperplasia. A summary of the alterations is listed in Table 1.

Discussion

The alterations in the livers of the 37 hormone-treated cows showed features of chronic toxic hepatitis with characteristics of cholestasis. The cows originated from six different herds, the only factor they had in common was the presence of stanozolol residues in the urine. The pluriparous adult meat-type cows were in good general condition.

In humans, severe hepatotoxicity because of stanozolol abuse has been described (Ishak, 1979; Thein et al., 1995). Severe cholestasis, sometimes combined with renal failure, is described in human athletes after stanozolol abuse (Creagh et al., 1988; Yosida et al., 1994). The 17-α-alkylated steroids, which are notorious for their hepatotoxicity, used in human sport doping are the oral drugs ethylestrenol, fluoxymesterone, methandrostenolone, methyltestosterone, oxymetholone and stanozolol (Thein et al., 1995). Most of these drugs are found in illegal cocktails used for fattening cattle (intern results RIKILT). Direct liver cell toxicity was demonstrated in liver cell cultures with 17α-alkylated steroids whereas non-alkylated steroids had no effect (Welder et al., 1995).

A wide range of hepatotoxic effects of androgenic anabolic steroids have been described including cholestasis, peliosis hepatis and hepatic neoplasm (Johnson et al., 1972; Nadell and Kosek, 1977; Ishak, 1979; Turani et al., 1983; Wakabayashi et al., 1984; Sheffer et al., 1987; Creagh et al., 1988; Graham and Kennedy, 1990; Soe et al., 1992; Dossing and Sonne, 1993; Gurakar et al., 1994; Yosida et al., 1994; Handelsman, 1995; Knopp et al., 1997; Chitturi and Farrel, 2001). Especially, 17α-alkylated androgens are known for inducing cholestasis (Stang-Vos and Appell, 1981), resulting in reduced biliary excretion of bile acids. Androgens are suggested to inhibit the Na, K, ATPase activity of hepatocytes by altering the fluidity of the liver cell membrane (Gurakar et al., 1994). Bile acid uptake and bile flow is also reduced. Androgens also increase the intrahepatic concentration of carcinogens that would be excreted by bile. By means of focal necrosis, lacunae occur filled with blood caused by damaged sinus endothelia. Pericholangitis followed by fibrosis is a secondary reaction (Stang-Vos and Appell, 1981). Peliosis hepatis is a dilatation of liver veins and sinusoids because of the destruction of intercellular bindings (Soe et al., 1992). Some authors differentiate between parenchymatic peliosis due to loss of hepatocytes and phlebectatic peliosis due to dilatation of hepatic veins (Wakabayashi et al., 1984). Peliosis is found in association with anabolic androgenic steroids (Wakabayashi et al., 1984; Yosida et al., 1994). The pathogenesis of this lesion is thought to be because of steroid-induced injury to endothelial cells (Balazs, 1988) or bile infarct induced hepatocellular necrosis leading to dilated sinusses (Wakabayashi et al., 1984).

Peliosis is associated with siderosis and bile stasis (Nadell and Kosek, 1977). Death may occur because of liver rupture or haemorrhage. In cattle, another form of peliosis is described: Saint George disease, which is caused by toxic plants of Pimelia species (Seawright, 1984). These cattle develop profuse diarrhoea with cardiopulmonary dysfunction because of severely increased blood volume. A more common lesion in cattle is telangiectasis, which is a cavernous ectasia of sinusoids (Jubb et al., 1993). This lesion is not thought to be functionally significant and considered as a normal finding at slaughter.

Apoptosis of the biliary epithelium, as was seen in some of the cows, is not described in humans in relation to anabolic steroids. Apoptosis is seen in response to hypoxic, ischaemic, oxidative and drug-induced cellular injury. Cholangiopathies are characterized by both cholangiocyte loss and proliferation and various degrees of portal inflammation, fibrosis and cholestasis (Strazzabosco et al., 2000). Apoptosis can be induced by direct action of cytotoxic chemicals (Yoshida et al., 2001), or by altered bile composition (Strazzabosco et al., 2000) as is seen in steroid-exposed patients. Stanozolol is also suggested to induce apoptosis in muscle cells in humans (Abu-Shakra et al., 1997).

Hepatic tumours associated with anabolic steroids are adenomas and carcinomas (Johnson et al., 1972; Goldfarb, 1976; Ishak, 1979; Creagh et al., 1988).

Patients treated with alkylated and non-alkylated anabolic androgenic steroids developed proliferation of bile ducts, with or without cystic dilatation, peliosis, atypical hyperplasia of liver cells and liver tumours (Turani et al., 1983). Oral anabolic

steroids are the most hepatotoxic in humans. The administration route in cattle was not known. In illegal practice, injection, oral and pour-on application is known to be used. In the stanozolol-treated cows, all characteristics described as hepatotoxic effects of 17α-alkylated anabolic androgenic steroids in humans are observed, except for liver tumours.

Induction of tumours is seen in long-term treated rats and humans (Boada et al., 1999). Cattle, illegally treated for growth promotion, are suggested to be treated during the last months before slaughter, a period that may be too short to induce tumours. Moreover, only a relative small number of cows were investigated; it is possible that more thorough investigation of a larger number of treated animals will reveal liver tumours in cattle.

Because, apart form the history of illegal stanozolol abuse, no clinical data of the cows were available, it is not possible to attribute all changes to the illegal hormone treatment. Moreover, the cows have probably been treated with a cocktail, and apart from stanozolol more anabolic steroids may have been used. Management factors, viral and bacterial infections, former caesarean sections and especially feeding regime may also be responsible for the lesions described.

Striking similarities with data from hepatotoxicity found in human body builders using similar agents, however, suggests a major role of stanozolol as causative agent.

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