

AN EVALUATION OF THE OUT COME OF BULL CASTRATION BY INTRA-TESTICULAR INJECTION OF ETHANOL AND CALCIUM CHLORIDE

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SUMMARY

This study evaluated intra-testicular injection of ethanol and calcium chloride for chemical-castration in 12 mix bred young bulls (250-400 kg). Ten ml absolute ethanol and ten ml calcium chloride at 30% concentration per testis were injected intratesticularly and the testicles were removed with the open surgical technique about 60 days for histopathologic evaluation. Testicular swelling was evident in both group bulls following injection and reached peak within 48 hours. While testicular volume decreased significantly ($P<0.05$) in ethanol group after 3 weeks, no significant change occurred in calcium chloride group. The testicles underwent atrophy at the 60th day in ethanol group with no marked alteration in calcium chloride group. Though only 3 bulls were sterile, other bulls maintained androgenesis.

It was concluded that intra-testicular injections of ethanol and calcium chloride administration may not be accepted as a suitable alternative to the open surgical technique for castration in bulls.

INTRODUCTION

Chemosterilization is tried in males monkeys, hamsters, rabbits, rats and dogs by intratesticular injection of some agents such as ferric chloride (Kar et al.,1965), danazol (Dixit et al., 1975), BCG (Das et al.,1982), zinc tannate (Fahim et al.,1982), glycerol (Weinbauer et al., 1985, Immegart 2000), glucose, NaCl (Heath et al., 1987, Russell 1987 et all.) DBCP (Shemi et al.,1988), lactic acid (Fordyce et al.,1989), zinc arginine (Fahim et al.,1993), sodium fluoride (Sprando et al.,1996), formaline (Bakır et al.,2002) and calcium chloride (Samanta 1998, Jana et al. 2002), potassium permanganate+glacial

acetic (Giri et al., 2002). In male ruminants intratesticularly lactic acid (Hill et al. 1985) tannic acid and zinc sulphate (Feher et al. 1985) alpha-hydroxypropionic acid (Cohen et al. 1995), Formalin (Ijaz et al. 2000) Castrate-Quin 14 (Soerensen et al. 2001) have been tried, but because of many complications following the use of these chemicals, an effective chemosterilizing agent has yet to be established.

The purpose of the study was to determine the efficacy of intratesticular injection of ethanol and calcium chloride on chemosterilization outcome of the bulls.

MATERIALS AND METHODS

In this study, 12 mixbreed young bulls (250-400 kg) were used. They were divided randomly and equally into two groups called EG (ethanol group) and CCG (calcium chloride group). Ten ml absolute ethanol and ten ml calcium chloride at 30% concentration per testis were injected intra-testicularly. Trans-scrotal testicular ultrasonography was performed with 7.5-5 Mhz linear probe (Pie medical-scanvet 200) prior to the intratesticular injection and once a day for week following intratesticular injection and then one week intervals up to the end of the study. During this examination, the ultrasonographic appearance of a testis was evaluated and its height (h), width (W) and length (l) was measured. The volume of the testis was estimated using $\text{Volume} = (\pi/6) \times (w \times h \times l)$ equation. Sement examination and serum testosterone concentrations was performed prior to and 15, 30 and 60 days after intratesticular drug injection. The testicles were then removed the open surgical technique about 60 days after drug injection for histopathologic evaluation. Statistical analyses was performed with student t-test.

RESULT

Testicular swelling was evident in both group bulls following injection and reached peak within 48 hours. While testicular volume decreased significantly ($P < 0.05$) in EG after 3 weeks, no significant change occurred in CCG ($P > 0.05$). The testicles underwent atrophy at the 60th day in EG but marked alteration was not observed in CCG (Figure 1). In 2 CCG cases, testicular swelling was associated with orchitis and scrotal sloughing. At necropsy, their testicular tissues were found to had necrotized and abcess formation (Fig-2). These problems were not recognized in any EG cases.

Ultrasonographic examination following drug injection by revealed that the testicular tissue of both group animals had a diffuse echotexture and increased echogenite (Fig-3). The necrotized regions were differentiated ultrasonographically from the normal testicular tissue with a hypoechogenic area (Fig-4)

In this study, mean baseline value of serum testosterone concentration was determined as 13.2 ± 2.6 pg/ml in EG and 13.0 ± 2.4 CCG which reduced significantly $P < 0.05$ in EG and remained almost the same in CCG ($P > 0.05$). Intratesticular injection of etanol produced a more gradual fall: from baseline 13.2 ± 2.6 pg/ml to 5.7 ± 3.9 , 3.5 ± 0.7 and 2.6 ± 0.7 pg/ml 15, 30 and 60 days, respectively. On the other hand calcium chloride remained almost the same : 12.9 ± 1.8 , 13.0 ± 2.2 and 12.9 ± 2.4 pg/ml 15, 30 and 60 days, respectively. In the sement examination only 3 case had inactive sperm in EG, the remaining case had active sperm..

In the microscobic examination of EG, severe diffuse tubular necrosis along with varying degree of inflammatory response was the main finding observed (Fig-5). Inflammatory cells were consisted of mainly mononuclear cells. Intertubular edema, fibrosis, hemorrhage were also detected. Some of the necrotic cells showed desquamation, or even calcification. Intertubuler vessels were severely congested. Although similar lesions were noted in CCG bulls, the severity and distribution of the lesions were not so pronounced as in EG group.

DISCUSSION

Chemical, surgical, and mechanical castration, were tried in bulls for chemical castration in male ruminants intratesticular lactic acid (Hill et al. 1985) tannic acid and zinc sulphate (Feher et al. 1985) alpha-hydroxypropionic acid (Cohen et al. 1995), Formalin (Ijaz et al. 2000) Castrate-Quin 14 (Soerensen et al. 2001) have been used. A few studies on the clinical use of absolute ethanol have been reported. These studies involved in the treatment of simple renal cysts, tumors and renal angioinfarction (Ellman et al., 1981 Livraghi et al., 1986) and benign prostate hyperplasie (Zvara et al., (1999). The actual physiologic effect of this treatment has previously been speculated. Necrosis and infarction leading to fibrosis, shrinkage, or sloughing of tissue are presumed to be its mechanism of action (Zvara et al.,1999). In this study; severe diffuse tubular necrosis along with varying degree of inflammatory response was the main

finding observed (Fig 5).

For chemical sterilization ethanol and formaldehyde mixture is also used (Plant et al. 1979, Gardner 1980). Gardner (1980) used intratesticularly a solution consisting of 3.6% formaldehyde in 90% ethanol for sterilization purpose in 10 bulls where 3 showed active sperms after 85 days. In the present study, absolute ethanol was used in 6 bulls while 3 became sterilized others maintained androgenesis despite marked testicular atrophy.

Optimum effective doses of calcium chloride in rat were considered to be between 10-20 mg, however, the most desired result obtained with 20 mg (Jana et al., 2002). These authors and Samanta (1998) found marked necrosis in the seminiferous tubules during the use of this agent. In a study; the effect of calcium chloride on serum testosterone levels following intratesticular administration was investigated (Mitra and Samanta 2001) and found a marked reduction in serum testosterone level in bulls.

The result of the present study, however, appear to disagree with the former one. Because, the alteration in serum testosterone level in CCG was minimum and histopathological disorders were not serious. Thus, this agent has in fact no effect on the chemosterilisation of the bulls.

It was concluded that intra-testicular injections of ethanol and calcium chloride administration may not be accepted as a suitable alternative to the open surgical technique for castration of bulls.



Fig 1: Left CCG and right testicles underwent atrophy at the 60th day in EG.

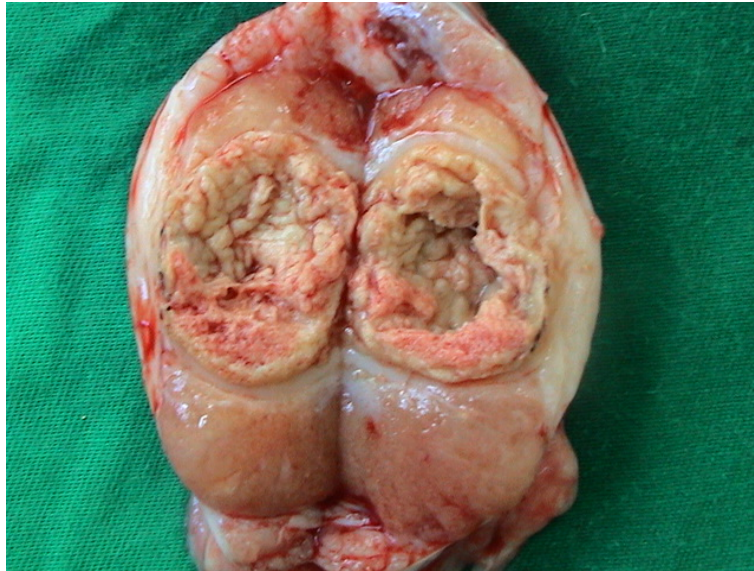


Fig 2: The testicular tissues had necrotized and abscess formation in CCG.

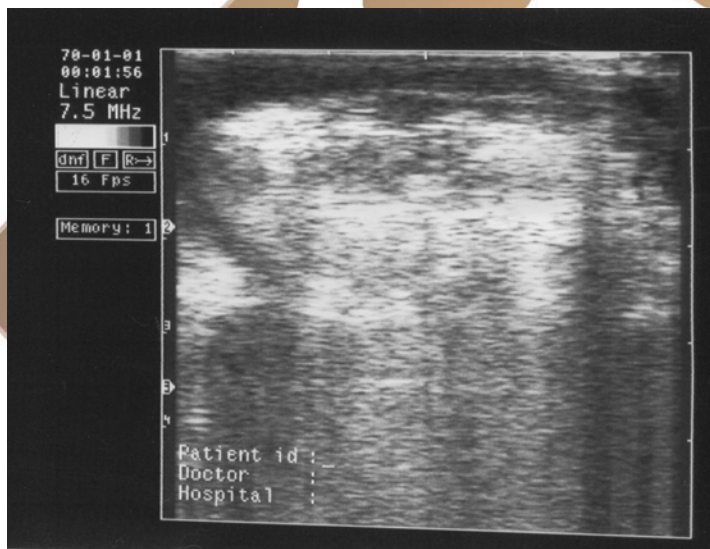


Fig 3: The all animals of both groups to present diffuse echotexture and increased echogenicity after intratesticular injections.



Fig 4: The testicular tissue with hypoechoic appearance in the necrotic area in EG.

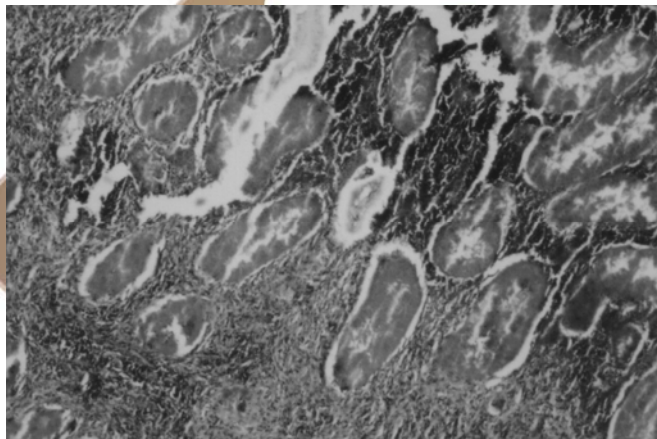


Fig 5: Diffus tubular necrosis and post necrotic mononuclear cell infiltration. (HEX20)

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