

EFFECT OF INTRA TESTICULAR INJECTION OF FORMALIN ON SEMINIFEROUS TUBULES IN AWASSI LAMBS

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ABSTRACT

The effect of an intra testicular injection of neutral buffered formalin solution on seminiferous tubules was studied in Awassi lambs. Animals injected intra testicularly with 1 ml of 10% formalin in the right testes (treated group; n = 10) showed a decrease in size ($p < 0.01$) and weights ($p < 0.05$) of the testes with- or without- tunica vaginalis parietalis, one month after treatment compared to the control group (normal saline; n = 4). Light microscopic examination showed that the number of seminiferous tubules in each field was higher ($p < 0.05$) in the testis of treated group. In the treated testes, seminiferous tubules were more prominent owing to the presence of large quantity of connective tissue. An increase in vascularity and connective tissue mass with the presence of limited number of macrophages in interstitial tissue was also observed. In conclusion, formalin causes reduction in size and weight of testes by destroying seminiferous tubules. Thus formalin possesses the potential to be considered for chemical castration.

Keywords: Testis, formalin injection, lambs.

INTRODUCTION

Chemical castration is one of the methods to undergo experimental castration on different animal species. A large number of drugs and chemicals have been tested for this purpose. These include, non-steroidal anti-inflammatory drugs in male rats (Loscher and Blazaki, 1986), bromocriptine in the male blue fox (Smith et al., 1987), lactic acid in rats and dogs (Sailasutra et al., 1988; Nshimura et al., 1992), hydroxypropionic acid in calves (Mora et al., 1991) and silver nitrate and lactic acid in rats and rabbits (Lee, 1987). It has been reported that the injection of 4.5% chlorhexidine digluconate into the caudae of the epididymis in cats results in lasting oligospermia or azoospermia (Pineda and Dooley, 1984) and the injection of 0.6 ml of 1.5 % chlorhexidine gluconate in 50% dimethyl sulfoxide in dogs has lead to total disappearance of spermatozoa from ejaculate for 91 days (Pineda, 1978).

Recently, chemical castration has been tried in men using medroxy-progesterone 17-acetate (Berlin, 1997). Furthermore, cytotoxic effect of cisplatin, methotrexate and endoxan have been described in albino rats testes (Galal et al., 1994) and the effect of opiates or heroin and other chemotherapeutic drugs on mouse testis cells as well (Mendelson et al., 1975; El-Hossiny et al., 1988).

Formalin is mainly used as a fixative. Chemically, it reacts with the side groups of the amino acids to form chemical bonds between adjacent proteins (Lamberg and Rothstein, 1978). Formaldehyde is an intermediary metabolite of formalin, which is highly reactive with nucleophilic biological compounds. It is rapidly metabolized in animal body into formic acid, carbon dioxide and one carbon pool, which may be incorporated into the proteins and/or nucleic acids (Heck, 1982). In cases of skin dermatitis formaldehyde combines with proteins and produces a hapten - protein complex, which sensitize T-lymphocytes and subsequently produce allergic contact dermatitis (Shelly and Lennart, 1977).

To our knowledge no previous studies have been reported on the effect of formalin on seminiferous tubules. Therefore, this study investigates the effect of a single intra testicular injection of 10% neutral buffered formalin solution (formalin) on testicular tissue, especially the seminiferous tubules.

MATERIALS AND METHODS

Experimental procedures

Fourteen clinically normal Awassi lambs aged four months with average weights of 26.8 ± 2.1 kg each were used in this experiment. They were fed a diet

consisting of concentrates and hay, with fresh water *ad libitum*. Five ml of Xylazine hydrochloride (2%) was injected into the testicle by inserting the needle perpendicularly through the skin into the parenchyma of the testicle in each of the experimental animals. After ten minutes, ten of the animals were each injected intra testicular (right testis) with 1 ml of formalin (treated group). The remaining four lambs were injected each with 1 ml of normal saline (control group). In both groups, the injection was given in a way so as to distribute the injecting material uniformly in the middle of the testis.

One month later, after local infiltration anesthesia using 5 ml Xylazine HCl 2%, the right testes were removed along with 1 cm length of the spermatic cord from all the lambs by open castration method. Each testis was grossly examined and weights of the testes with the tunics were recorded (weight one). The tunica vaginalis parietalis was then removed completely from each testis and the weight (weight two) was recorded. One extremely emaciated animal from treated group was excluded from the study because both of its testes were very small in size.

Histological analysis

Samples taken from different regions of the testes and epididymides were placed in 4% cold glutaraldehyde solution in cacodylate buffer pH 7.2. Other samples were placed in 10% neutral buffered formalin solution. Consequently, standard histological procedures were followed to prepare thin, semi thin and ultra thin sections for examination under light and electron microscopes (Zeiss 10 CR electron microscope). Haematoxylin and eosin stains were used for light microscopy. Number of tubules per field was counted in ten fields of each section and the number of cell layers in the walls of ten different tubules was also recorded for each specimen using 40 X, magnification. Methylene blue was used to stain semi thin sections. For contrast staining of ultra thin sections, heavy metals like uranyl acetate and lead citrate were used for ultra structural examination under electron microscope. Microphotographs of the selected fields were also taken for record.

Statistical analysis

Data were analyzed using t - test to compare mean testicular size and weight between control and treated testes. Mann - Whitney test was used to compare the means of number of seminiferous tubules per field and cell layers in the walls of the tubules of the control and treated testes (Daniel, 1991). Data shown in the text are means \pm SD.

RESULTS

Size of the treated testes (length 2.5 ± 0.4 cm; circumference 3.8 ± 0.9 cm) was smaller ($p < 0.01$) than the control (length 4.9 ± 0.3 cm; circumference 5.8 ± 1.1 cm; Fig. 1). Weight one of the testes was smaller ($p < 0.05$) in the treated group (16.60 ± 5.79 gm) as compared with the control (42.38 ± 8.46 gm). Similarly, weight two of the testes was lighter ($p < 0.05$) in the treated group (11.0 ± 3.77 gm) as compared with the control (37.23 ± 7.01 gm). The number of seminiferous tubules in each field was higher ($p < 0.05$) in the testis of treated group (12.94 ± 3.23) as compared with the control (6.28 ± 0.44). However, number of cell layers in each seminiferous tubule showed no difference between the treated (2.6 ± 0.42) and control groups (2.1 ± 0.75).

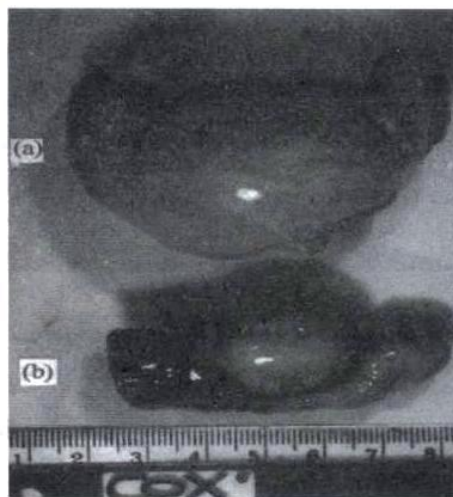


Figure 1. Photograph of a lamb testis injected with normal saline (a) compared with the treated testis injected with 10 % neutral buffered formalin solution (b).

Most of the seminiferous tubules of treated testes had a single cell layer composed of supporting cells (Sertoli cells) and a few large size gonocytes, and were surrounded by a heavy mass of connective tissue. However, most of the seminiferous tubules of control testes had more than one cell layer with normal distribution of the tubules and less connective tissue around them. Abundant gonocytes were also present in tubules of the control testes (Fig. 2). Seminiferous tubules in the treated testes were more prominent owing to their thick basement membranes and the presence of medium to heavy mass of connective tissue when compared to the control spermatogenically active tubules (Fig. 3 & 4).

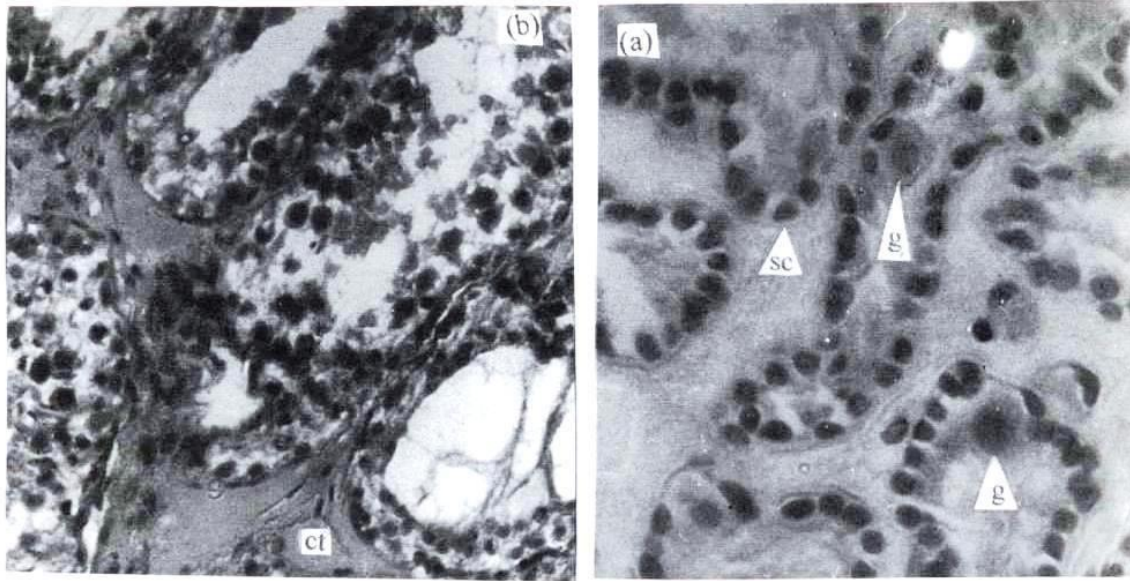


Figure 2: Microphotographs of a lamb testis injected with normal saline (a) and a treated testis injected with 10% neutral buffered formalin solution (b). A large size gonocyte (g) supported by Sertoli cells (sc) are shown in seminiferous tubule of the control testis. Also, note abnormal seminiferous tubule development and the presence of a heavy mass of connective tissue (ct) around the seminiferous tubules in the treated testis. Sections were stained with Haematoxylin and Eosin and examined under light microscope. Magnification, 700 X.

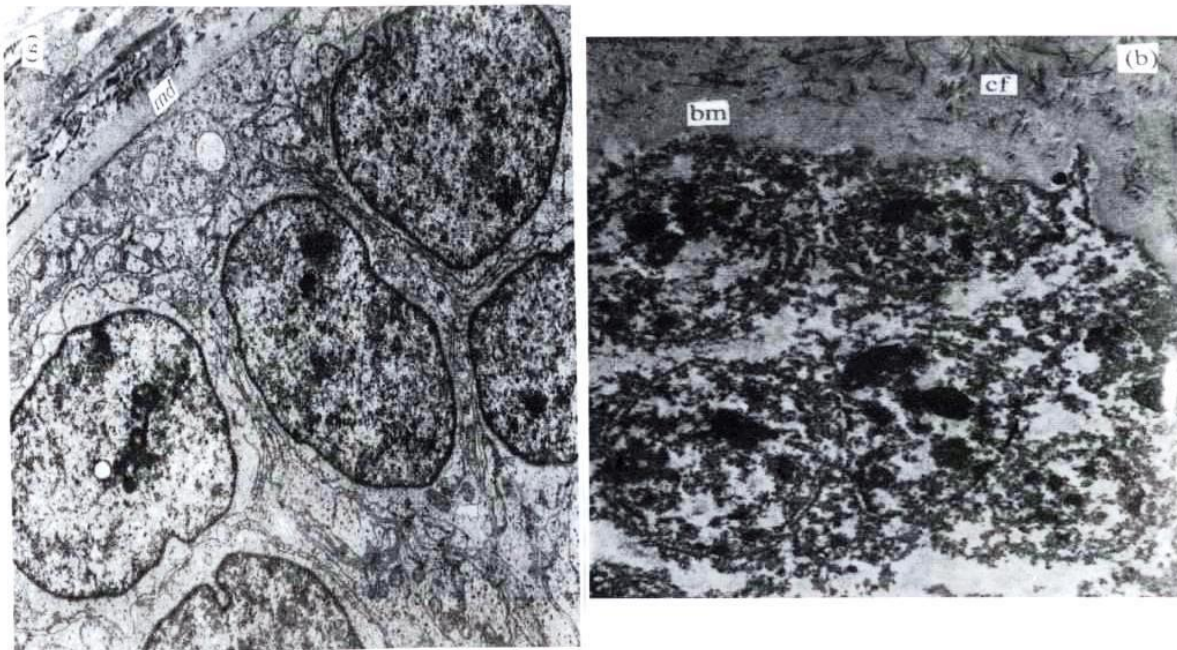


Figure 3: Electron microphotographs of seminiferous tubules of a lamb testis injected with normal saline (a) and a treated testis injected with 10% neutral buffered formalin solution (b). Basement membrane (bm) in the treated testis is thick, distorted and surrounded by large amounts of collagen fibers (cf) as compared to the control. Magnification, 6250 X.

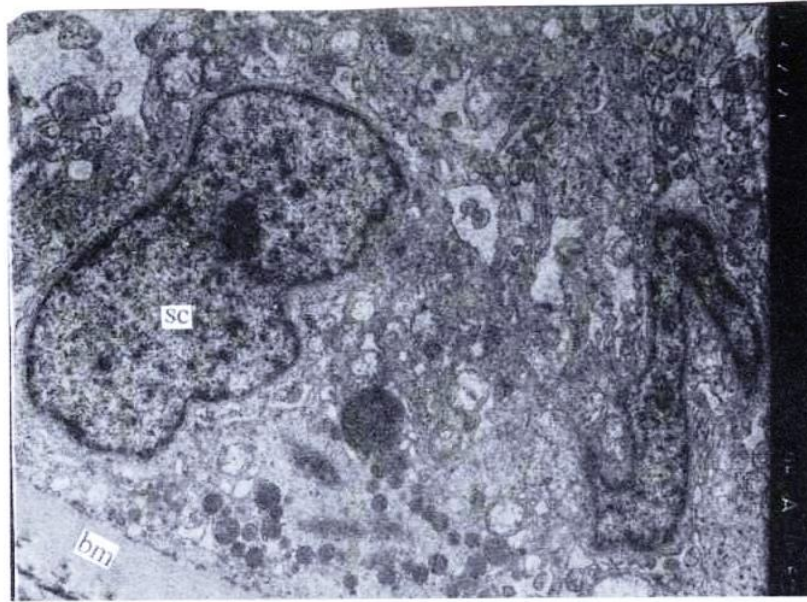


Figure 4: Electron microphotograph of a seminiferous tubule of a lamb testis injected with normal saline. A Sertoli cell (sc) appears resting on a normal basement membrane (bm). Magnification, 7875 X.

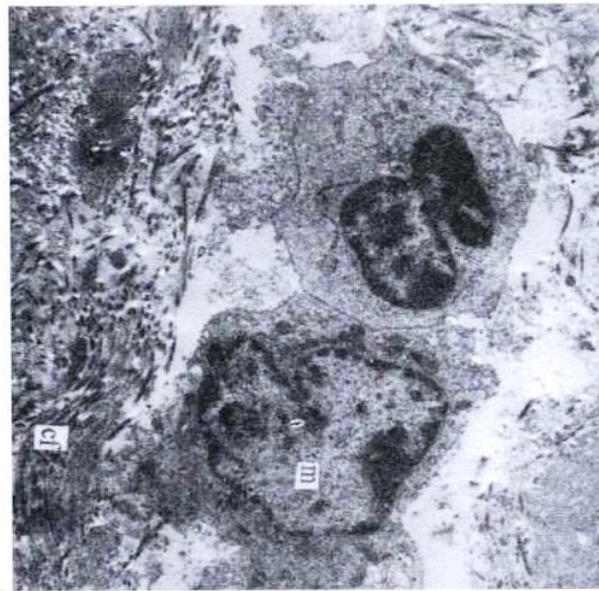


Figure 5: Electron microphotograph of a lamb testis injected with 10% neutral buffered formalin solution. Macrophages (m) are present in the interstitial tissue with abundant collagen fibers (cf). Magnification, 6250 X.

Irregularities in the seminiferous tubules structure, differences in their diameters and of the interstitial tissue were observed in the treated testes and varied from animal to animal. These irregularities included less developed seminiferous tubules with basal cells and a few large nuclei in the lumen of the tubules. In the interstitial tissue, an increase both in vascularity and connective tissue mass with the presence of limited number of macrophages was noted (Fig. 5). The presence of relatively few scattered Leydig cells between the tubules surrounded by connective tissue was also observed (Fig. 6).

Epididymides appeared distorted in the treated group. The blood vessels were congested and the height of the epithelium was relatively low. In one case, red blood cells were seen inside the lumen of the epididymis. Normal histological picture of the epididymis was observed in the control group. No spermatozoa were observed in all specimens examined regardless of the group.

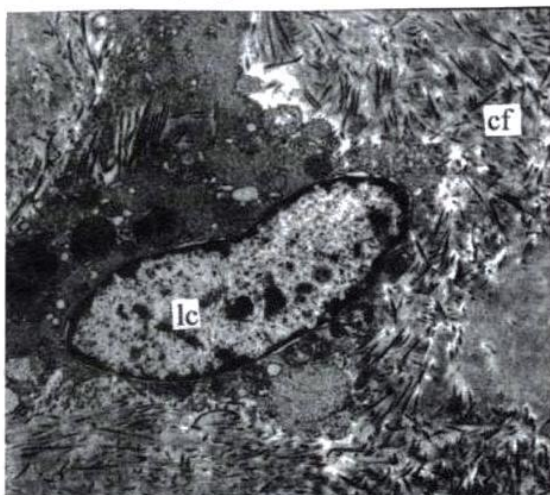


Figure 6: Electron microphotograph of a lamb testis injected with 10% neutral buffered formalin solution. A Leydig cell (lc) appears surrounded by a large amount of collagen fibers (cf) in the interstitial tissue. Magnification, 6250 X.

DISCUSSION

In this study, formalin was used locally on the seminiferous tubules because it is known to cause denaturation of proteins *in situ* in contrast to systemic toxins that has to be absorbed and distributed to distant organs to produce their effects (National Research Council, 1981). In the present study, formalin resulted in loss in weights of treated testes when compared to the weight of control testes. Weight difference of the

testes with tunica vaginalis parietalis (weight one) and their weight without tunica vaginalis parietalis (weight two) was not due to edema and fluid accumulation between these tunics. Only in a few cases treated testes were larger and their weight one was heavier than the average weight one of the control testes. This increase in size and weight seems to be due to edema and accumulation of fluid between the tunica vaginalis visceralis and parietalis because formalin is considered as a medium type irritant and probably drops of the solution may have dispersed under the tunic during insertion and/or withdrawal of the needle.

In the present study, formalin caused destruction of the seminiferous tubules and/or had resulted in reduced growth rate and division of the cells inside them. Destruction of cellular elements was clear as cells were replaced by connective tissue especially collagen fibers (Fig 5 and 6). It also resulted in a decrease in the rate of development of the seminiferous tubules. The number of seminiferous tubules as well as the number of cell layers per field in treated testes were less as compared to the control. However, the number of cell layers was very limited in both groups (restricted to few gonocytes, spermatogonia and supporting cells) as the animals used were pre pubertal.

Interstitial macrophages are functionally associated with Leydig cells (Tahka, 1986). Furthermore, macrophages are also stated to be steroidogenic and possess common surface antigens in Leydig cells of rat testis (Bergh, 1982). It has also been reported that macrophages are normally present in rat's testis and absent in ram's testis under normal conditions (Pollanen and Maddocks, 1988). However, in this study the limited numbers of macrophages present in the seminiferous tubules may be a response to the destruction of the tubules. In conclusion, formalin causes reduction in size and weight of testes by destroying seminiferous tubules, the functional units of testis. Thus formalin possesses the potential to be considered for chemical castration. However, more work has to be done to evaluate its effect on the testes of sexually active pubertal males.

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