Histochemical study of the effect of heat on the function of male reproductive organs of rats

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Abstract:

The literatures mentional that the male reproductive organs are under the effect of androgens which secreted from the testes (Greenspan and Baxter 1994) .

To know the role of scrotum in decreasing of testicular temperature we used twenty mature male rate . The animals were randomly divided into two groups , ten animals each (Control and treated) . Both testes of the treated animals sutured to the abdominal wall while the control animals were shamly operated .After one month all animal were killed and the testes , prostate and seminal vesicle were removed and put in liquid nitrogen (- 196c) . frozen section made and stained according to the stander histochemical methods .

The results showed an increase in acid phosphatase activity in the treated tissues compared with the control . Alkaline phosphatase enzyme activity were unstable , while the succinate dehydrogenase enzyme activity decreased markedly in the treated tissues compared with the control tissues .

The present observation confirmed the role of androgen in regulation of male organs function .

دراسة كيمياء نسيجية لتأثير الحرارة على عمل الجهاز التناسلي الذكري للجرزان

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الخلاصة:

اشارت البحوث السابقة على ان عمل الجهاز التناسلي الذكري يكون تحت تأثير هرمون التستوستيرون المفرز من الخصيتين من حيث تكوين ونضج الحيوانات المنوية وكذلك افراز الفدد الملحقة به .

ولغرض معرفة دور كيس الصفن على عمل الخصيتين تم استخدام عشرون من ذكور الجرذان البالغة وقسمت عشوائيا الى مجموعتين المجموعة الاولى (مجموعة المعالجة) فيها عشرة حيوانات حيث اجريت لها عملية جراحية تم فيها اخفاء الخصى في البطن اما المجموعة الثانية (مجموعة السيطرة) وعددها عشرة حيوانات حيث اجريت لها جراحة خادعة (ham operation) بعد اربعة اسابيع تم قتل الحيوانات واستئصال الخصى وغدد البروستات والحويصلات المنوية ووضعت في النتروجين السائل للتجميد وعمل منها شرائح مجمدة وصبغت الشرائح بعدها حسب الطريقة الهستوكيميائية النموذجية .

اوضحت الدراسة الكيميائية النسيجية بأن هنالك زيادة في معدل انزيم الفوسفاتيز الحامضي لحيوانات مجموعة المعالجة وفي كل الانسجة التي درست للمقارنة مع مجموعة السيطرة ،اما انزيم الفوسفاتيز

القاعدي فقد كان متذبذب النتائج ولكن بصورة واضحة جدا بعمل انزيم السكسينيتديهايدروجينيز حيث قل تركيزه في انسجة الحيوانات المعالجة للمقارنة مع انسجة حيوانات السيطرة.

Introduction:

Enzymes are found in all living cells . They are biological catalysts and their synthesis is regulated in the nucleus being stimulated or inhibited according to physiological needs . Cellneeds dephosphorylated glucose for its energy an hydrolytic enzyme (phosphatase) which introduces a water molecule and separates the phosphoric radical allowing the cell to have its much needed pure glucose . Alkaline phosphatase has different isozyme in the germ cells of the testis (1) , as well as human placental tissues (2) . Narisawa et al (1992) found that embryonic acid phosphatase is expressed in M-phase of spermatogenic cells during postnatal development and they suggested a role of this acid phosphatase isozyme during meiosis (3) . During cellular activity usually the alkaline phosphatase activity increased (4) . such function was observed by Nazawa et al (1980) in early embryonic stage and uterine reserve cells (5) , more over this result was found in the intestinal epithelial cells (6) .

The acid phosphatase is a catalyze phosphoric esters hydrolysis at ph 5.0 . Gatie et al (1987) found that the acid phosphatase enzyme activity increased in the middle segment of the epididymis of the guinea pig (7). The succinate dehydrogenase enzyme involves in the transformation of succinic acid in to fumaric acid in the krebs cycle reaction . Moniem (1972) mentioned that the succinate dehydrogenase enzyme distributed in the epithelial cells of the ram , rabbit , rat and hamster epididymidis (8) . To confirm the previous finding and the possible effects of heart on the histochemical contents of the male reproductive tissue, the present study aimed .

Materials & Methods:

Twenty sexually mature male rats were used in this study. The animals were maintained at 26ºC , 14/10 L/D. Water and feed were provided *ad libitum* . The animals were randomly divided in to control and treated group , ten animals each . Under ether anaesthesia a midline abdominal incision was made , and both testes were drawn through the inguinal canal and sutured to the abdominal wall . Sham operations were made to the control animals . After one month , the animals killed and the testes , prostates and seminal vesicles were kept in the liquid nitrogen (- 196ºC) . Frozen section were cut on the cryostat (- 15ºC) at 15mic and allowed to dry for 1 hr . dried section treated with fresh incubation medium prepared by mixing a solution containing 5 mg Na-alpha-naphthyl phosphate , dissolved in 0.25 ml of dimethyl formamide (DMF) with a solution containing 5mg fast blue dissolved in 5.0 ml at 0.1M acetate buffer , ph 5.0 containing 75mg polyvinyl pyrolidine (PVP) / ml for 45min . at 37ºC. (modified method from pearse 1960) (9) . Stained

section were fixed in formal saline for 3 min . slides were rinsed in water and mounted in Farrants medium . A brown colour indicated the presence of acid phosphatase (ACP) activity . The method for determination of alkaline phosphatase (ALP) activity was similar to the ACP procedure except for the use of a carbonate – bicarbonate buffer PH 10 : 12.5 mg of fast blue in 5ml buffer and incubation at room temperature for 45 min . A dark reddish brown colour indicate the presence of ALP . The technique used for the succinate dehydrog- enase (SDH) was modified from the method of Nachlas *et al*. (1957) (10) . Equal volumes of two solution were mixed just before use ;0.2M . Phosphate buffer (PH 7.6) containing 75mg/ml of polyvinyl pyrrolidine and 1mg nitro blue tetrazolium (NBT) per 1ml water . Fresh frozen section were incubated in the medium for 45 min at 37ºC. All sections were fixed in formal saline for 5 min . then washed in water and mounted in Farrants medium.A purple-blue colour (diformazan) indicate succinate dehydrogenase(SDH) activity .

Results & Discussion:

The result showed that the acid phosphatase activity was prominent in the testicular tissue of the treated animals compared with control (Figure – 1 – a-) . The heat stress caused an increase in the cellular activity which lead to increasing acid phosphatase activity(Figure–1).

This finding is in agreement with (4) in increasing acid phosphatase activity when there is increasing in cellular activity . The present observation support the suggestion of (11) in the role of acid phosph- atase in the meiotic process . On the other hand , the acid phosphatase enzyme showed a different levels of activity in prostatic tissue section . This result could be confirm the suggestion of (12) when they suggested that the acid phosphatase activity decreased in middle G1 stage and become undetectable activity in S stage of cell cycle . The acid phosphatase present in the control (Figure -1-c-) prostatic tissues in a diffused from while in the treated tissues (Figure) -1-d-) showed a localized activity .The acid phosphatase enzyme activity showed a marked increase in the treated seminal vesicle tissue (Figure -1-f-) compared with control tissue (Figure -1-e-) . The present finding concluded that the seminal vesicle cells faced a stress (cryptorchidism) which leads to increase their activities as a form of defense before epithelial damage.

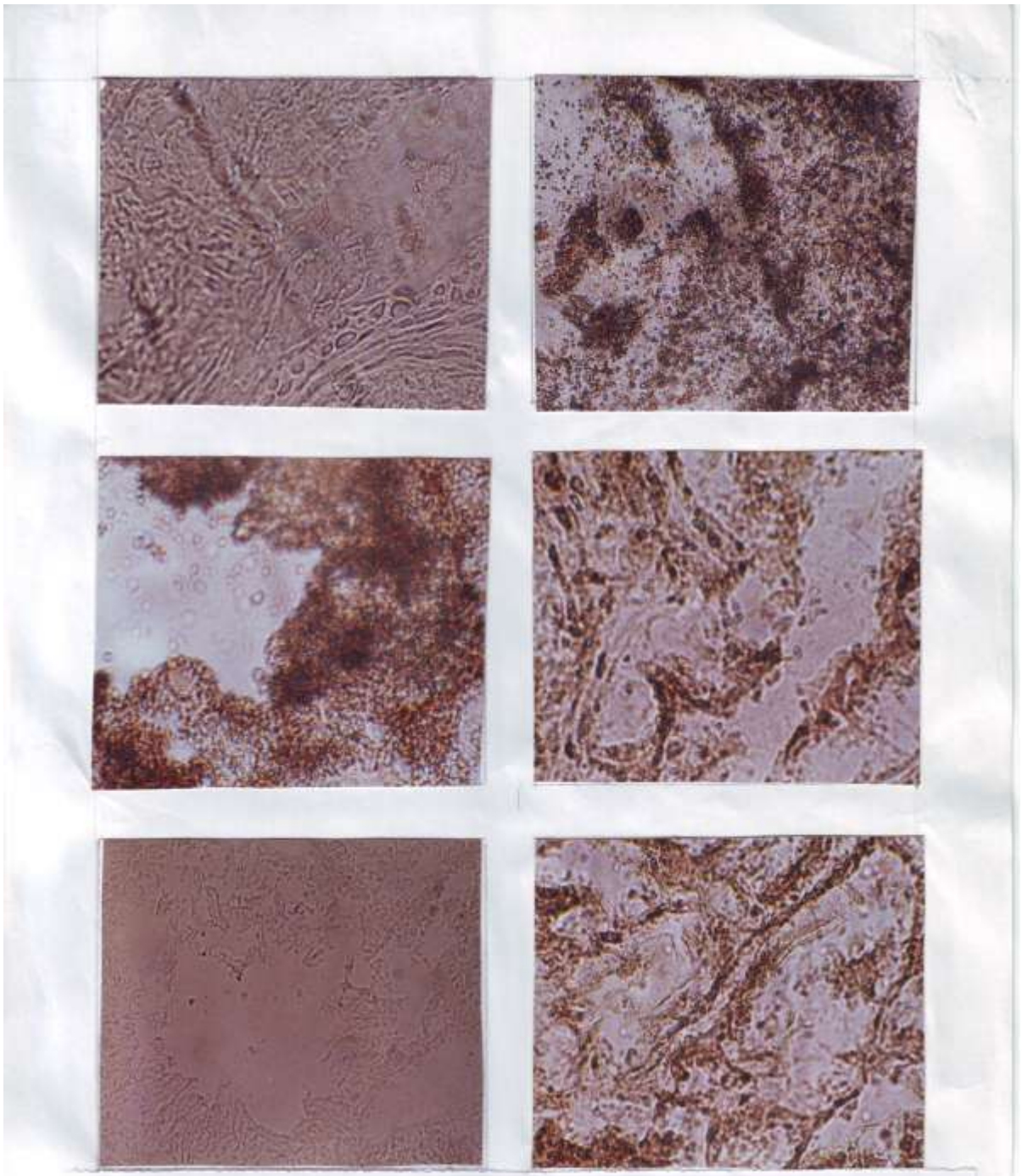

Figure-1-A-control testicular frozen section shows a weak ACP activity x 480. B-treated testicular frozen section with marked ACP activity x 480.C-control prostatic frozen section shows adiffused form of ACP activity x 480 . D-treated frozen prostate section with localized ACP activity x 480. E-control seminal vesicle frozen section shows a weak ACP activity x 480. F-treated seminal vesicle frozen section shows a strong AP activity.x480.

These result will support the postulation of (13) who said that the acid phosphatase was involved in the intracellular digestion of endogenous and phagocytosed exogenous compounds containing phosphate residues. Alkaline phosphatase enzyme showed unstable activity in the different studied tissue . The alkaline phosphatase enzyme detected in a diffused form in the control testicular tissue (Figure -2-a-) compared with the treated testicular tissue (Figure -2-b) which showed a reduction in the enzyme activity . Keel and Abney (1980) stated that artificial cryptorchidism caused a reduction in the androgen level due to damage of the Leydig cells(14). Previous studies have frequently confirmed that the male organs function were androgen dependent (15;16) which are supported by the present observation in reduction of alkaline phosphatase enzyme activity .While prostate tissue showed no marked differences in alkaline phosphatase enzyme activity between control (Figure-2-c- n) and treated (Figure -2-d) tissue . These finding would be dependent upon the functional integrity of the androgen sensitive epithelial cells of prostate. A reduction in the alkaline phosphatase enzyme activity were seen in the treated seminal vesicle tissue (Figure -2-f-) compared with control tissue (Figure-2-e-).The present observations confirmed the finding of (17) when they stated that the alkaline phosphatase enzyme is heat sensitive and reduced during germ cells division. All the male tissue which examined in the present study showed a reduction in the succinate dehydrogenase enzyme activity in treated groups compared with control. There were a slight reduction in the succinate dehydrog-enase activity in the testicular treated section (Figure-3-b) compared with control section (Figure-3-a-) . Prostates and seminal vesicles tissue section showed a marked reduction in the succinate dehydrogenase activity in treated section (Figure-3-d&f-) compared with control section (Figure-3-c&e) .

Generally , the male organs function under the effect of androgen which mainly secreted from Leyding cells in the testes (18).The cryptochidism treatment caused a damage to the testicular tissue (19) which concluded the present results in retardation in the male organ functions demonstrated as reduction in the histochemical enzymatic contents .

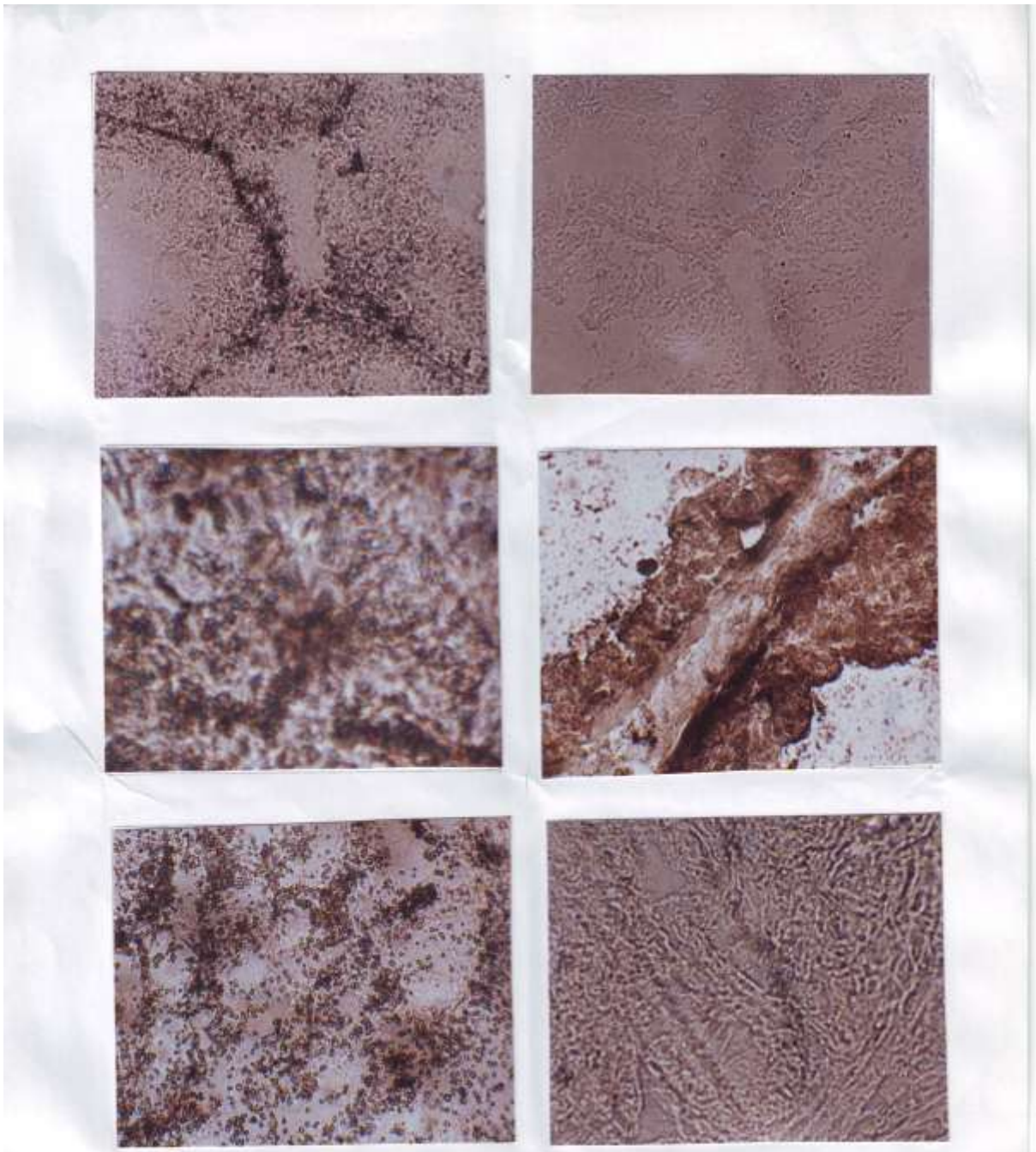

Figure-2-A-control testicular frozen section shows a marked ALP activity. x480. B-treated testicular frozen section shows no activity to ALP enzyme.x480. C-control prostatic frozen section shows a clear ALP activity.x480. D-treated prostatic frozen section shows a marked activity of ALP enzyme.x480. E-control seminal vesicle frozen section shows a clear ALP activity.x480. F-treated seminal vesicle frozen section with no clear ALP activity.x480.

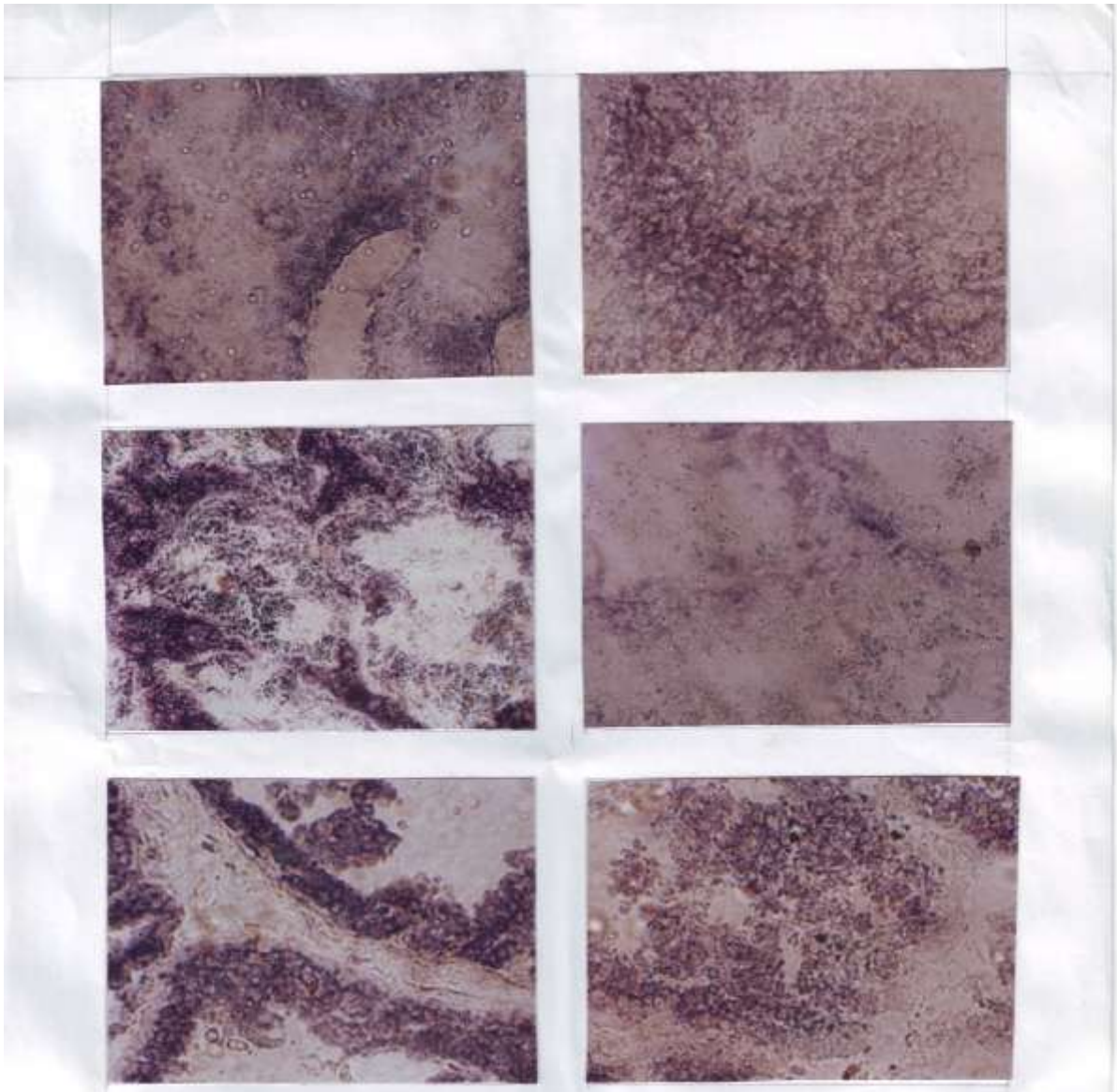

Figure-3-A-control testicular frozen section shows a localized SDH activity. x480. B-treated testicular frozen section with a reduction in the SDH activity.x480. C-control prostatic frozen section shows a marked SDH activity.x480. D-treated prostatic frozen section with a reduction in the SDH activity.x480. E-control seminal vesicle frozen section with a marked SDH activity.x480. F-treated seminal vesicle frozen section shows a reduction in SDH activity.x480.

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