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تأثير الزايوانول على بعض الغدد الصم والغدد الذكرية فى الخراف غليظة الذيـــل

الخصيـة

جمال كامل ، أحمـد حسـن ، فيصـل الحمصى*

فى هذا البحث تم التقييم الكمى والوصفى للخصية بحساب كل خلية ثم نسبتها الى عـــدد خلايا سرتولى مع قياس قطر الانابيب المنوية فى الحيوانات المعاملة بمادة الزايرانول مع الغـــير معاملـــة . أكدت النتائج أن مادة الزيرانول تؤدى الى زيادة مؤكدة فى وزن الحيوان وتعطل حركة الحيوانات المنوية كما أنهـــا تقلل من عـــدد ها .

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THE EFFECT OF ZERANOL IMPLANATION ON SOME ENDOCRINE GLANDS AND GONADS IN FAT-TAILED LAMBS II. THE TESTES

(With 4 Tables and 15 Figures)

By

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SUMMARY

Quantitative and qualitative analysis of the testis after zeranol implantation resulted in a change in the rate of frequency of the stages 7,8 and 1 of the seminiferous epithelial cycle. Also the ratio of germ cells/Sertoli cells (Sertoli cell ratio) was relatively lower as well as the neutral lipids appeared in the form of larger droplets in the Sertoli cells in comparison with that of the control testis.

Moreover, the number, size and shape of the interstitial cells were greatly affected and exhibited signs of decreased cellular activity in the implanted tested testis.

It is conduluded that, zeranol implantation significantly increased body weight and improve gain. Although it did not affect the synchronized initiation of successive cycles of the seminiferous epithelium it delayed the release and decreased the number of the spermatozoa.

INTRODUCTION

The primary goal of the livestock producers is overincreasing efficiency of animal protein. The most economical management practices available to the animal producers has been the use of growth promoting implants. The growth promoting effect of zeranol implants has been investigated by ROSS and BROWN (1970); SHARP and DYER (1970, 1971) and BORGER et al, (1971). The relationship between zeranol, pituitary size and feedlot gain was demonstrated in a recent trial conducted by LESPERANCE, (1973) on bulls and steers. Recently, CORAh (1980) studied the effect of zeranol implantation on the sexual development of beef bull.

Our first report (HASSAN et al, 1981) delt with the histochemical features of the pituitary gland after zeranol implantation in fat-tailed lambs. However, this second report is a further study including a quantitative and qualitative analysis of the testes in zeranol implanted lambs compared with that of normal ones.

MATERIAL and METHODS

The present work was carried out at the animal production station,Faculty of Agriculture,Assiut University Egypt. Thirty-nine fat-tailed lambs, aging about eight monthes and weighing about 50 Kgs, were divided into three groups. The animals of group (I) were considered as control, while animals of groups (II) and (III) were implanted subcutanously at the backside of the ear with three doses each of 12mg. Zeranol at 40 days intervals. The animals of groups I and II were fed on concentrate mixture containing 70.45% starch value and 14.42% digestable protein, while that of the group III containing 70.44% starch value and 7.92% digestable protein. Feeding staffs were adjusted once a week according to the average livebody weight of each group.The refusals of feeding staffs were weighed every day and feed intake was estimated for each group. Lambs were kept in two semi-open sheds. At the end of the experiment, three animals of each group represent the average weight of the group were selected and slaughtered. The testes were dissected out immediatly, weighed and small peices from each testis cutted and fixed in neutral formalin and Bouin's fluid.

Material fixed in formalin were cutted by cryostate at 20 um. thickness and stained with Sudan Black B for demonstration of fat. The Bouin fixed tissue was dehydrated and embedded in paraffin, then sections at 5Um.were stained with:

(a) Iron Haematoxylin and Eosin.

(b) PAS-Haematoxylin (McManus, 1948).

The identification of the various stages of the seminiferous epithelium cycle was based on the morphological changes of the germ cell nuclei and the local arrangement of the spermatids.Each stage of the seminiferous epithelium cycle was identified when the entire cross section of the seminiferous tubule include germ cells in the same stage. Twenty-four rounded cross sections of the tubules from each testicle reprosenting all the stages of the cycle were examined. The number of all various types ff cells (spermatogonia, spermatocytes I,II, spermatids and Sertoli cells) occupied the entire cross sections of these tubules was calculated and the Sertoli cell rations (SCR) were estimated. The total number of counted germ cells of each of the different types was divided by the total number of sertoli cell in the same cross sections of tubules. The resultant values are referred to as Sertoli cell ratios.

The diameter of the seminiferous tubules was measured in unifixed cryostate sections with an eye-piece micrometer scale which was caliberated with stage micrometer to the nearest micron.

The percentage of cross sections of tubules at agiven stage of the seminiferous epithelial cycle was taken as a figure for the relative frequency of this stages.

The cellular association which recognized during the seminiferous epithelial cycle will be described for each stage. The spermatogonia were classified into type-A and type-B. The intermediate-type was calculated together with type-A. The preleptotene and leptotene phases were also calculated together. As the final phase of the meiotic prophase (diakinesis) is very short, it calculated together with the pachytene. The Sertoli cell ratios of spermatogonia, primary and secondary spermatocytes and spermatids are presented in table 1.

In control testes, eight stages could be recognized during the seminiferous epithelial cycle. These stages are described as follows:

Stage (1):

(Fig. 1). It start from the end of the spermatozoa release into the lumen until the begining of the spermatied nuclei elongation. It characterised by abundant spermatogonia (type-A), leptotene, pachytene and spermatids with rounded nuclei. The nucleus of the Sertoli cell was irregular or triangular in shape with deep identations. It contain large multivesicular nucleolus. The Sertolli cells contain few, small sized sudenophilic droplets at the basal part of their cytoplasm, (Fig. 11). The frequency of this stage is 22.3% (Table 2).

Stage (2):

(Fig. 2). This stage began from the elongation of the spermatidnuclei up to the formation of the bundles of spermatids. On the basement membrane spermatogonia (type-A) was abundant. Two generations of primary spermatocytes are present; zygotene and pachytene. The frequency of the stage is about 11.1%.

Stage (3):

(Fig. 3). This stage start from the formation of the first elongated spermatid bundles in the Sertoli cell cytoplasm up to the appearance of the metaphase of the first maturation division. Spermatogonia (type-A) are still present. This stage is characterised by the appearance of diplotene phase on a higher level than the zygotene spermatocyte.Few diakinesis figures are observed. The relative frequency of this stage is about 17.2%.

Stage (4):

(Fig. 4). The end of the meiotic prophase coincides with this stage during which the metaphase, anaphase and telophase rapidly occure and the secondary spermatocytes are clearly observed. They have rounded nuclei. Each nucleus contain about six chromatin particles. The secondary spermatocyte then divided to give rise to spermatids. On the basement membrane the intermediate- as well as type-A spermatogonia are demonstrated. Zygoten are still present. The relative frequency of this stage is about 10.1%.

Stage (5):

(Fig. 5). This stage start from the end of the second maturation division up to the appearance of the fine dustylike chromatin in the nuclei of the young spermatid (type-a). This stage is characterised by the presence of type-A and intermediate-type of spermatogonia as well as zygotene phase of primary spermatocytes. However, two generations of spermatids are recognized; young spermatid which arised from the secondary spermatocytes of the previous stage and the bundles of elongated spermatids (spermatid type-d). The average frequency of this

stage is about 5.2%.

Stage (6):

(Fig. 6). This stage is characterised by the presence of type-A and type-B spermatogonia. Also pachytene, spermatids (type-b) with their dusty chromatin and the bundles of elongated spermatids (type-d) embedded in the Sertolian cytoplasm are observed. These bundles of elongated spermatids start to migrate towards the lumen of seminiferous tubules characterising the end of this stage. The rate of occurance of this stage is about 13.6%.

Stage (7):

(Fig. 7). This stage lasts from the beginning to the end of the centripetal migration of the elongated spermatids, towards the lumen of the seminiferous tubules. The different types of cell association of this stage are nearly the same like that of stage six. However, the only difference is that, the elongated spermatids tend to move toward the lumen of the seminiferous tubules. The rate of occurance of this stage is about 9.7%.

Stage (8):

(Fig. 8). This stage starts from the end of the migration of the spermatids up to their complete release from the Sertolian cytoplasm as spermatozoa into the lumen of the seminiferous tubules. It characterised by the presence of spermatogonia (type-A and B), are present. Also Preleptotene and pachytene spermatocytes as well as spermatid type-b are present. The relative frequency of this stage is about 10.8%.

The interstitial (Leydig) cells of the control animals are large in size, rounded or polyhedral in shape with a centeral nucleus and acidiphilic cytoplasm rich in small lipid droplets (Fig. 9).

In Zeranol implanted animals, the testes showed a significant decrease in weight as compared with the control ones (Table 3). Moreover, the diameter of the seminiferous tubules are sharply decreased. However, the Zeranol implanted lambs had a higher final livebody weight than the control lambs (Table 4).

In Zeranol implanted testes, all stages of the seminiferous epithelial cycle are present. The total number of the Sertoli cells and the Sertoli cell ratios are sharply decreased (Table 1).

Zeranol implantation resulted in change in the relative frequency of a number of stages of the seminiferous epithelial cycle (Table 2). The statistical analysis of the data from groups II and III showed no significant difference between the control and the treated testes with respect to the frequency of the eight stages of the seminfierous epithelial cycle. The frequency of the stage 7 was increased. However, the frequency of stages 8 and I are decreased. A careful analysis of the seminiferous tubules in these three stages (7, 8 and I) revealed the following:

Stage (7):

(Fig. 13). This stage was extend to the appearance of preleptotene spermatocyte which was observed in stage 8 of the control testes.

Stage (8):

(Fig. 14). Characterised by the appearance of leptotene spermatocytes and the centripetal migration of the elongated spermatids.

Stage (1):

(Fig. 15). Characterised by the appearance of a few zygotene figures and the young spermatids start to elongate.

With the exveption of the previously mentioned stages (7,8 and I) the other stages have a similar association. Moreover, the Sudanophilic droplets of the Sertoli cells are greatly increased in number and size, and extended towards the lumen of the seminiferous tubule (Fig. 12).

The interstitial cells are very few in number and appeared small in size with small deeply stained nuclei (Fig. 10).

DISCUSSION

Two priniciples methods for quantification of the cells of the seminiferous tubules have introduced. One is based on the development of the acrosomic system (CLERMONT and LEBLOND, 1953). With this system CLERMONT and LEBLOND (1955) defined twelve stages in the cycel of the seminiferous epithelium of the ram and bull. The other method is bassed on the morphological changes of germ cell nuclei and local arrangement of spermatids (ROOSENRUNGE, 1962 and ORTAVANT, 1959) who defined eight stages in the seminiferous epithelial cycle of ram. In this work the last method was choosen for it permit a rapid identification of variations in the principle events of spermatogenesis. A method using the Sertoli cell as a constant (ROWLEY and HELIER, 1971 and SKAKKEBAEX and HELLER,1973) was also applied in the present work. This may be of value because the Sertoli cells, unlike germ cells, are very resistant to changes caused by hormones (CLERMONT and MORGENTALER, 1955 and LOFTS, 1965). Moreover, division of these cells have not been observed in mature animals (ROWLEY and HELLER, 1971 and ROOSEN-RUNGE, 1962).

In the present investigation, it was particularly useful to apply the Sertoli cell ratio for the measurement of alterations in.the testes of zeranol implanted lambs.

According to ORTAVANT et al. (1977) spermiogenesis can be devided into three periods: rounded spermatids (stages 5-1), nuclear elongation (stages 2-4) and elongated spermatids (stages 5-8). In the present work, the same classification has been followed, but for precice analysis the period of rounded spermatids (stages 5-1) was subdivided into two periods: rounded spermatids (type-a) in stages 4 & 5 and rounded spermatids (type-b) with dusty-like chromatin in stages (6-1). The other periods are classified as zpermatids type-c and type-d respectively.

The eight stages of the seminiferous epithelial cycle observed in the control testes afe similar to those mentioned by ROOSEN-RUNGE (1962); ORTAVANT (1959) and ORTAVANT et al, (1977). Moreover, the relative frequency of these stages in control testes are in accordance with the results of ORTAVANT (1959) in ram.

The weight of the testes in zeranol implanted lambs are significantly lowered. Similar observation were observation were obtained by HALL et al, (1977) in lambs and CORAH (1980) in beef bull. In addition, the diameter of the seminiferous tubules are smaller than that of the control animals.

In the testes of zeranol implanted animals (groups II & III), the total number of Sertoli cells was not affected, however, the Sertoli cell ratios of all types of germ cells showed a marked decrease compared with normal ones. So, it can be stated that, the number of germ cells produced during spermatogenosis is greatly decreased. The interstitial cells are less prominant and less active.These findings greatly support the results of the previous study HASSAN et al,(1981) who concluded that,the FSH-and ICSH-cells are significantly decreased in number and exhibited signs of reduced cellular activities in the pituitary gland of zeranol implanted lambs.

Zeranol implantation resulted in a change in the frequency of the stages 7, 8 and 1 of the seminiferous epithelial cycle. On the basis of these observation it can be concluded that, zeranol selectively interfered with the movement of spermatozoa from the Sertoli cells to the lumen of the seminiferous tubule. Such an interference would be expected to increase the frequency of the stage 7 and decrease the frequency of the stage immediatly following it (Stages 8 & 1). Statistically, there is no significant difference concerning the frequency of the eight stages of the seminiferous cycle between the control and zeranol implanted lambs. Therefore, it can be stated that, zeranol implantation did not affected the synchronized initiation of the sucessive cycles, but rather delayed the release of spermatozoa.

It is worthy to state that, zeranol implantation in fattailed lambs increased the final livebody weight without any significant changes in the fat content of the carcass and have the lowest cost of food/kg. gain (EL-HOMMOSI,1980). Thus it becomes very obvious from this data that zeranol implantation must not be used with males intended to be as herd sires.

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LEGEND OF THE FIGURES

Figures (1-8). Cross sections of seminiferous tubules at the stages of cycle (1-8) respectively, in the testes of control lambs. ALL figures X 1000, and stained with PAS. Hx technique.
A = type-A spermatogonia. I = intermediate type of spermatogonia.
B = type-B spermatogonia. Pr = Preleptoten.
L = leptotene. Z = zygotene
P = Pachytene. D = diplotene. Cll. secondary spermatocyte.
Sa, Sb, Sc and Sd = spermatids type-a,b,c and d respectively.
Sz= spermatozoa.

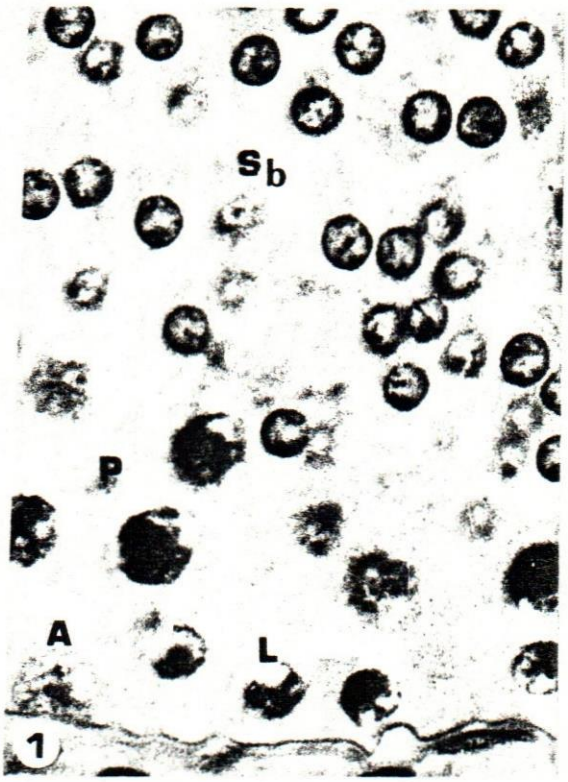
Figures (9-11). Cross sections of seminiferous tubules at different stages (7,8 and I) of the cycle in the testes of zeranol implanted lambs. All figures X 1000, and stained with PAS. Hx technique.
Pr= prelaeptotene. L = Peptotene.
Z = zygotene. Arrow shows elongating spermatid nucleus.

Figure 12. Section in the testes of control lambs showing normal intersititial cells (arrow). (PAS. Hx. 250).

Figure 13. Section in the testes of zeranol implanted lambs showing affected interstitial cells (arrow). PAS. Hx. X 250).

Figure 14. Cross section of seminiferous tubules of control lambs showing small amount of sudanophilic granules (Sudan black B. x 250).

Figure 15. Cross section of seminiferous tubules of zeranol implanted lambs showing increased amount of sudanophilic droplets. (Sudan black B. X 250).



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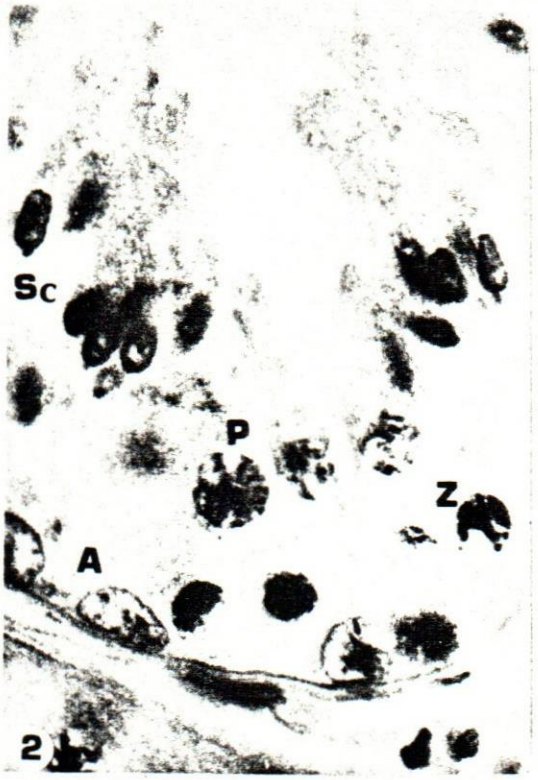

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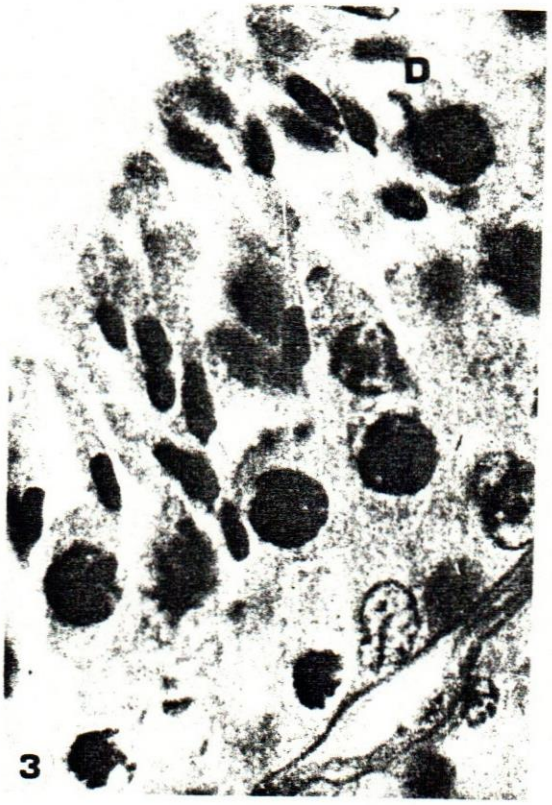

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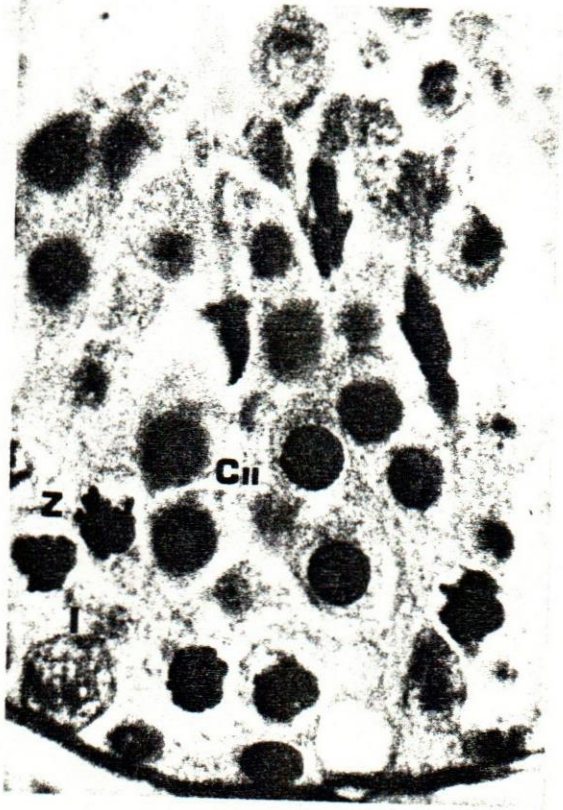

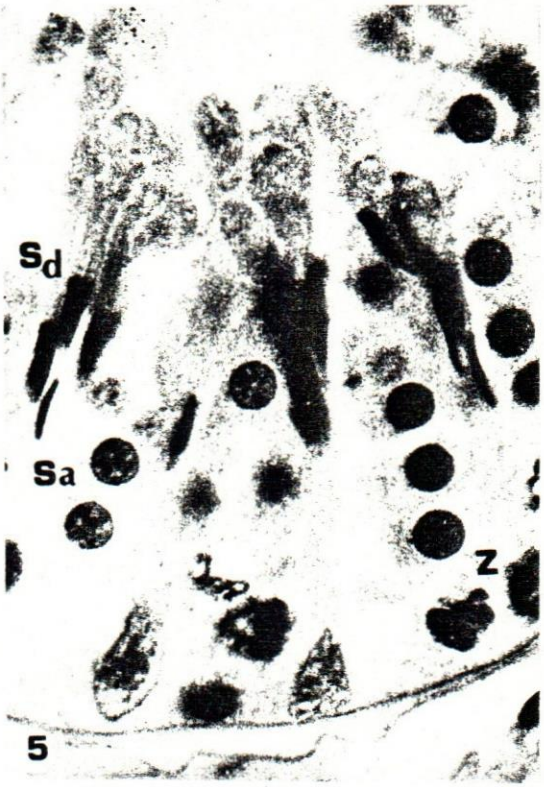
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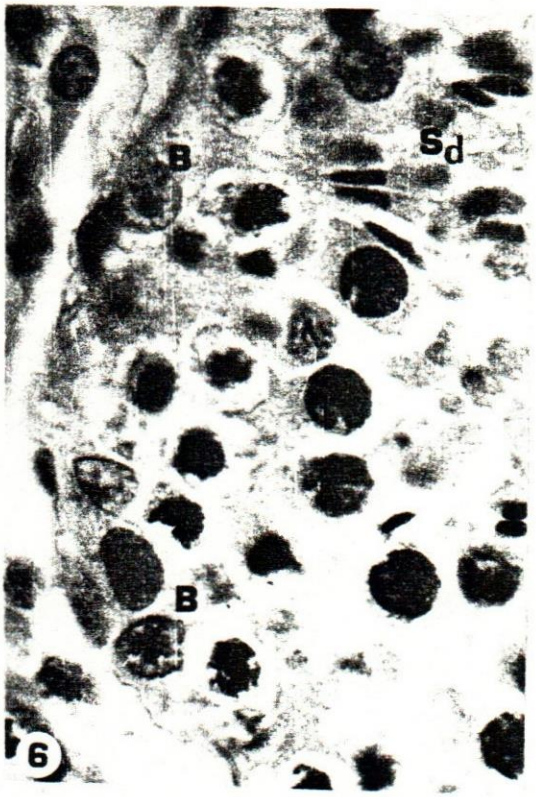
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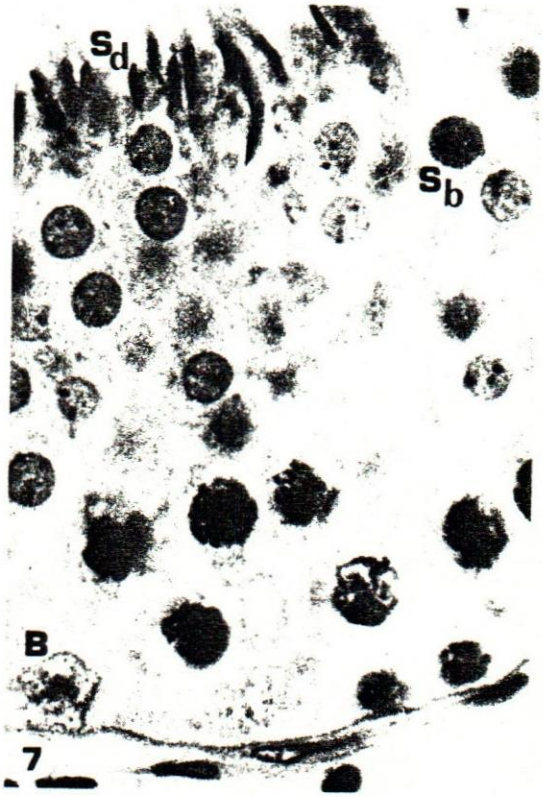
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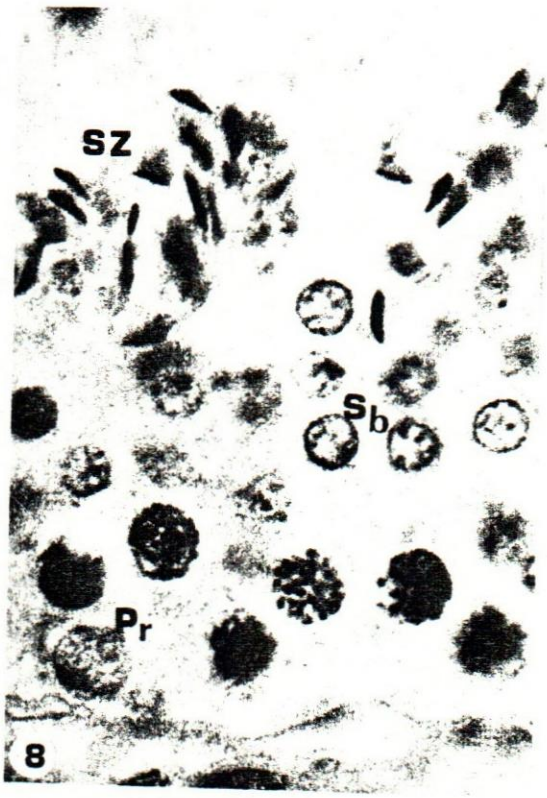
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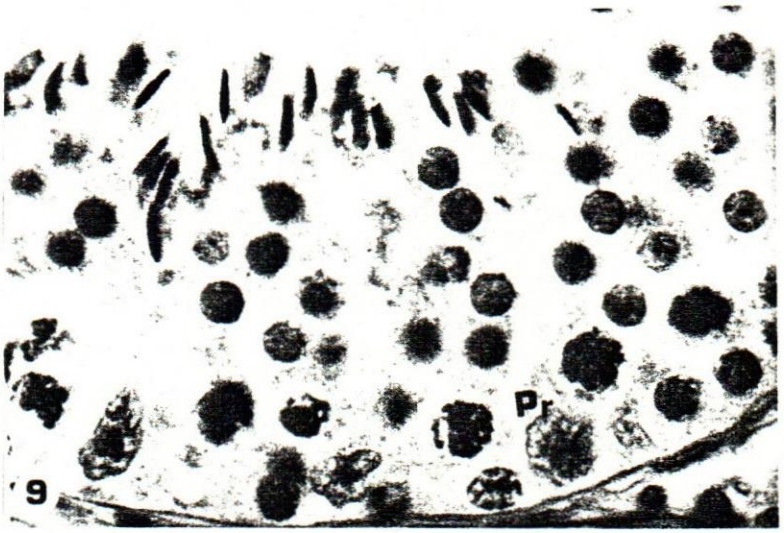
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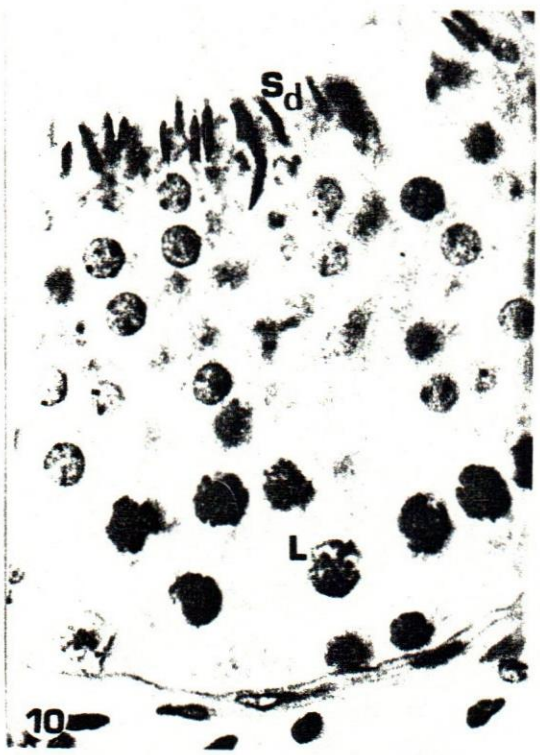
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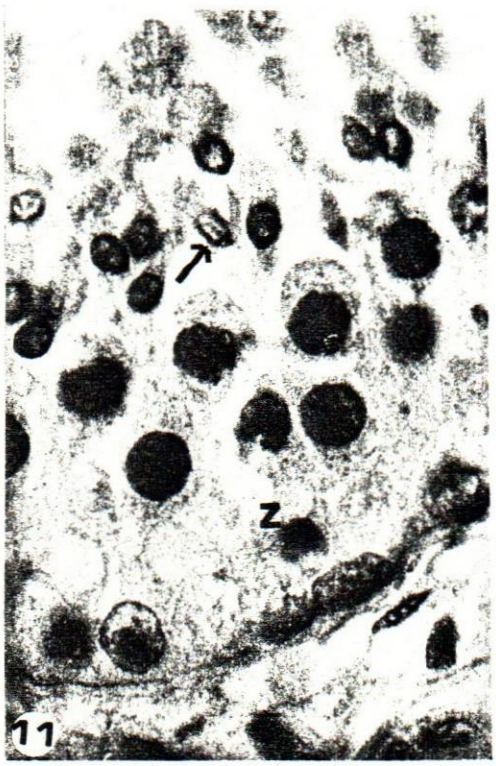
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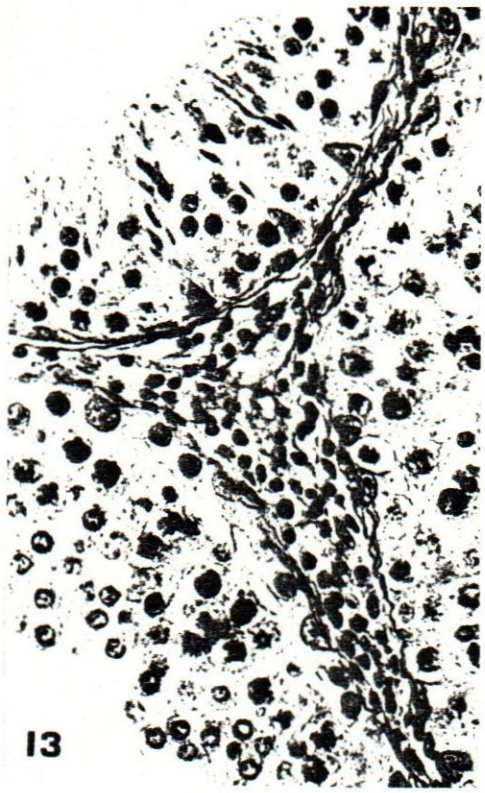

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