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Study effect of lead acetate on sperms abnormalities and histopathological alternates of testes tissue

دراسة تأثير خلات الرصاص على تشوهات النطف والتغيرات النسجية المرضية لنسيج الخصية في الذكور البالغة للفئران البيض

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Abstract:

This study is relevant in assess the genotoxic effects of lead acetate using sperm abnormalities assay, and study the histopathological changes induced by exposure of lead acetate in testicular tissue of albino male mice, between the treated and the negative control groups. Sixteen mature male mice were used and divided into four groups, the first group kept as a control, the second, third and fourth groups were given18mg/kg b.w of lead acetate daily by oral dosage for one, two and three weeks respectively. The Results showed a statistically significant differences ($p < 0.5$) represented in increase the number of abnormal sperms in treated animals for one, two and three weeks compared with control group. This results showing a different types of sperm abnormalities as tail less sperm, head less sperm, hook less sperm, amorphous head sperm, pin head sperm, triangle head sperm and eight head sperm. Administration of lead acetate induced enormous generation of Reactive Oxygen Species **(ROS).** The interaction of some of these **(ROS)** with the genetic material effect on the differentiation of the spermatogonial cells during spermatogenesis may be responsible for these observations.

As well as diverse histopathological changes were observed in the testis of male mice had been induced by lead acetate for different periods. The histopathological change was represented in relative decrease in spermatogenic activity, , intercellular spaces was appeared between seminiferous tubules and lack of leydig cells number compared with normal state of control group.

الخلاصة:-

هدفت الدراسةِ الحالية إلى بحث التأثيراتَ السمية الوراثية لأحد أهم العناصر الثقيلة ذات الصلة بحياة الإنسان وهو عنصر الرصاص المأخوذ بشكل خلات الرصاص على تشوهات نطف ذكور الفئران البيض البالغة باستخدام اختبار تشوهات النطف. كما درست التغييرات النسجية المرضية للنسيج الخصوي في المجاميع المعاملة بخلات الرصاص بتركيز 18 ملغم/كغم من وزن الجسم ومجموعة السيطرة ولثلاثة أسابيع، إذ تم اخذ ستّة عشرَ فأر ذكر بالغ قسمت إلى أربع مجموعاتِ كل مجموعة تضم أربعة حيوانات، المجموعة الأولى استخدمت كسيطرة سالبة أما المجموعات الثانية والثالثة والرابعة فقد تم تجريعها خلات الرصاص فمويا بتركيز 18 ملغم/ كغم يوميا ولمدة أسبوع واحد وأسبوعين وثلاثة أسابيع على التوالي. بينت النَتائِج المتحصل عليها وجود فروق معنوية ($p <0.5$) في التشوهات الحاصلة لنطف الفئران بين المجموعات المجرعة لخلات الرصاص ومجموعة السيطرة ، تمثلت هذه التشوهات بالنطف الفاقدة للذنب والنطف الفاقدة للرأس والنطف الفاقدة لكلاب الرأس والنطف اللاشكلية ونطف مثلثة الرأس ودبوسيه الرأس ونطف بشكل رقم 8 بالانكليزي .

كما أظهرت نتائج الفحص ألمجهري لنسيج الخصية وجود تغييرات نسجية مرضية في المجموعات المعاملة بخلات الرصاص لمدة أسبوع واحد وأسبوعين وثلاثة أسابيع بالمقارنة مع مجموعة السيطرة ، وقد تمثلت تلك التغييرات بالانخفاض النسبي لنشاط عملية تكوين الحيامن ، كما ظهرت مسافات بينية بين النبيبات المنوية بالإضافة إلى انخفاض أعداد خلايا لايدك. وهذه النتائج تبين سمية عنصر الرصاص وقدرته على إنتاج الجذور الحرة المسببة للتأثيرات السلبية.

Introduction:

Not detected any useful role vital component lead while its adverse effect has been documented by many researchers in animals and humans alike , and its impact was on the behavioral aspects, physiological and biochemical aspects **[1].** The mechanism of lead genetic toxicity in eukaryotes did not know until the present time but thought that it was because its indirectly affect on DNA and caused the harmful effects either by influence on the stability of chromatin or interference with the reform system **[2].**

Also **[3]** noted that lead induces oxidative stress , like other heavy metals in the animal by raising the ratio of lipid peroxidation LPP dramatically. It is believed that the damage resulting from oxidative stress which linked with presence of this element has been returned to its toxicity **[4]**. Imbalance between the production of reactive oxygen species process, and the low percentage of free radical scavengers in reproductive system cause oxidative stress, and this is the reason behind of toxicity of lead, so an increase ROS play an important role in the function of the reproductive system and infertility **[5]**. lead is a poisoning agent for the male reproductive system **[6]**. Toxicity of this element in male reproductive system occur as a result of deposition in different member, especially in testes, epididymis, vas deferens, seminal vesicle and seminal ejaculate which led to influence on the vitality of sperm and preparation as well as the reduction of activity including live sperm**[7].** In addition the animal and clinical studies have shown that defects occurring in the genesis of sperm process (spermatogenesis) occurs as a result of exposure to toxic **[8].**

Therefore, the abnormal shape of the sperm is the guide on the disorder of the sperm genesis process (spermatogenesis) **[9].** It is one of the most detected parameters of natural fertilization or fetal development is a natural sperm shape standards**[10].** Hence, the shape of abnormal sperm crosses correctly serious imbalances in the process of sperm formation (spermatogenesis)**[11].** It is well known that the phases of sperm formation process (spermatogenesis) be adversely affected by many factors, including pollutants, and one of these contaminants is lead **[12].** Lead acetate led to atrophy in testes due to toxic alternates in the testicular tissue **[13].** In another side **[14]** reported the increased production of reactive oxygen species within the cell and process of lipid peroxidation possibly because of exposure to lead has led to tissue damage. More importantly, it was noted recently that lead compounds induce oxidative stress in different tissues by generating ROS **[15]**.

Material and method:

Material

Lead acetate

lead acetate (3-hydrate lead acetate) had brought from storage of collage of science/ university of Karbala, that supported from the company(BDH, limited pool/ England) as a powder form in concentration 99% and take the dosage from it 18mg /kg and dissolved by distilled water and gavage orally to mice.

Experimental animals

The male mice were kept in Gages for at least 7days to acclimate to laboratory conditioning with an temperature of 24±2C^{o} in the animal house in pharmacy collage, during the period of the experiment. Male mice were 6-8 weeks old and weighed 23±2g. Water and food was locally prepared and consists of available constituents which fulfill the mice dietary require.

Experiment design

Sixteen experimental animals (mice) were divided into four groups containing four mice in each group. All groups administered orally.

1. group one:- this group treated with normal saline for one week.
2. group tow:- this group treated with 18 mg /kg lead acetate for one week.
3. group three:- this group treated with 18 mg/kg lead acetate for two weeks.
4. group four:- this group treated with 18 mg/kg f lead acetate for three weeks.

Procedures

The male mice were sacrificed by killed with chloroform, Male mice sperm abnormality was tested according to the method of **[16]** with some changes. The slides were examined under microscope and different abnormalities were recorded.

Histological study

The testes were fixed in 10% formalin and embedded in paraffin. Four µm thick sections were prepared and stained with hematoxylin and eosin dyes. The specimens were examined under an light microscope, the images were diagnosed for histological study**[17]**.

Statistical analysis

Statistical comparisons were made using the ANOVA test for comparison of data in the control group and the experimental groups. The data were expressed as mean ± SEM (standard error of means) and uses the low significant difference (LSD).

Result & discussion

The results of statistical analysis were showed that there are a significant differences ($p < 0.05$) between dosage period for (one week, two weeks, three weeks) with 18mg/kg of Lead acetate compared with negative control for each abnormality of sperms.

The result of the oral gavage for one week, two weeks and three weeks with 18 mg/ kg b.w of lead acetate led to raise the mean of abnormalities in Tailless sperm, Head less sperm, Amorphous head sperm, Triangle head sperm and Hook less head sperm. While the eight number shape and pin head abnormalities, did not posses any significant differences ($p > 0.05$) compared with treatment for one week period and with negative control for that abnormalities as showed in table (1).

Table (1):mean of mice sperms abnormalities which treated with 18mg/kg lead acetate for one, two and three weeks.

Duration of exposure / Abnormalities	Control M± SE	First week M± SE	Second week M± SE	Thired week M± SE	$LSD_{0.05}$ For duration of exposure
Tail less sperm	C 2±12 A	A 7.6±38.75 A	B 2.2±29.5 A	B 3±27 A	2.548
Head less sperm	C 3.2±12.5 A	A 0.94±34.75 B	B 2.8±28 A	B 1.9±27 A	
Amorphous head sperm	B 3.1±7.5 Bc	A 1.9±23 C	A 0.95±21.5 B	A 3±21 B	
Triangle head sperm	C 1.9±5.75 C	A 3.1±15.5 D	B 1.2±10 D	B 1.6±12 D	
Hook less head sperm	D 3.8±9.5 Ab	A 6.4±26.25 C	B 2.6±23.5 B	C 1.6±20 B	
Number 8 sperm	C 3.1±6.75 C	C 1±9 E	A 1.1±22 B	B 2.4±15.75 C	
Pin head sperm	B 0±0 D	B 0±0 f	A 2.1±16.25 C	A 1.6±16 C	
$LSD_{0.05}$ Abnormalities	3.371				$LSD_{0.05}$ Interferences 6.742

* The capital letter horizontal differences refer to found significant differences ($p<0.05$) .
* The small letter vertical differences refer to found significant differences ($p<0.05$).
M : mean
SE: standard error

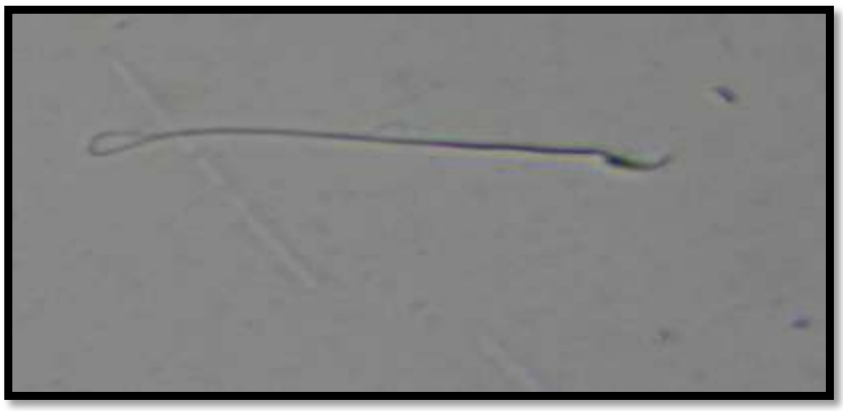

Fig (1) show normal sperm(400X, methylene blue)

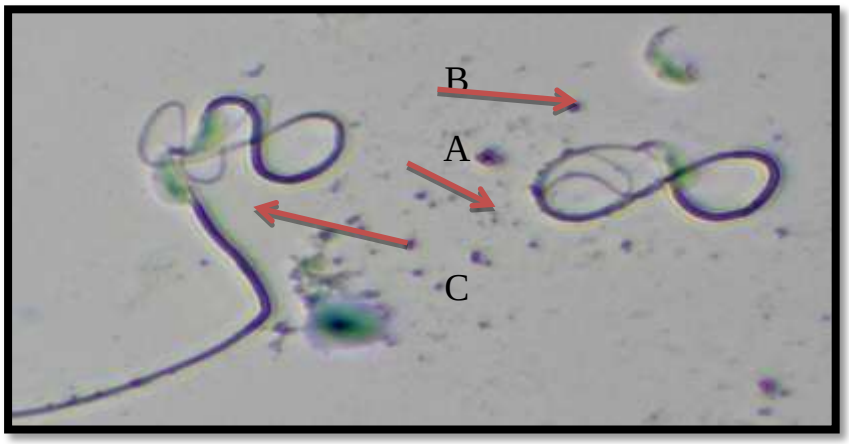

Fig (2) show sperms abnormality of male mice treated with 18mg/kg lead acetate (400 X, methylene blue Daye)

A- eight shape sperm B- Tailless sperm C- amorphous head sperm

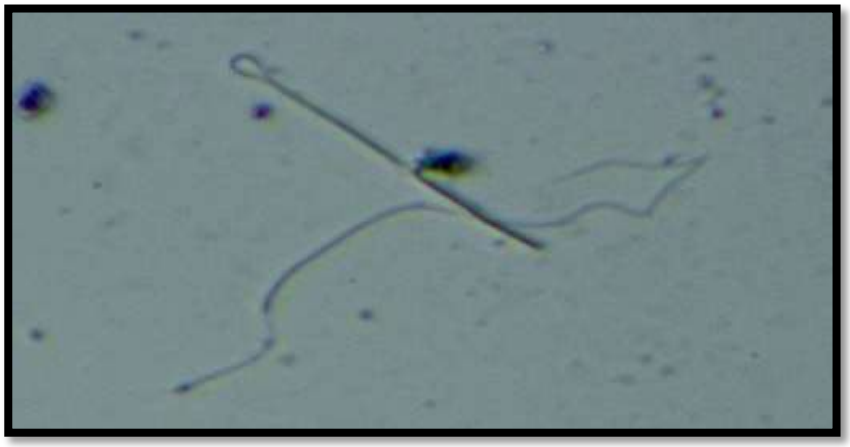

Fig (3)head less sperm of male mice treated with 18mg/kglead acetate (400X, methylene blue Daye)

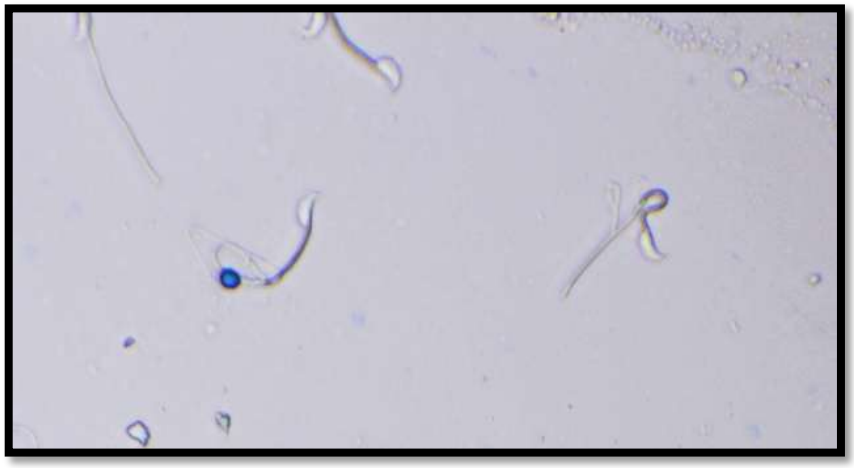

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Fig (4) hook less sperm of male mice treated with 18mg /kg lead acetate (400x, methylene blue Daye)

Fig (5) pin head sperm of male mice treated with 18m /kg lead acetate (2-3weeks) (400x,methylene blue daye).

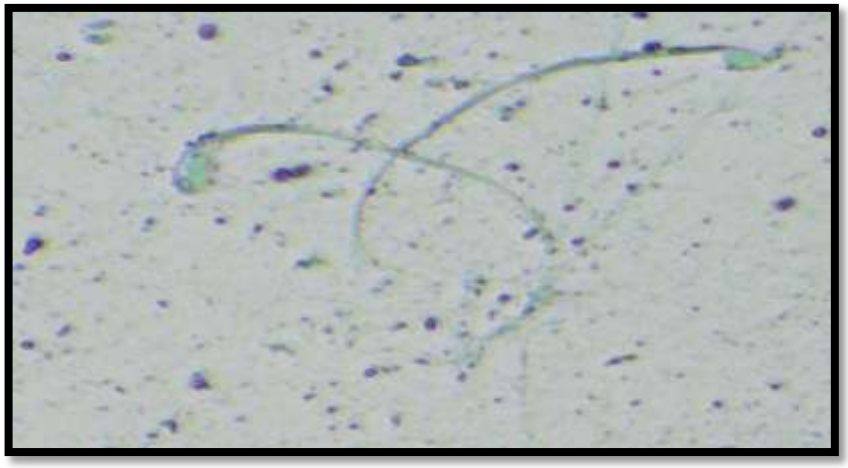


Fig (6) trangle sperm head of male mice treated with 18mg /kg lead acetate (400x,methylene bluue daye).

The result shows as in table (1) an increase in level of abnormalities of sperms and differences in types of these abnormalities observed in lead-treated mice. lead Exposure Led to raise the proportion of lipid peroxidation LPP significantly in testicular tissue that was provide a link the

production of reactive oxygen radicals excessively with LPP after treatment with lead, therefore the possibility of induction of genetic change in the germ cells due to the production of ROS with help of lead. factually lead compounds do not show any adverse effect directly on the DNA **[18]**. In addition , it is known that the ions of lead discourage DNA polymerase one of the main enzymes involved in DNA repair process **[19].** And thus ROS was caused the changes in DNA with stimulation of lead which is not possible to repair Moreover, the information available in the recently revealed the relationship between the occurrence of genetic mutation and ROS resulting from metals **[20].** induced genetic damage in males germ cells was caused unnatural sperm head and these abnormalities perhaps created by the access point mutation or a small deletion mutation. These distortions may occur as a result of changes in the mechanism of genetic, cytotoxic or physiological work, changing in DNA of testicular tissue which followed by disrupts the process of discrimination of spermatozoa**[21]**.

Histopathological Examination:

Microscopical examination of histopathologic changes of animals that were gavaged 18 mg/kg of lead acetate for three weeks were observed in testicular tissue and the results of this exam showed, intercellular spaces was appeared between seminiferous tubules and lack of leydig cells number in the second and third week, as well as decrease in spermatogenic activity caused decrease in sperms numbers observed in three weeks of administration for lead acetate fig **(8), (9)** and **(10)**compared with normal state of control group fig **(7).** These results correspond with observations of **[22]** which confirmed that lead works as an assassin for the sperm in the event of prolonged exposure to this element in male rabbits. Also, **[23]** had Observed a serious changes in testicular tissue of male rabbits which exposed to lead acetate included vacuolar degeneration, necrosis and atrophy of seminiferous tubules. These findings indicate that lead was aimed a sperms in the epididymis and spermatogenesis in testes tissues. These results support reports which prove that lead acetate seriously affect on male reproductive system and testes in male rats treated with lead acetate for three months. Also in another related study, Exposure to lead for long periods led to the depletion of germ cells in the seminiferous tubule. These results may be return to the process of programmed cell death (Apoptosis), or because of the failure of differentiation. Same results obtained in mutant mice**[24].** It also may be associated with an imbalance in the formation of sperm (spermatogenesis) and changes such as the remaining objects process, involving multiple lipid droplets in mice was treated with lead **[25].**

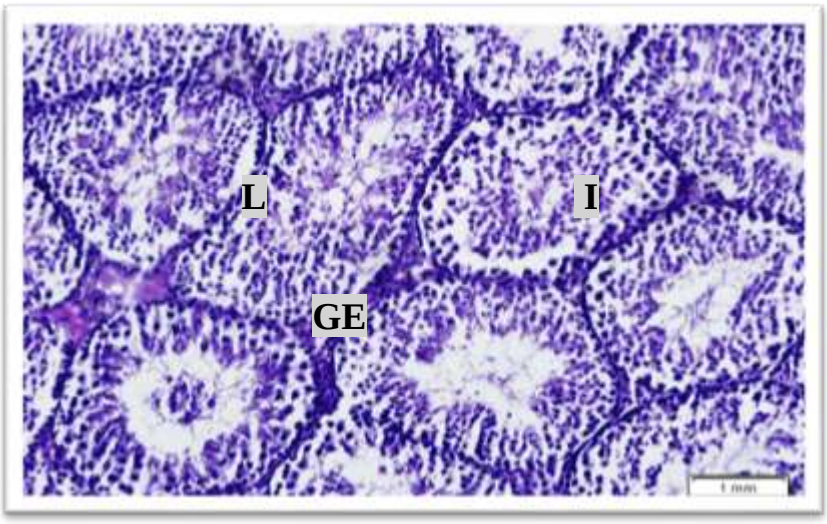
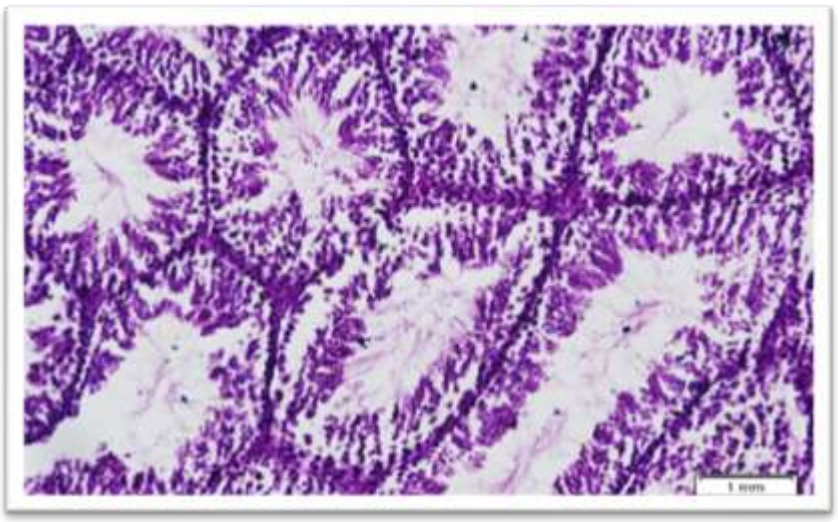
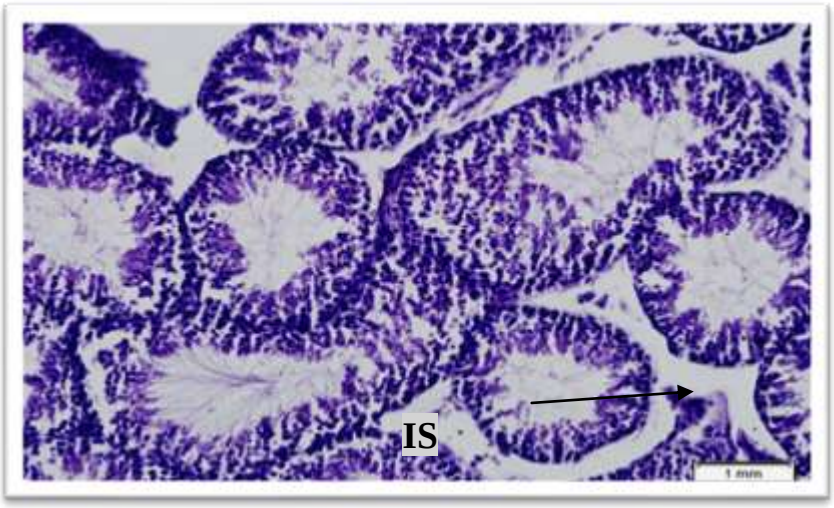


Fig (7) shows section of control group (200x H&E)

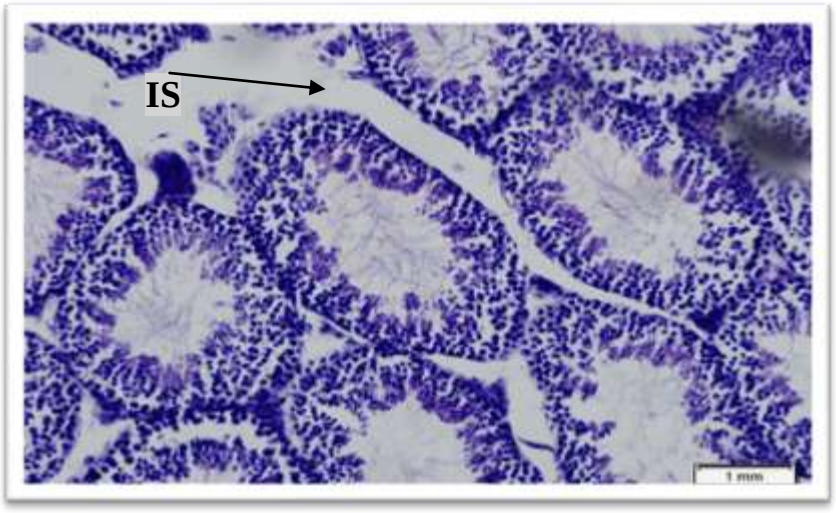
GE: Germinal epithelium, I:Interstitium, L:Central lumen



Fig(8) shows spermatogenic activity caused decrease in sperms numbers, (200X H&E) First week



Fig(9) testis tissue show intercellular spaces (IS) & decrease of leydig cells (200X, H&E) second week



Fig(10) testis tissue show intercellular spaces (IS) & decrease of leydig cells (200X, H&E) third week

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