Follow up of the pancreatic structural alterations in a rat model of andropause induced by bilateral orchiectomy after different time periods

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ABSTRACT

Introduction: The pancreas and male gonads shared similar utilities.
Aim: To follow-up the pancreatic structural alterations after orchiectomy on different times.
Materials and Methods: Twenty-four rats were allocated into sham-operated (Group I, n=6) and orchiectomized (Orx) (Group II, n=18). After 2 weeks of rest, the Gr II was equally re-distributed into subgroups IIa, IIb, and IIc depending on sacrifice time (3, 6 and 9 weeks, respectively). The pancreatic specimens were processed and stained with H&E and were immunostained with anti-insulin and glucagon proteins and anti-single-strand DNA (ss-DNA). Body masses, serum testosterone, fasting plasma glucose levels, oxidant and antioxidant markers were measured. The optical density of insulin immunopositive reaction and immunopositive area % of insulin, glucagon, and ss-DNA were realized with image analyzer. Data were statistically analyzed by ANOVA's and Tukey's test.
Results: This study revealed that the orchiectomized rats were hyperglycemic. The oxidative stress was significantly elevated while the antioxidant enzyme was significantly decreased. Structurally, in Gr IIa, the acini were deformed and shrunken. The acinar cells had less basophilic cytoplasm with small and darkly basophilic nuclei, while the islets cells appeared deeply acidophilic with small darkly stained nuclei. The apoptotic cells were observed in some acinar cells and in cells peripherally located in islets. The positive area% of the β cell was non-significantly decreased, whereas the positive area% of α cell was significantly decreased. Additionally, the positive area% of apoptotic cells was significantly increased. Gr IIb and GrIIc revealed the same structural changes as in GrIIa.
Conclusion: Orchiectomy-induced testosterone deficiency affects the exocrine and endocrine pancreatic portions, both with a risk for pancreatogenic diabetes (type III). Therefore, we should be more cautious before performing orchiectomy in humans or animals.

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Key Words: Andropause, orchiectomy, pancreas, rats.

INTRODUCTION

Andropause (a decline in serum total testosterone level less than 3.5 ng/mL or in free testosterone (T) level less than 72 pg/mL) has become a true health problem as the T level falls progressively from the late 20's or even, mid 20's and continues across the rest of men life. Despite decline of T level occurs slowly and subtly[1, 2], many adverse effects including, cognitive decline, erectile dysfunction, a decrease in lean mass and bone density or even osteoporosis, and mild anemia are documented[3, 4].

In concert, the pancreas and male gonads shared similar utilities. The sex-determining region Y (Sry)-box-containing (Sox) factors especially SOX9 has been identified in pancreogenesis. SOX9 is responsible for endocrine differentiation and maintenance of the embryonic and adult pancreatic ductal state. The roles of SOX9 in both the developing and mature pancreas have been covered in the review of Seymour[11]. Moreover, testosterone exerts a natural protective antioxidant and anti-apoptotic activities in many tissues especially the pancreas[11]. Recently, pancreatic β cell dysfunction is proven to be
responsible for male sexual dysfunction in humans and animals. Excessive production of radical oxygen species (ROS) in testicular cells is documented in diabetic patients. Increased ROS and apoptosis can induce male infertility. Also, DM increases germ cells apoptosis and interrupts spermatogenesis[13]. Decreased testosterone levels, DM type II, and even insulin resistance were found to be linked. So, some investigators considered steroid hormone therapy as a new approach for the treatment of DM[14, 15]. Even, prevention of obesity and DM by dehydroepiandrosterone (DHEA) administration is considered as a line of treatment[16]. In the meantime, DHEA is currently widely accepted as a “treatment for aged men”[17]. Finally, several cross-sectional studies with different methodologies could prove an inverse relationship between levels of serum free testosterone and fasting insulin hormones[18, 19]. The previous data spurred us to mimic andropause (testosterone deficiency) in rats via surgical orchiectomy and follow up the structural alterations that may occur in the pancreatic structure and function after different time periods.

MATERIALS AND METHODS

Animals

The experimental protocol was performed according to the guidelines of the ethical committee of Zagazig University, Egypt. Middle-aged male Wištar rats (n=24; 10-12 months old; 441.95±98.82 g mean body mass), housed in the Breeding Animal House, in the Faculty of Medicine, Zagazig University under a strict care at a temperature of 25 ± 2°C and 12:12 h light/dark cycle were utilized. The animals fed on a soy-free diet and water ad-libitum.

Bilateral orchiectomy procedure (scrotal approach)

We followed the method described by[20]. After anesthesia with 20 mg/kg dose of sodium thiopental (Pentothal), a small incision was made at the tip of the scrotum. The blood vessels and vas deferens were ligated with 4-0 absorbable surgical thread. Then, the testis, epididymis and a piece of vas deferens were removed. Afterwards, the remaining tissues were returned into the scrotal sac and the skin incision was sutured with non-absorbable surgical thread. The same procedure was repeated for the left testis. In the sham-operated group (Gr I), the testicular fat pads were only excised. The animals were singly separated and carefully monitored to avoid chewing sutures. The rats were given 25 mg/ kg/12h metamizole sodium as an analgesic and received IM injection of 4000 IU/kg/24 h ampicillin for 2 days. The wound dressing was also applied every day for a week. All rats were rested for two weeks under specified-pathogen-free conditions.

The experiment

Rats were allocated into two main groups after one-week-acclimatization. Sham-operated group (Group I, n=6) underwent only mobilization of testicular fat pads and orchiectomized group (Group II, n=18) underwent bilateral orchiectomy and epididymectomy via scrotal approach. After resting for two weeks, the Gr II was re-distributed into three equal subgroups: IIa, IIb, and IIc, depending on different sacrifice times: 3, 6 and 9 weeks, respectively.

Biochemical analysis

All chemicals and kits were purchased from Al Gomhouri co, El Mohafza st, Zagazig, Egypt. After 8-hours fasting, blood samples (6 ml /animal) were collected by retro-orbital puncture just before sacrifice by decapitation at daytime, same hour (5 p.m.). Blood samples were divided into two parts; the first part was left to clot overnight at room temperature, then centrifuged at 3000 rpm for 10 min for separation of serum and the second part was collected in heparinized tubes 10 min at 4000 rpm and then, aspirate off the plasma and the erythrocyte lysate was prepared for estimation of superoxide dismutase (SOD). Separated plasma and sera samples of all the animals were stored at −80°C until used.

Measurement of serum testosterone level

Serum testosterone level was measured with the use of an enzyme-linked immunosorbent assay kit [Testosterone (mouse/rat) ELISA Kit, Cat No: MBS843463; MyBioSource] according to the kit manufacturer’s instructions and expressed as ng/mL.

Measurement of plasma glucose level

Fastig plasma glucose level was measured using glucose oxidase method (Spinreact, Girona, Spain). The values were expressed as mg/100 ml.

Measurement of oxidative and antioxidant markers

Following the manufacturer’s instructions, SOD (an antioxidant marker) and Malondialdehyde (MDA); a lipid peroxidation marker levels were measured using commercially available kits (Cat No. K335-100; and Cat No. K739-100; Biovision Inc, San Francisco, USA, respectively). Colorimetrically, the absorbance of SOD was at 450 nm and the absorbance of MDA was at 532 nm.

Histological analysis

The pancreatic specimens were immediately fixed in 10% neutral buffered formalin, dehydrated in ascending grades of ethanol, cleared in xylol and embedded in paraffin wax. Finally, 3 µm thick paraffin sections were cut and stained with Hematoxylin and Eosin (H&E)[21].

Immunohistochemistry

The immunohistochemical staining with anti-insulin protein, anti-glucagon protein, and anti-ssDNA was performed to detect the β and α cells and apoptotic cells, respectively. In brief, the streptavidin-biotin immunostaining procedure was performed according to manufacturers’s instructions. The paraffin sections of about 3 µm in thickness were deparaffinized and
Effects of bilateral orchectomy on the pancreas

RESULTS

General observations

The orchietomized rats appeared tired and exhausted. Low activity of rats and tendency to sleep especially after meals were noticed. In Table 2, the body masses of the Gr IIa were statistically decreased by comparison with the Gr I. While, the Gr IIb and Gr IIc showed a significant increase of body masses when compared with Gr IIa.

Biochemical results

Table 3 showed the effects of bilateral orchectomy after different time periods on biochemical parameters. Compared to the Gr I, fasting plasma glucose levels of Gr IIa, IIb, and IIc were significantly increased while, serum free T levels were significantly decreased.

The antioxidant and oxidative stress results

In Table 3, compared to Gr I, SOD level was significantly decreased in the Orx groups. On the other hand, the level of the oxidative stressor MDA was significantly increased in Gr IIa when compared with the Gr I. In Gr IIb, the increment of MDA level was significant when compared with the Gr I and non-significant with Gr IIa. In Gr IIc, the increment of MDA level was significant when compared with both of Gr I and Gr IIa.

Histological evaluation of pancreatic tissue

H&E stained sections

Histological examination of the control pancreas revealed a distinctive multi-lobulated organ. The lobules were separated by delicate connective tissue septa that housed blood and lymph vessels. Each lobule was formed of multiple coalesced acini and intermingled pale stained areas of islets of Langerhans (Fig. 1A and 1B). The acinar cells were truncated pyramidal with apical acidophilic and basal basophilic cytoplasm. Their nuclei were rounded and basal with prominent nucleoli (Fig. 1B'). In the islets of Langerhans, the central cells were either gathered around the blood capillaries as follicles or irregularly clustered. The centrally located cells had slightly dark basophilic rounded nuclei and acidophilic cytoplasm. The fewer peripherally located cells were closely adjacent together in cords. Their nuclei were oval and darkly basophilic (Fig. 1B and 1B').

In Gr IIa, the acini appeared deformed and shrunken (Fig. 2A). The acinar cells were distended. Their cytoplasm was mainly acidophilic with faint basal basophilia. Their nuclei were small and darkly basophilic (Fig. 2B). The islets cells were deeply acidophilic and their nuclei were small and dark. The blood capillaries were dilated (Fig. 2C). In Gr IIb, the acini appeared less arranged (Fig. 2D). Their cytoplasm was acidophilic and their nuclei were darkly basophilic (Fig. 2E). The islets cells appeared distended with pale acidophilic cytoplasm and pale basophilic nuclei (Fig. 2F). Examination of the pancreatic tissues from Gr IIc revealed more shrinkage of acinar cells with less basal basophilic cytoplasm and darkly basophilic stained nuclei (Fig. 2G and 2H). Small aggregations of the islets cells that had pale acidophilic cytoplasm and pale basophilic nuclei were seen (Fig. 2I).

Immunostained sections

The insulin-positive reaction was localized in the cytoplasm of centrally located cells (Fig. 3A, B, C, and D). The reaction was intense in the Orx groups (Fig. 3B, C and D). Statistically, optical density of insulin immunopositive reactions was significantly increased in the Orx groups by comparison with the Gr I. The mean area % of insulin immunopositive reaction was non-significantly decreased in Orx groups when compared to Gr I (Table 4).

Image analyses and morphometric study

The image analyzer computer system Leica Qwin 500 (Leica Ltd, Cambridge, UK) in the Image Analyzing Unit of the Pathology Department, Faculty of Dentistry, Cairo University, Egypt, was used for estimation of the insulin optical density, areas % of immunoreactivity for insulin, glucagon and ssDNA in the pancreatic islets in all examined groups. Three non-overlapped high-power fields (>400) from three immunostained different sections of each animal (n=6 per group) were examined. The immunopositive areas were masked with a blue color in a binary manner by using the color detection in a standard measuring frame area equal to 118476.6 µm².

Statistical analysis

All the experimental obtained data were statistically analyzed by Analysis of Variance (ANOVA) using SPSS program version 16 (Chicago, USA). Data are presented as the mean ± standard error (SE) and followed by Tukey's post hoc test to compare means. Differences were considered to be significant when P value ≤ 0.05.
The immunopositive reaction with anti-glucagon protein was restricted in the cytoplasm of the peripherally located cells that formed a mantle in all studied groups (Fig. 3E, F, G and H). The mean area % of glucagon immunopositive reaction was significantly decreased in Gr IIa by comparison with Gr I. While, Gr IIb and Gr IIc showed non-significant decrement when compared with Gr IIa (Table 4). To localize the apoptotic cells, immunostaining with anti-ss-DNA was performed. In the Gr I, the apoptotic nuclei were hardly seen (Fig. 3I). On the contrary, the Orx groups showed numerous apoptotic nuclei in islets especially in the peripherally located cells and in the nuclei of some acinar cells (Fig. 3J, K and L). The mean area % of ssDNA immunopositive reaction in the Gr IIa was significantly higher than that in Gr I. Compared to Gr IIa, the mean area % of ssDNA immunopositive reaction of the Gr IIb and Gr IIc increased non-significantly (Table 4).

**Table 1**: A list of antibodies, sources, dilutions, antigen retrieval and temperature used

<table>
<thead>
<tr>
<th>Antibodies</th>
<th>Sources</th>
<th>Dilutions</th>
<th>Antigen retrieval, temperature, time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-insulin protein (ab216418)</td>
<td>Abcam, USA</td>
<td>1:200</td>
<td>Citrate buffer (pH 6.0), 95°C, 20 min.</td>
</tr>
<tr>
<td>Anti-glucagon protein (ab48287)</td>
<td>Abcam, USA</td>
<td>1:500</td>
<td>Citrate buffer (pH 6.0), 105°C, 20 min.</td>
</tr>
<tr>
<td>Anti-rabbit ss-DNA</td>
<td>IBL –Fujioka, Japan</td>
<td>1:200</td>
<td>No antigen retrieval and heat used</td>
</tr>
</tbody>
</table>

**Table 2**: Statistical analysis of body mass of the studied groups

| Body mass before surgery (g) | 441.95±98.82 |
| Body mass after surgery (g)  | 447.40±5.35  | 399.40±5.85* | 462.60±15.20* | 474.00±5.64* |

Mean ±SE; n=6/group; significant difference when P ≤ 0.05; *significant when compared with control group (Gr I); #significant when compared with Gr IIa.

**Table 3**: Statistical analysis of biochemical parameters of the studied groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Gr I</th>
<th>Gr IIa</th>
<th>Gr IIb</th>
<th>Gr IIc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum free T level (ng/mL)</td>
<td>2.54±0.20</td>
<td>0.04±0.014*</td>
<td>0.025±0.005*</td>
<td>0.022±0.006*</td>
</tr>
<tr>
<td>Plasma glucose level (mg/dL)</td>
<td>92.3±1.41</td>
<td>142.36±1.75*</td>
<td>144.54±1.51*</td>
<td>146.98±1.70*</td>
</tr>
<tr>
<td>Serum SOD level (U/ml)</td>
<td>157.5±2.32</td>
<td>128.33±2.74*</td>
<td>122.5±.99*</td>
<td>111.0±1.15**</td>
</tr>
<tr>
<td>Serum MDA level (nmol/ml)</td>
<td>8.33± 0.33</td>
<td>16.17 ±0.48*</td>
<td>17.7± 0.60*</td>
<td>19.17 ±19.17*</td>
</tr>
</tbody>
</table>

Mean ±SE; n=6/group; significant difference when P ≤ 0.05; *significant when compared with control group (Gr I); "significant when compared with Gr IIa.
Effects of bilateral orchiectomy on the pancreas

Table 4: Statistical analysis of the insulin optical density, area % of insulin, glucagon and ss-DNA immunopositive reactions in the islets of pancreas of all studied groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gr I</td>
</tr>
<tr>
<td>Insulin optical density</td>
<td>2.51±0.005</td>
</tr>
<tr>
<td>Insulin +ve area %</td>
<td>13.1±3.20</td>
</tr>
<tr>
<td>Glucagon +ve area %</td>
<td>6.07±0.73</td>
</tr>
<tr>
<td>ssDNA +ve area %</td>
<td>0.001±0.000</td>
</tr>
</tbody>
</table>

Mean ±SE; n=6/group; significant difference when $P \leq 0.05$; * significant when compared with control.

Fig. 1: Photomicrographs of a section in the pancreas of a control rat stained by H & E. (A) Showing multiple lobules (L) separated by thin connective tissue septa that housed blood and lymph vessels (circle). Each lobule composed of coalesced acini (AC) and intermingled with islets of Langerhans (I). (B) A higher magnification of the black boxed area in A. (B’) A higher magnification of the yellow boxed area in B showing the truncated pyramidal cells with apical acidophilic (arrow) and basal basophilic (dashed arrow) cytoplasm that contained basal rounded nuclei with prominent nucleoli (arrowhead). (B’’) A higher magnification of the green boxed area in B showing the central cells with slightly acidophilic cytoplasm (arrow) and slightly dark basophilic rounded nuclei (arrowhead), while the peripheral cells have oval and darkly basophilic stained nuclei (closed arrow). Bars: A= 200 µm, B= 50 µm, B’ and B’’= 30 µm.
Fig. 2: Photomicrographs of pancreases of bilateral orchiectomized rats H&E stained. (A-C) Gr Ila. (B) A higher magnification of the yellow boxed area in A showing distended acini with apical acidophilic (arrow) and faint basal basophilic cytoplasm (dashed arrow) with small and darkly basophilic stained nuclei (arrowhead). (C) A higher magnification of the green boxed area in A showing islet cells with deeply acidophilic cytoplasm (arrow) and small darkly basophilic nuclei (arrowhead). Notice the dilated blood capillaries (dashed arrow). (D-F) Gr Iib. (E) A higher magnification of the yellow boxed area in D showing acini with apical acidophilic (arrow) and less basal basophilic cytoplasm (dashed arrow) with oval dark basophilic nuclei (arrowhead). (F) A higher magnification of the green boxed area in D showing the islet cells with pale acidophilic cytoplasm (arrow) and pale basophilic nuclei (arrowhead). (G-I) Gr Iic. (H) A higher magnification of the yellow boxed area in G showing acinar cells with declared less basal basophilic cytoplasm (dashed arrow) and small dark basophilic nuclei (arrowhead). (I) A higher magnification of the green boxed area in G showing small aggregation of islet cells. Their cytoplasm is pale acidophilic (arrow) and contains pale basophilic nuclei (arrowhead). Bars: A, D & G = 50 µm; B, C, E, F, H and I =30 µm.

Fig. 3: Photomicrographs of immunostained sections of pancreatic islets in all studied groups. (A-D) Showing insulin-positive reactions in the cytoplasm of centrally located cells. Notice the intense reaction in orchiectomized groups (B, C & D). (E-H) Showing glucagon-positive reaction restricted in the cytoplasm of peripherally located cells (arrow) and in cells forming the mantle (dashed arrows). (I) Showing negative reaction with anti-ssDNA in the control group. (J-L) Showing positive reaction with anti-ssDNA restricted to the nuclei of the peripherally located cells (arrows) and in some acinar cells (arrowhead) in orchiectomized groups. Bars= 50 µm.
DISCUSSION

This study revealed that orchidectomy affected both exocrine and endocrine pancreatic tissue. The undetectable T levels in the Orx groups were in accordance with previous researchers\(^\text{[10, 22]}\). They proved depletion of testosterone up to eight months postoperative. Despite production of testosterone by both testicles and adrenal glands, the reported data in a recent study on orchicimized rats was surprising, whereas their conclusion implies that T deficiency decreases expression of adrenal genes confined to lipids and cholesterol metabolism. Consequently, decreased cholesterol metabolism will lead to decreased T production by adrenal gland. More investigations are necessary to clarify the compensatory role of the adrenal gland after orchidectomy\(^\text{[23]}\).

The glucagon-like peptide-1 (GLP1) is an incretin hormone secreted from entodistine. Via androgen receptor in β cells, dihydrotestosterone activates it. Consequently, GLP1 will stimulate insulin secretion and inhibit glucagon secretion\(^\text{[29]}\). The observable hyperglycemia in the investigated Orx groups was in accordance with\(^\text{[23]}\), who reported a significant insulin resistance in elderly men with reduced T level. Consequently, these patients will suffer from metabolic syndrome and type II diabetes which considered a risk factor for cardiovascular diseases.

In clinical studies with long-term ADT, the incidence rates of hyperglycemia, hyperinsulinemia, and metabolic syndrome were augmented. Even in T-deficiency independently, whether it is from the metabolic disorders or obesity, impaired fasting glucose and glucose intolerance were recorded\(^\text{[24, 26, 27]}\). Xia and colleagues correlated between low serum T level in elderly and insulin resistance\(^\text{[28]}\). Surprisingly, normal blood glucose levels with hyperinsulinemia in patients with prostate cancer submitted to testectomy was documented\(^\text{[29]}\). Moreover, in orchidectomy adult rats, normoglycemia was noted after eight months of ablation. Modifications of body composition after T deficiency, including accumulation of visceral fat which consequently affected glucose metabolism might be the cause\(^\text{[22]}\). Initial defects in insulin secretion regardless the causes are associated with increases in glucagon secretion that followed by impaired glucose tolerance\(^\text{[20]}\).

Oxidative stress is a basic process involved in many tissue injuries. Unfortunately, the pancreatic β cells possess very low expression levels of antioxidant enzymes\(^\text{[31]}\). In the Orx animals of this study, an imbalance in the ROS system was detected. These data were coincided with Son et al\(^\text{[20]}\), who proved that orchidectomy in mice accelerated oxidative injury in the brain. They claimed that T is a strong antioxidant. It could protect the tissue against oxidative damage even in stress conditions. In this work, the oxidative alterations were in line with the significant increase in single-strand breaks of DNA observed in orchicimized rats.

Since insulin is considered as a local trophic factor via insulin-acinar portal system, a lack of local insulin secretion due to T insufficiency was found to induce acinar atrophy\(^\text{[32]}\). In our work, the shrunken and deformed acini that lined by less basophilic acinar cells with small dark basophilic nuclei indicated low activity of exocrine pancreas in Orx groups. Recently, Talukdar and Reddy\(^\text{[33]}\) clarified the association between exocrine pancreatic insufficiency and hyperglycemia in humans. Vice versa, impairment of the exocrine pancreas will lead to mal-digestion of fat and protein. Consequently, the secretion of incretins which largely depends on normal fat and protein digestion will be affected\(^\text{[34]}\). Furthermore, any exocrine pancreatic disorders including; inflammation, tumors, or cystic fibrosis can induce DM named type 3 (a pancreatogenic diabetes)\(^\text{[35]}\). Unfortunately, exocrine pancreatic insufficiency did not depend on the duration of diabetes\(^\text{[35, 36]}\).

In group IIa of this work, the increased density of insulin immunostaining was coincided with accumulation of insulin secretion inside granules and its failure of secretion after T-deficiency\(^\text{[32]}\). Whereas, T hormone proven to increase glucose-stimulated insulin secretion via the interaction between the extra-nuclear androgen receptor and the GLP-1 receptor in β cells. Gao and his colleagues considered the hypercholesterolemia as another cause of hypertrophy of β cells in rabbit\(^\text{[41]}\). Alexandersen and Christiansen\(^\text{[26]}\) reported increased low-density lipoprotein fraction in T deficiency due to hypogonadism.

Interestingly, α cells are more affected than β cells in the investigated Orx groups. Immunopositive staining with anti-ssDNA (a nuclear apoptotic marker) was mostly in cells peripherally located in the islets. These apoptotic cells are not restored even 10 weeks postoperatively. This suggested that decreased of α cells index occurred was due to breaks in single stranded DNA. In accordance, Inoue\(^\text{[35]}\) reported decreased of all pancreatic parameters in castrated mice when compared to sham-operated mice. Decrement of epidermal growth factor secreted from rat submamillary salivary gland as a result of decreased quantity of androgen receptor following testectomy might be the cause\(^\text{[46]}\).

Several animal studies have proved that testosterone acts as a vasodilator on coronary, the thoracic aorta and pulmonary arteries\(^\text{[41]}\). Contradictory, in this work, dilation of islets blood vessels was noted in Orx group.

CONCLUSION

Orchietomy-induced testosterone deficiency affects both exocrine and endocrine pancreatic portions and is associated with a risk for pancreatogenic diabetes (type III). Therefore, we should be more cautious before
performing orchiectomy in humans or animals.

CONFLICT OF INTEREST

There are no conflicts of interest.

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Effects of bilateral orchiectomy on the pancreas


المملح العربي

متابعه التغيرات التركيبية للبنكرياس في نموذج الإياس الذكري للجرذ الناجم عن استئصال كلا الخصيتين بعد فترات زمنية مختلفة

الملخص

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المقدمة: يشارك البنكرياس والغدد التناسلية في نفس المنافع.

المقدمة: متابعه التغيرات التركيبية للبنكرياس بعد فترات زمنية مختلفة من عملية استئصال الخصيتين.

المقدمة: مواد وطرق البحث: تم تقسيم أربعين من الجرذان إلى مجموعتين متساويتين حيث وردت نتائج متفاوتة في المراجعة. بعد أسبوعين من الراحة، تم إعادة توزيع المجموعة II، (IIb وIIb) بسيطة على وقت التضحية (3 و9 و6 أسابيع، على التوالي). وتم تمرير لعينات البنكرياس وصبغها بصبغة الهيماتوكسيلين والإيوسين والصبغة المناعية المضادة لبروتينات الإنسولين والجلوكاجون، والحمض النووي المنفرد أحادي السلسلة. تم قياس كتلة الجسم، مستويات هرمون التستوستيرون في الدم والجلوكوز صام في البلازما، وحفرات التوارد والحمض النووي المنفرد أحادي السلسلة. كما تم قياس الكثافة البصريه لتفاعل المؤكسدات ومضادات الأكسدة. 

النتائج: أرتفاع ذو دلالة إحصائية في الإجهاد التأكسدي في حين أن الأنزيم المضاد للأكسدة انخفض بشكل ذو دلالة إحصائية. تركيبا، كانت العنيبات البنكرياسية مشوهه ومنكمشه، واحتفظ الخلايا المكونة للعنيبات على هيئه مزدوجة، في حين أن النوى صغيره مبطنة داكنة. وتحتوي على نواة صغيره نحتالة داكنة. 

النتائج: ظهرت خلايا الجزر البنكرياسية شديدة الحمضية وتحتوي على نواة صغيره حاكمة لينا. وقد أنخفضت النسبة المشهية للمنطقه الإيجابية للإنسولين والجلوكاجون والحمض النووي المنفرد أحادي السلسلة. بالإضافة إلى ذلك، كانت هناك زيادة ذات درجة سlight. 

الاستنتاج: نزع هرمون التستوستيرون عن طريق استئصال الخصيتين يؤثر على أجزاء كل من الغدد القنويه والغدد الصماء للبنكرياس. ووربط بمخاطر السكري الذكري البديل التأكسدي (النوع الثالث). وعلاوة على ذلك، فقد تكون الإجهاد التأكسدي وتحديم الحمض النووي المنفرد أحادي السلسلة من الآليات المحتملة. إذا يجب أن تكون أكثر حذرًا قبل إجراء عملية الإخصاء في الإنسان أو الحيوان.