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Original Article

## Stereological, Morphometric and Morphological assessment of Changes Induced by Bilateral Epididymal Lipectomy in Mouse Testicular Histoarchitecture

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### Article Info

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### Abstract

**Introduction:** Therapeutic strategies such as chemotherapy can affect gonadal adipose depots. The present study was conducted to assess stereological, morphometric and morphological changes in mouse testicular histoarchitecture structure following bilateral epididymal white adipose tissue lipectomy (BiEWATx)

**Methods:** In this experimental study, adult male mice were divided into three equal groups (n=6). In the lipectomy group, following induction of anesthesia, both epididymal white adipose tissues (EWATs) were harvested by a posterior abdominal incision, with adequate precautions to prevent any damage to neural and vascular structures of the testis. In the sham group, after induction of anesthesia, an incision was made in the posterior abdomen and after manipulation without removal of the adipose tissue, the incision was stitched. In the control group, animals received 100 mg per kilogram of body weight of intraperitoneal ceftriaxone at the time of opening the abdomen in the other two groups. After 35 days, testis of all animals were harvested and histological assessments were subsequently performed.

**Results:** Bilateral epididymal white adipose tissue lipectomy (BiEWATx) led to a significant reduction in diameter, the height of the germinal epithelium, cross-sectional area and numerical density of seminiferous tubules, the number of sections of seminiferous tubules per unit area of testis, and severe morphological changes in the testis tissue compared to the control and sham groups.

**Conclusion:** It appears that BiEWATx can provide the grounds for structural changes in the mice testis.

### Introduction

Continuity of metabolic activities of the cells that provide for body temperature maintenance and advances in biosynthetic pathways require stable sources of energy. Accordingly, the energy-consuming functions such as gametogenesis and steroidogenesis that are not vital to the survival of the organism are suspended in case of energy shortages (1, 2). As previous studies have shown, reduction of body fat as a result of nutritional deprivation (3, 4), exposure to cold (5) and increased activity (6) impairs reproductive activity. In this regard, it is well known that white adipose tissue (WAT) as a multiple functional, dynamic, endocrine organ plays an important role in the regulation of reproductive processes through bioactive peptides secretion, and the gonadal white adipose tissue (gWAT) has a special effect on nearby gonadal functions through production of local effective compounds (7). However, although several studies have approved the role of gWAT in reproductive function of female laboratory animals (8-10) and women (11, 12), but the effects of these energy reserves on male reproductive activity especially

spermatogenesis and sexual behavior are not well defined.

In addition, recent theories have been proposed in relation to reproductive damages caused by some chemotherapy drugs by reducing epididymal adipose tissue (13) and it has also been shown that physiological and pharmacological weight loss can affect the epididymal adipose tissue of mice (14).

Accordingly, since different therapies can affect gWAT and also due to the increasing efforts to use new and effective therapeutic strategies, the present study was conducted in the absence of detailed histologic studies to assess Stereological, morphometric and morphological changes induced by bilateral epididymal lipectomy in mouse testicular histoarchitecture.

### Material and Methods

This controlled randomized experimental study used 18 adult male mice with a mean age of 8 weeks and approximate weight of 25 grams procured from the center of breeding and keeping laboratory animals in the Department of Veterinary Medicine, Orumiyeh University, Orumiyeh, Iran. Animals were kept under standard conditions (light/dark cycle: 12 hours,

humidity:  $50\pm 10\%$ , and temperature:  $25\pm 2^\circ\text{C}$ ). All animals were fed similarly with corn, wheat, barley and pellets with equal proportions and had free access to water. All the ethical issues related to the use of laboratory animals in this study was also based on the guidelines approved by the Ethics Committee of Department of Veterinary Medicine at Orumiyeh University.

Following a week of adaptation to the environment, animals were randomly divided into three groups ( $n=6$ ). The mice were put under anesthesia by intraperitoneal injection of ketamine (40 mg/kg) and xylazine (5 mg/kg) for surgery and after surgical preparation, animals underwent posterior abdominal incision. The animals received a dose of intraperitoneal ceftriaxone (100 mg per kilogram of body weight) at the time of surgery to comply with the principles of infection control in surgery of laboratory animals (15). Animals in the control group received 100 mg per kilogram of body weight of intraperitoneal ceftriaxone at the time of opening the abdomen in the other two groups. In the sham group, after induction of anesthesia, an incision was made in the posterior abdomen and after manipulation (without removal) of adipose tissue, the incision was stitched. In the lipectomy group, following induction of anesthesia, and a posterior abdominal incision, EWAT was harvested with adequate precautions to prevent any damage to neural and vascular structures of the testis, and eventually, the abdominal incision was stitched in two layers. It is noteworthy that all doses, timing and surgical techniques used in this study were designed in consistence with previous studies in this area (7, 16).

According to the duration of spermatogenesis in the mouse, 35 days after surgery, the mice were euthanized and the testis were harvested in sterile conditions (16, 17). After fixation, the testicular tissue samples were transferred into special containers with characteristics and molded with liquid paraffin after tissue passage. Then, using a microtome,  $6\ \mu\text{M}$  sections of paraffin blocks were made and finally, Hematoxylin and Eosin staining method was used to stain samples. Evaluation of diameter and height of germinal epithelium of testicular seminiferous tubules in the intervention groups was performed using a reticle eyepiece. The number of sections of seminiferous tubules per unit area of testis was counted using a grid eyepiece. The cross-sectional

area of seminiferous tubules ( $A_C$ ) was calculated using the following formula:

$$A_C = \pi D^2 / 4 \pi$$

where  $\pi$  was equal to 3.142, and  $D$  was the mean diameter of seminiferous tubules. The numerical density of seminiferous tubules ( $N_V$ ) was determined according to the following equation:

$$N_V = N_A / (D+T)$$

where  $N_A$  was the number of sections of seminiferous tubules per unit area of testis,  $D$  was the mean diameter of seminiferous tubules, and  $T$  was the section thickness (18).

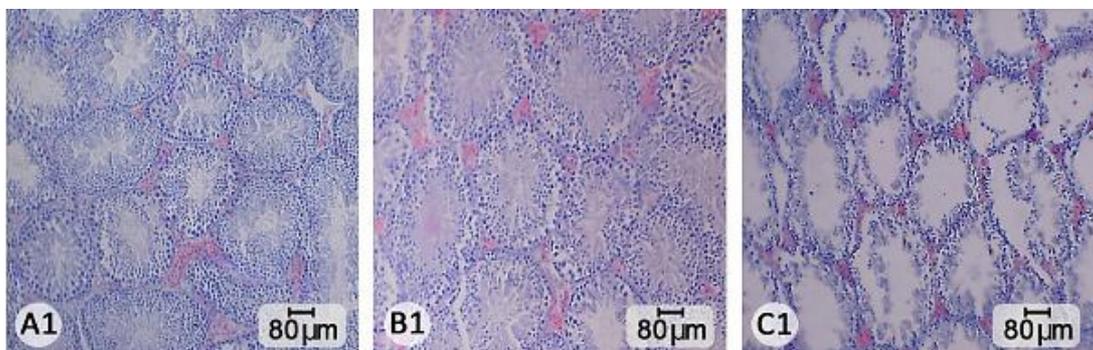
Results were expressed as Mean $\pm$ SD and due to the continuous quantitative nature of data, the number of evaluated independent groups, and the subsequent assurance of normal distribution of data, the results were analyzed using SPSS software Version 18. ANOVA and Tukey's multiple comparison tests were used for comparison between groups. The significance level between groups was considered as  $P < 0.05$ .

## Results

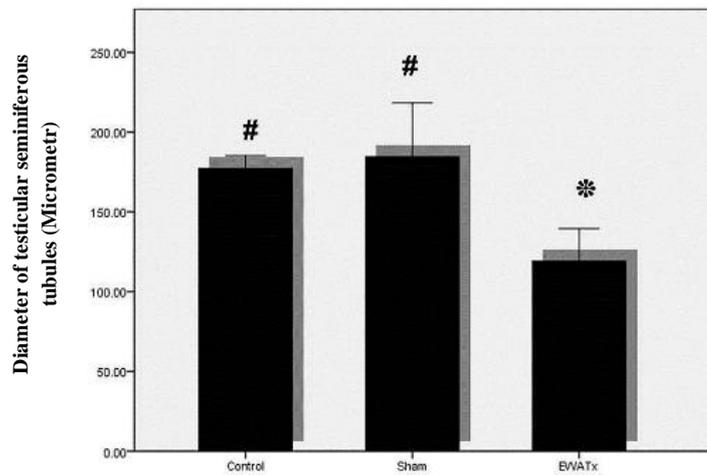
Histological evaluations of testicles in different intervention groups showed that bilateral epididymal lipectomy caused significant morphological changes in the testicles (Figure 1-C1). A significant reduction and degeneration of spermatogenic cells along with disruption and vacuolation of germinal epithelium was evident in the wrinkled testicular seminiferous tubules of animals. In the control (Figure 1-A1) and sham (Figure 1-B1) groups, testicular seminiferous tubules with normal tissue structure were covered with spermatogenic cells at different stages of maturity.

Statistical analysis of histomorphometric evaluation of testis revealed that the mean diameter of seminiferous tubules (Graph 1) and the height of germinal epithelium of the tubules (Graph 2) decreased significantly ( $P < 0.001$ ) following bilateral epididymal lipectomy compared to the control and sham groups.

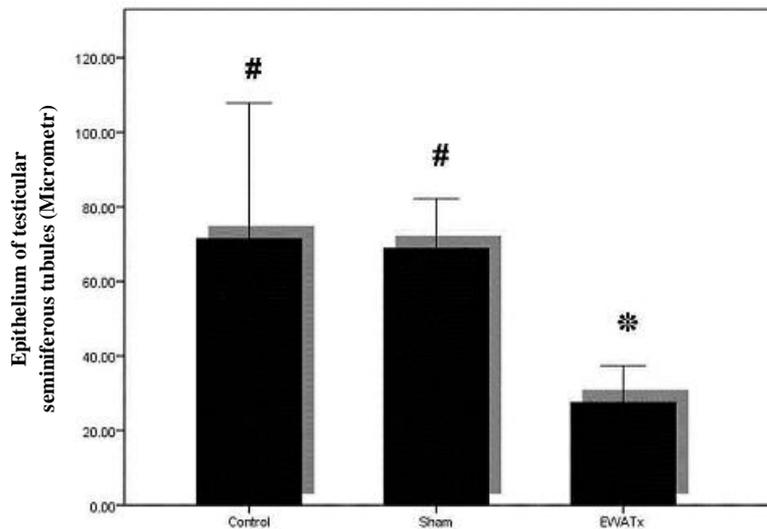
Stereological studies also revealed that bilateral epididymal lipectomy led to a significant decrease ( $P < 0.001$ ) in seminiferous tubules cross-sectional area (Graph 3) and the number of sections of testicular seminiferous tubules per unit area of testis (Graph 4) as well as a significant decrease ( $P < 0.001$ ) in the numerical density of seminiferous tubules (Graph 5) compared to the control and sham groups.



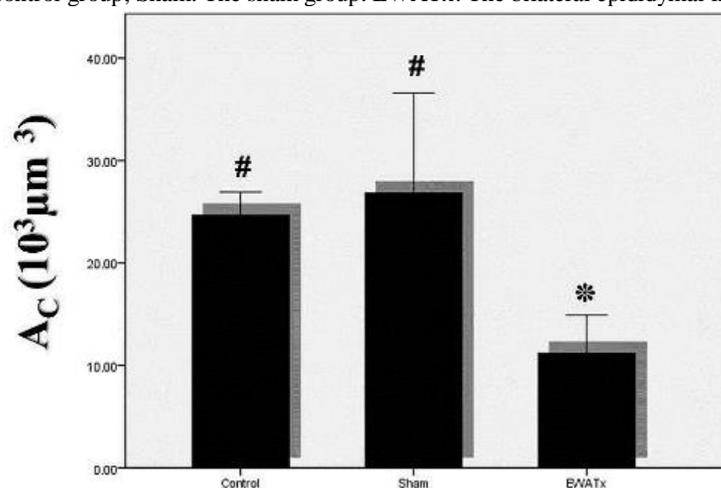
**Figure 1.** Cross-section of the testis: In the control (A1) and sham (B1) groups, the germinal epithelium of seminiferous tubules had normal tissue structure and the seminiferous tubules had and active spermatogenesis. Bilateral epididymal lipectomy (C1) led to severe hypoplasia and degeneration of spermatogenic cells along with disruption and vacuolation of germinal epithelium of seminiferous tubules.



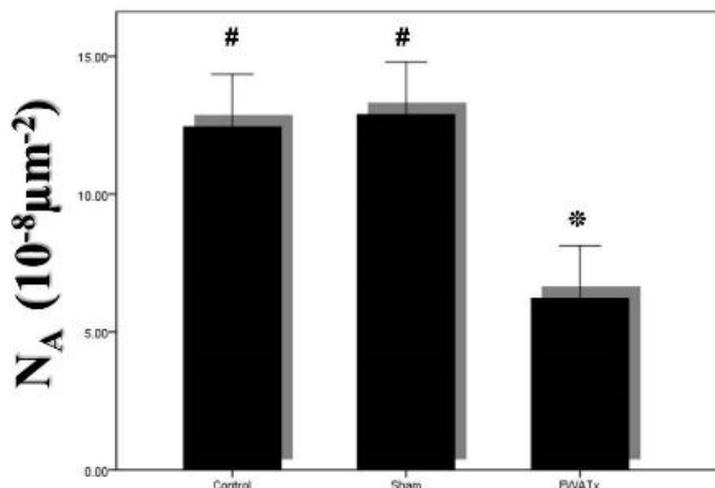
**Graph 1.** Comparison of mean diameter of testicular seminiferous tubules in the intervention groups  
Dissimilar markers indicate significance at P<0.001.  
Control: The control group; Sham: The sham group; EWATx: The bilateral epididymal lipectomy group



**Graph 2.** Comparison of mean height of germinal epithelium of testicular seminiferous tubules in the intervention groups  
Dissimilar markers indicate significance at P<0.001.  
Control: The control group; Sham: The sham group; EWATx: The bilateral epididymal lipectomy group



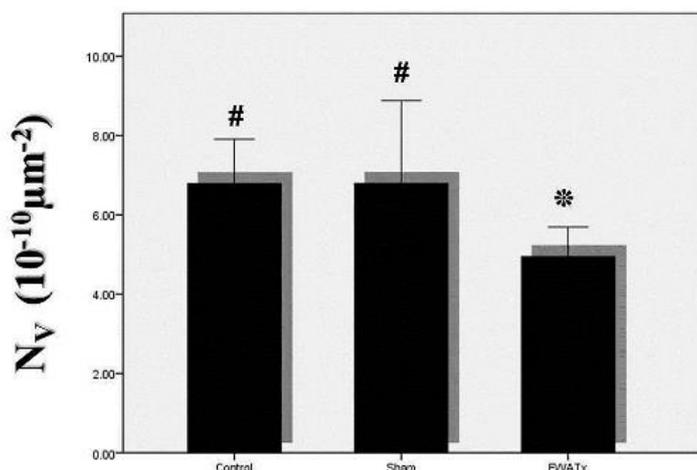
**Graph 3.** Comparison of cross-sectional area of testicular seminiferous tubules in the intervention groups  
Dissimilar markers indicate significance at P<0.001.  
Control: The control group; Sham: The sham group; EWATx: The bilateral epididymal lipectomy group; Ac: Cross-sectional area of testicular seminiferous tubules



**Graph 4.** Comparison of mean number of sections of testicular seminiferous tubules per unit area of testis the in the intervention groups

Dissimilar markers indicate significance at  $P < 0.001$ .

Control: The control group; Sham: The sham group; EWATx: The bilateral epididymal lipectomy group  $N_A$ : Number of sections of testicular seminiferous tubules per unit area of testis



**Graph 1.** Comparison of mean numerical density of seminiferous tubules in the intervention groups

Dissimilar markers indicate significance at  $P < 0.001$ .

Control: The control group; Sham: The sham group; EWATx: The bilateral epididymal lipectomy group  $N_V$ : Numerical density of seminiferous tubules

**Discussion**

The present study showed that bilateral epididymal lipectomy in mice causes severe tissue damage in testis, confirming other reports in the field that emphasized the role of EWATx in inhibiting spermatogenesis and sperm production (19, 20). Moreover, it appears that some chemotherapy drugs such as adriamycin also provide the ground for spermatogenic disorders by reducing epididymal adipose tissue, which is in line with this study (13).

Previous studies have shown that adipose cells are capable of expressing androgen receptors and androgens affect the function of adipose tissue through regulating their receptors (21). Also, the expression of these receptors in the EWAT of mice and serum testosterone levels reduction following the decreased expression of androgen receptors of EWAT in these animals is approved (22). Accordingly, due to the fact that spermatogenesis in human and rodents is severely impaired in the event of defects in endocrine protection

(23), abnormalities in spermatogenesis after EWATx can be associated with low levels of serum testosterone in this study. Moreover, given the important role of the Sertoli cells in structural evolution and maturation of germ cells in the testis tissue (24), it is suggested that disorders in testosterone dependent binding of Sertoli cells to germ cells can disrupt spermatogenesis. As recent empirical evaluations have revealed, EWAT lipolysis would be associated with reduced expression of androgen receptors and thus reduced serum levels of testosterone in mice (22).

On the other hand, since inhibin, as the main inhibitor of FSH secretion, is secreted by Sertoli cells (25), obvious vacuolation and degeneration of Sertoli cells following bilateral epididymal lipectomy in this study could have provided for FSH level increase. Previous reports have also approved the role of bilateral epididymal lipectomy in increasing FSH amounts which might be a reflection of the testis tissue damages and spermatogenic disorders (7).

In addition, it appears that testicular blood supply disorders might also inhibit spermatogenesis and tissue injuries due to bilateral epididymal lipectomy in mice testis. As it is well known that the testicular pampiniform plexus injury suppresses spermatogenesis because of increased testicular temperature (26).

Finally, several studies have been carried out in this field that supported the fact that spermatogenic disorders emergence following bilateral epididymal lipectomy is not associated with the reduction of energy reserves since the graft of harvested subcutaneous EWAT did not result in spermatogenesis improvement (13). Accordingly, it seems likely that EWAT provides for the improvement of spermatogenesis with the help of secretion of lateral nutritional and growth factors that directly affect the adjacent testis tissue (7, 13). In this regard, theories have been proposed recently about the possibility of presence of a venous-arterial transmission device between EWAT and the consistent testis (27). Also, compounds such as unsaturated fats with several double bonds, androgens, specifically testosterone, estrogen and leptin, all found in fat cells, are suggested as mediators of local epididymal fat performance (27-29).

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## Limitations

Lack of measurement of antibody titers and intratesticular testosterone, as well as failure to assess the secretory activity of Sertoli cells especially for survival and self-renewal of spermatogonial stem cells were among the limitations of this study.

## Conclusion

It appears that lack of secreted substances with the function of localized epididymal adipose mass following bilateral epididymal lipectomy can provide for significant stereological, morphometric and morphologic changes in the mice testis that eventually lead to spermatogenic disorders and reduced fertility. Revealing the exact mechanism of EWAT in maintaining the normal process and regulation of spermatogenesis requires larger experimental studies and clinical trials to be planned.

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