Int. J. Morphol.,
33(4):1371-1376, 2015.

Histomorphology of Liver, Testicle and Body Fat in Farm Rabbits Fed with an Obesogenic Diet During the Peripuberal Period

Histomorfología del Hígado, Testículo y Grasa Corporal en Conejos de Granja Alimentados con una Dieta Obesogénica Durante el Período Peripuberal

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MEJÍA, H. N. G.; CÓRDOVA, I. A.; ARAGÓN, H. J.; FLORES, P. F. I. & PÉREZ-MARTÍNEZ, M. Histomorphology of liver, testicle and body fat in farm rabbits fed with an obesogenic diet during the peripuberal period. ***Int. J. Morphol., 33(4)***:1371-1376, 2015.

SUMMARY: Two groups were formed in order to evaluate the effect of an obesogenic diet on body and liver weight, body fat accumulation and the histometric characteristics of liver and testicles of New Zealand rabbits during the peripuberal period. One group received a standard farm diet and the other received a standard farm diet with canola oil added. During the study, weight of both groups was recorded. At the end of the study, the rabbits were euthanized and liver, testicles, and visceral, scapular and scrotal fat were weighed. Tissue samples were obtained from liver and testicles to carry out histometric analysis. Body weight, body mass index, liver and visceral fat weight were significantly different (P<0.01) in the obesogenic diet when compared to the control group. Notwithstanding, there were no significant differences between both groups in testicular weight, and scapular and scrotal fat (P>0.01). Histometry of the liver of the obesogenic diet group showed the greater total area and greater nucleus area of hepatocytes in the square lobe, right lobe and left lobe medial segment (P<0.01). Histometry of both testicles of individuals from the obesogenic diet group had a greater germinal epithelial thickness and intertubular intersticium (P<0.01). It is concluded that New Zealand male rabbits that received an obesogenic diet during 12 weeks had a higher liver and body weight, as well as, an important increased visceral fat. Furthermore, said diet caused histometric changes in liver and testicles. This set confirms that the domestic rabbit is a good model for the study of the body fat accumulation process associated to the consumption of an obesogenic diet and its effects on liver and testicles.

KEY WORDS: Histometry; Rabbits; Liver; Testicle; Weight; Peripuberal period.

INTRODUCTION

Currently in domestic pet animals, such as the dog and the cat, the disorders related to overweight and obesity states constitute a growing problem in veterinary clinical practices (German, 2006).

Obesity is defined as an excessive accumulation of fatty tissue as a consequence of ingesting a diet with higher caloric content than what is required. The effects on health, associated to the accumulation of adipose tissue, depend on factors such as the breed, age, and time of ingestion of an obesogenic diet (Bach *et al.*, 1996). The increase of visceral adipose tissue is associated to glucose intolerance and insulin resistance, which in turn are directly correlated to the content of triglycerides in the liver (Jakobsen *et al*., 2007; Korenblat *et al*., 2008).

Liver steatosis is the accumulation of lipids in the hepatocyte cytoplasm and it is associated with progressive hepatic deterioration accompanied with inflammation, and chronic stages with different degrees of hepatic fibrosis (Teli *et al*., 1995; Birkner *et al*., 2005).

Some studies have shown that overweight and obesity may be a key factor in infertility etiology (Erdemir *et al*., 2012; Kort *et al*., 2006) and it is related with a reduction of the number of spermatozoa (Hammoud *et al*., 2008).

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Likewise, adipose tissue in the scrotum of obese individuals is accompanied with increased temperature in the scrotum and with oxidative stress; this change in the testicular micro-environment may cause a reduction in spermatogenesis and harm the sperm structure (Kasturi *et al*., 2008). Furthermore, it has been reported that obese individuals have spermatic alterations accompanied with a reduction in fertility and there is evidence that his could be in part due to a higher activity of the aromatase enzyme with the consequent increase in conversion of testosterone into estrogens, this associated to metabolic syndrome (de Boer *et al*., 2005; Kastury *et al*.).

The objectives of this study were to evaluate the effect of an obesogenic diet on liver weight, body fat accumulation, and the histometric characteristics of liver, and testicles in New Zealand rabbits during the peripuberal period.

MATERIAL AND METHOD

Eighteen, clinically healthy, 15 weeks old New Zealand breed males, with an average body weight of 2.8 kg, were used. They were kept under a controlled light-darkness cycle (16 h light : 8 h darkness) in individual metal cages. All efforts were made to minimize both animal suffering and the number of animals used. The study carried out followed Mexican Law for the Protection of wild and domestic animals, and the Mexican Official Standard NOM-062-ZOO-1999. This research study was approved by the Ethical Committee of the Faculty of Veterinary Medicine at UNAM (MC-2013-7). Two groups were randomly established before the beginning of the experiment; both groups had a two week period of acclimatization during which they received a standard farm diet and ad libitum access to water. After two weeks, a group was fed 180 g per day of a finishing stage standard farm diet with 13 ml canola oil added during 12 weeks, and the control group was fed only 180 g per day of a finishing stage standard farm diet during 12 weeks.

Beginning on day "0" of the experiment, until its end, every seven days animal weights were recorded, as well as measurements were taken of height and body length using a flexible metric tape (dates not shown) in order to estimate body mass index, according to the model used for cats (Appleton *et al*., 2001).

At the end of the experiment, the animals in both groups were euthanized with an intravenous overdose of sodium pentobarbital (90 mg/kg) having previously been tranquilized with an intramuscular injection of acepromazine (1 mg/kg). Liver and testicles of each animal were then separated and weighed on a digital scale. Both organs were measured in thickness, length and width with a Vernier caliper. Tissue fragments were obtained and set in a buffered 10% formalin solution for histological study by the paraffin inclusion technique. Visceral fat was separated and weighed (perirenal fat of kidneys, and from the omentum and mesentery), as well as scapular and subcutaneous fat in order to estimate body fat condition. Data are shown as total weight (g) in wet base (Zhao *et al*., 2007). For the histological analysis, 6 to 7 μm thick cuts were performed using and automatic microtome and later stained with the Gomori Trichrome technique.

For the histometric analysis of the testicular tissue an optic microscope was connected to a digital microscopy camera and 10 microscope fields were captured per tissue sample, image analysis software (Motic Images Plus®, version 2.0) was used to measure the germinal epithelium, the intertubular interstitium and the diameter of the seminiferous tubules found in each microscopic field. The 40X objective was used to capture ten microscopic fields of the liver slices to determine total area, and nucleus area of hepatocytes that were peripheral to the central vein and ten hepatocytes peripheral to the portal space of the square lobe, right lobe and left medial lobe.

Data obtained from both groups were compared statistically (obesogenic diet *vs* standard farm diet) for each studied variable. Student's T test was used to compare body weight, liver weight, testicular weight and body fat weight, while the remaining variables under study were compared using Mann Whitney's U test. All results are shown as average ± SEM (Standard Error of Mean).

RESULTS

At the end of the study, animals with the obesogenic diet had a higher body weight (3.51±0.063 kg *vs.* 3.36±0.109 kg) when compared to the control group ($P<0.001$). Regarding body fat and liver weight at the end of the study, the group with the obesogenic diet had greater visceral fat accumulation and higher liver weight when compared to the control group ($P<0.001$). Nevertheless, there was no significant difference in scrotum and scapular fat between both groups ($P>0.05$) (Fig. 1).

The hepatocytes area in the portal space and central vein of the square lobe, in the group with the obesogenic diet, was significantly greater when compared to the group without the oil ($P<0.0001$ and $P<0.001$, respectively). The right hepatic lobe of the group with the obesogenic diet had hepatocytes with a greater total area in the portal space and the central

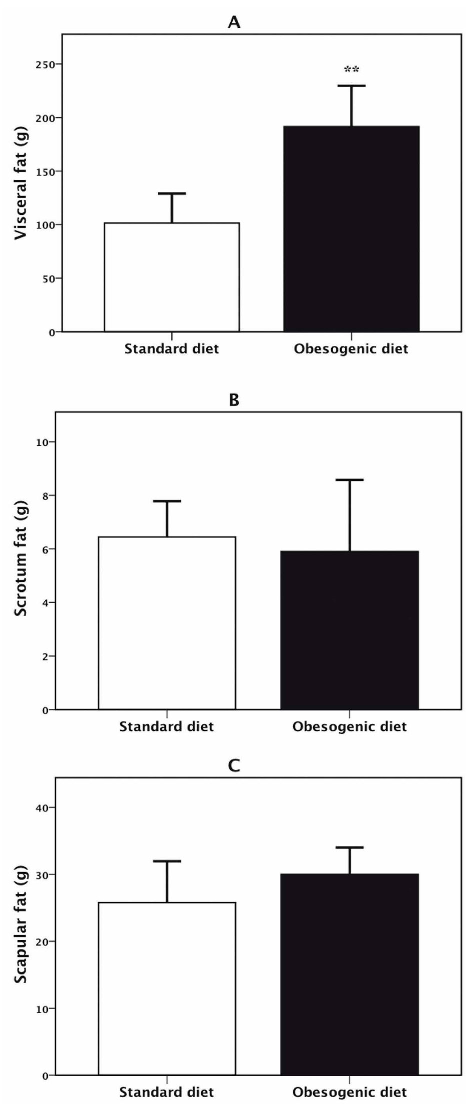

Fig. 1. Weight (average ± SE) of visceral fat (A), scrotum fat (B), and scapular fat (C) of rabbits fed with an obesogenic and a standard diet during 12 weeks. P<0.01 and P<0.001, Student T test n= 9/group. **P<0.001.

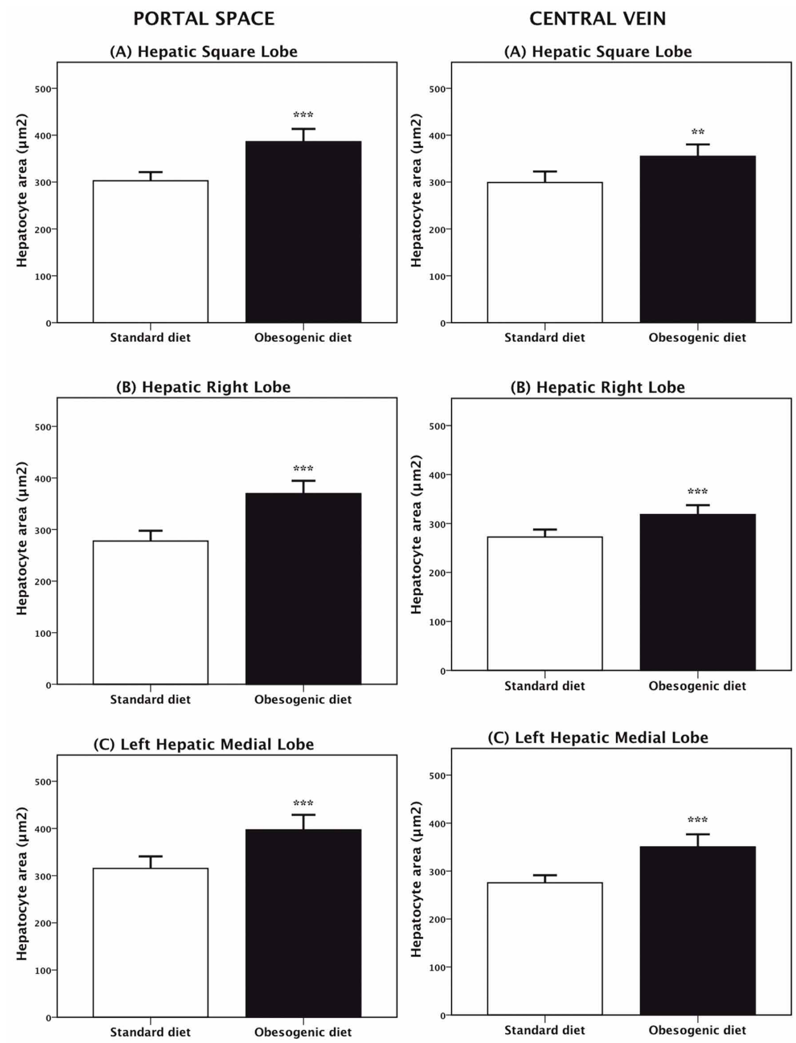

Fig. 2. Total area (average ± SE) of hepatocytes from different liver lobes of rabbits fed with an obesogenic diet during 12 weeks. A) Hepatocytes of the square hepatic lobe B) Hepatocytes of the right hepatic lobe. C) Hepatocytes of the left hepatic medial lobe. P<0.0001, Mann Whitney Test (n= 9/group). **P<0.001, ***P<0.0001.

vein in relation to the control group (P<0.001). Likewise, in the medial left lobe of the group with an obesogenic diet the hepatocytes situated in the portal space and central vein had a greater total area when compared to the control group (P<0.0001) (Fig. 2).

The area of hepatocyte nuclei of hepatocytes near the portal space of the square hepatic lobe, left medial lobe and right lobe were larger in rabbits with the obesogenic diet when compared to the control group (P<0.0001). Also, the area of the nuclei situated on the central vein of the square lobe (P<0.001) and the left medial lobe were greater in the animals with the obesogenic diet (P<0.01) (Fig. 3).

There was no significant difference in the weight of left or right testicles of the group with the obesogenic diet when compared to the control group (P>0.05). The values for the right testicle were: 3.77±0.32 g *vs.* 4.3±0.2 g and for the left testicle were 3.77±0.27 g *vs.* 4.20±0.24 g, respectively.

Furthermore, within the seminiferous epithelium of the area and perimeter of the intertubular interstitium of the testicle, it was found that the length of the seminiferous tubule of the right testicle was smaller in the animals of the obesogenic diet group (P<0.001) while the left testicle was greater in thickness of the seminiferous epithelium, as well as in the area and perimeter of the intertubular interstitium (P<0.0001 and P<0.01) (Fig. 4).

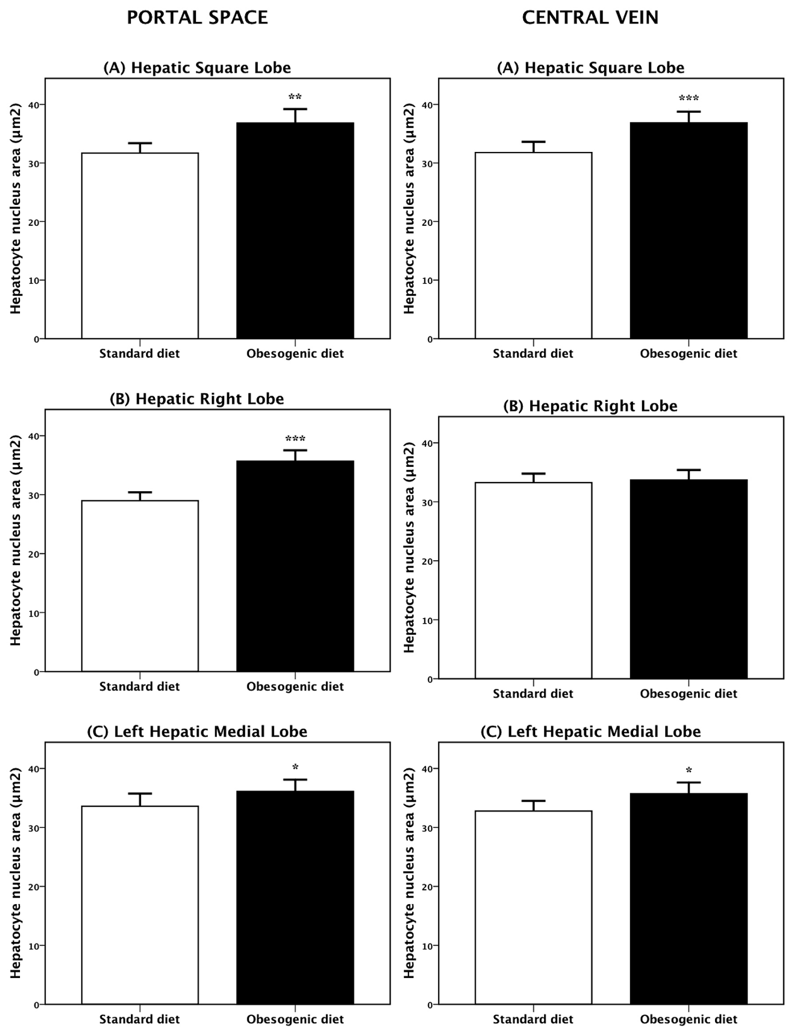

Fig. 3. Hepatocytes nucleus area (average ± SE) of rabbits with an obesogenic diet during 12 weeks. A) Hepatocytes of the square hepatic lobe. B) Hepatocytes of the right hepatic lobe C) Hepatocytes of the left hepatic medial lobe. P<0.0001, Mann Whitney. Test (n= 9/group). *P<0.01, **P<0.001, ***P<0.0001.

DISCUSSION

Some authors have reported that the addition of vegetable oil to rabbit diets in farm conditions improves the digestible energy content of feed as well as feed conversion efficiency (Parigi-Bini *et al*., 1974; Fernandez *et al*., 1994). Likewise, it is known that the incorporation of vegetable oil to the diet improves the digestibility coefficients of protein, fiber and nitrogen free extracts (Brenes Payá *et al*., 1978).

Few studies have analyzed body weight and its relationship with liver weight. In our study, the group that received the obesogenic diet had higher visceral fat (being almost double) and liver fat weights; situation that was not reflected in peripheral fat weight (scapular and scrotal fat); this result agrees with the observations by another author (Jeyakumar *et al*., 2009).

No statistically significant differences were found in testicle weight between the control and treatment groups, which is in agreement with the reports of studies carried out in obese laboratory rodents (Ghanayem *et al*., 2010). Nevertheless, we cannot dismiss the possibility of some effects on testicular weight since we consider that it is necessary to do this evaluation in rabbits fed obesogenic diets during a longer period than that of our study.

The increase in the total hepatocyte area that resulted from the histometric analysis of the liver of the group fed with vegetable oil may be explained in part by the infiltration of lipids into the hepatocytes. We must underline that livers of the animals within this group had more weight. Also, in this group the hepatocyte nuclei had a greater area in the square hepatic lobe, right lobe and left medial lobe. It is known that diets with high levels of saturated fat favor the accumulation of adipose tissue by increasing the number and size of adipocytes.

The seminiferous tubules of the group fed with vegetable oil had greater thickness and area of the germinal epithelium, indicating spermatogenic activity. Other studies have proposed that the histometric parameters evaluated of the seminiferous tubules such as: tubular diameter, seminiferous epithelium thickness and intertubular space maintain a direct relationship with spermatogenic activity (Pérez-Rivero *et al*., 2014).

In conclusion, New Zealand male prepuberal rabbits fed with an obesogenic diet during 12 weeks reached higher body and liver weights, showed increased visceral fat in relation to the control group. Furthermore, histometric changes were found in liver due to the fact that hepatocytes had a greater total area, as well as that of nuclei of hepatocytes within the square lobe, right lobe and left medial lobe, and there was greater thickness of the germinal epithelium and interstitial intertubular perimeter of the testicle. Jointly these results, confirm that the domestic rabbit is a good model for the study of the body fat accumulation process

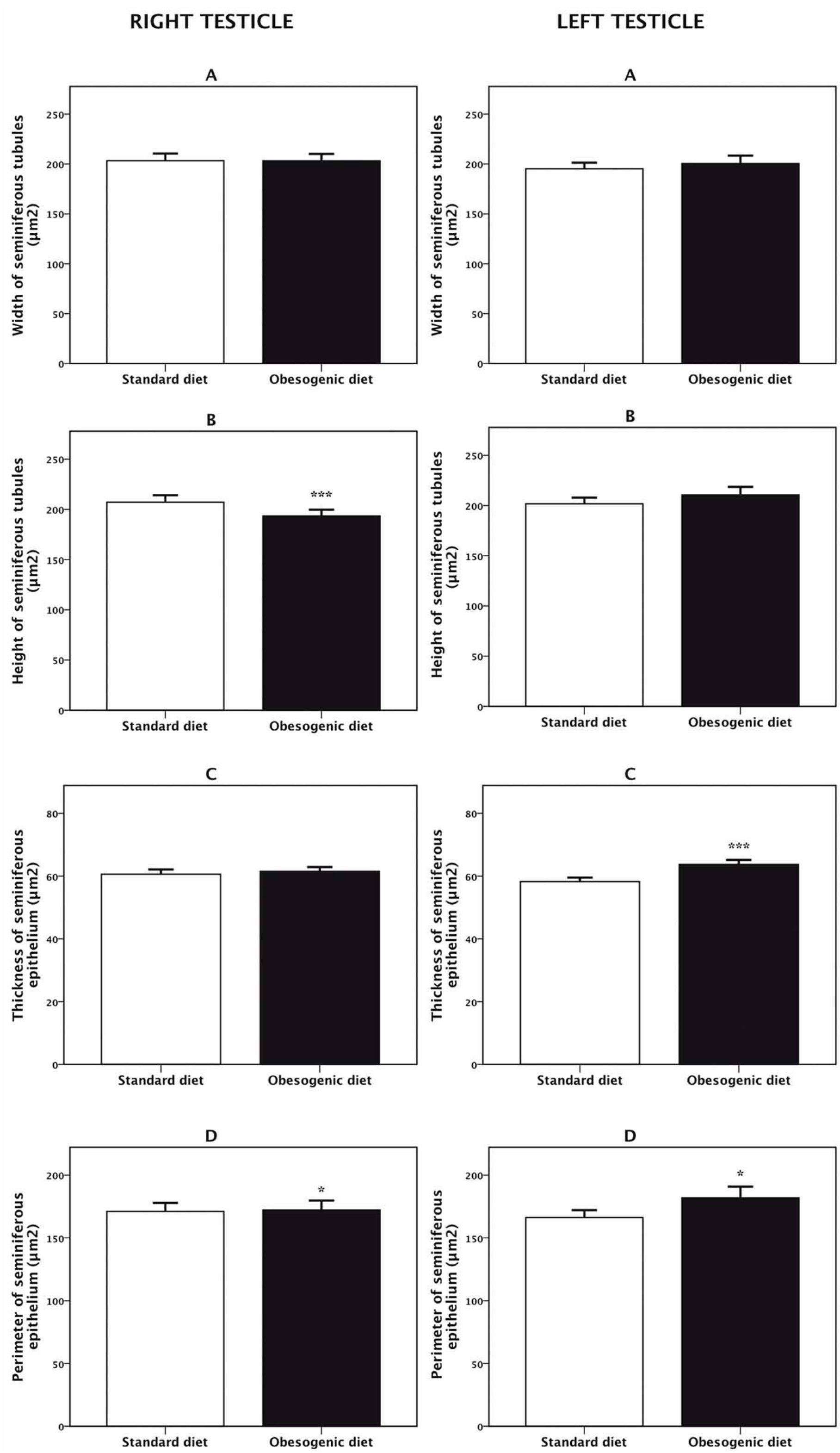

Fig. 4. Histometry (average ± SE) of the right and left testicles of rabbits fed with an obesogenic diet during 12 weeks. A) Width of the seminiferous tubules B) Height of the seminiferous tubules C) Thickness of the seminiferous epithelium D) Perimeter of the intertubular interstiium. P<0.0001, and P<0.01, Mann-Whitney. Test n= 9/group. *P<0.01, ***P<0.0001.

associated to the consumption of an obesogenic diet and its effects at the hepatic and testicular levels. Noteworthy is that at the end of the study the animals of the obesogenic diet group reached a higher body weight when compared to the control group. Nevertheless, currently there are no established criteria for the rabbit model, as it does exist for humans, which could allow us to define with precision at what point the increased weight can be considered as an obesity stage in this domestic animal. Moreover, it is necessary that long term effects be evaluated on the endocrine regulation of the testicle in relation to onset of puberty.

ACKNOWLEDGMENTS

We thank the Technician Francisco Lopez, ascribed to the Morphology Department, FMV-UNAM, for his aid in tissue processing.

MEJÍA, H. N. G.; CÓRDOVA, I. A.; ARAGÓN, H. J.; FLORES, P. F. I. & PÉREZ-MARTÍNEZ, M. Histomorfología del hígado, testículo y grasa corporal en conejos de granja alimentados con una dieta obesogénica durante el período peripuberal. ***Int. J. Morphol., 33(4)***:1371-1376, 2015.

RESUMEN: Con el propósito de evaluar el efecto de una dieta obesogénica sobre el peso corporal, hepático, acumulo de grasa corporal y las características histométricas del hígado y testículo de conejos Nueva Zelanda durante el período peripuberal, se formaron dos grupos; a uno se le proporcionó una dieta estándar de granja y el otro recibió una dieta estándar de granja adicionada con aceite de canola. Durante el estudio se registró el peso corporal de ambos grupos. Al final del estudio los conejos fueron eutanasiados y se pesó el hígado, testículos, la grasa visceral, escapular y escrotal. Se obtuvieron muestras de tejido hepático y testicular para realizar el análisis histométrico. El grupo con dieta obesogénica presentó diferencias significativas con respecto al grupo testigo, en las variables: peso corporal, índice de masa corporal, peso del hígado y de la grasa visceral (P<0,01). Por otra parte, no hubo diferencias significativas entre ambos grupos para las variables: peso de testículos, grasa escapular y escrotal (P>0,01). En la histometría del hígado el grupo con la dieta obesogénica presentó mayor área total y mayor área del núcleo de los hepatocitos en el lóbulo cuadrado, lóbulo derecho y lóbulo izquierdo segmento medial (P<0,01). En la histometría de ambos testículos del grupo con la dieta obesogénica se encontró mayor grosor del epitelio germinal y perímetro del intersticio intertubular (P<0,01). Se concluye que los conejos Nueva Zelanda machos que recibieron una dieta obesogénica durante 12 semanas presentaron mayor peso corporal, hepático y aumento de la grasa visceral. Además, presentaron cambios histométricos en hígado y testículo. Estos resultados en conjunto confirman que el conejo doméstico es un buen modelo para el estudio del proceso de acumulo de grasa corporal asociado al consumo de una dieta obesogénica y de sus efectos en el hígado y testículos.

PALABRAS CLAVE: Histometría; Conejos; Hígado; Testículo; Peso; Período peripuberal.

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Received: 23-03-2015
Accepted: 07-08-2015