Prenatal and/or postnatal high-fat diet alters testicular parameters in adult Wistar Albino rats

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Summary. Here, we evaluated the effects of a high-fat diet during the prenatal and/or postnatal period on the metabolic parameters and testes of 4-month-old Wistar rats. The experimental groups, composed of male Wistar rats, were: C/C (n=8), HF/HF (n=8) (pups with the same diet as their dams, after weaning), C/HF (n=8), and HF/C (n=9) (pups with a different diet after weaning, from that of their dams). The biometric parameters, blood glucose levels, serum levels, the gonadosomatic index, sperm parameters, testes, and genital fat deposits were evaluated. The HDL-c serum levels were significantly lower in the C/HF group (P=0.0100), whereas animals in the HF/C group presented hypertriglyceridemia (P=0.0005). The sperm concentration was lower in the HF/HF group than in the HF/C group (P=0.0072), and sperm viability was lower in all groups receiving a high-fat diet (P<0.0001). The tubular compartment was the smallest in the HF/HF group (P<0.0001). The diameter of the seminiferous tubule was the widest in the HF/C group (P<0.0001). The height of the seminiferous epithelium in all groups was lower than that in the C/C group (P<0.0001). Testosterone and steroidogenic acute regulatory protein (STAR) expression levels were lower in the C/HF group (P=0.0218 and P=0.0215, respectively). The HF diet, regardless of the administration period, induced a limited number of metabolic changes, and modifications in the histoarchitecture of the testes and sperm parameters. These data suggest that a HF diet may cause disturbances in spermatogenesis and fertility impairment in adulthood.

Key words: Prenatal, Postnatal period; High-fat diet, Testes, Rats

Introduction

Changes in the nutritional status during the gestation and/or lactation periods may result in permanent adaptations in the structure, physiology, and metabolism of various organs of the offspring during adulthood (Barker and Osmond, 1986; Rodriguez-Gonzalez et al., 2014). Clinical (Chavarro et al., 2014) and experimental studies (Campos-Silva et al., 2015) have revealed associations between obesity and changes in testicular morphology, as well as impairments in fertility, particularly due to a decreasing sperm quality and quantity. As a result of these findings, experimental models of nutritional programming are widely studied nowadays. Christante et al. (2013) observed that maternal obesity negatively affected gonocyte development and steroidogenesis during the first days of life in rats (Christante et al., 2013).

However, little is known about the implications of an excessive lipid intake during critical periods of development for sperm function and testicular morphology, in the adult offspring. Therefore, this study aimed to evaluate the effects of the administration of a high-fat diet during gestation, lactation, and/or the postnatal period on the glycaemia, lipid profile, testicular morphology, and sperm parameters in the adult

offspring.

Materials and methods

Study design

This research project was approved by the Ethics Committee for the Care and Use of Experimental Animals of the Institute of Biology of the State University of Rio de Janeiro (Protocol No. 0072014), in accordance with the guidelines of the Brazilian College of Animal Experimentation. The animals were kept under controlled temperature and lighting conditions (21±2°C; 12/12 h light/dark cycle) and received water and food *ad libitum*.

Nulliparous 3-month-old female Wistar Albino rats bred in our laboratory were mated overnight. Pregnant rats were divided into two groups (10 per group): control (C) and high-fat (HF) groups that received, a normal lipid diet (17 g of lipids per kg) or a HF diet (49 g of lipids from lard per kg) respectively, during pregnancy and lactation. After birth, the litter size was adjusted to six pups, to standardize the lactation process (Langley-Evans et al., 1996). On weaning, male offspring were monitored until four months of age, after being divided into four groups: C/C (n=8), C/HF (n=8), HF/C (n=9), and HF/HF (n=8), where the first letter corresponds to the maternal diet and the second letter corresponds to the diet of pups after weaning.

The dietary intake of the animals was recorded daily, and all diets were prepared following the recommendations of the American Institute of Nutrition (formulations AIN-93G and AIN-93M) (Reeves et al., 1993) (Table 1). The diets were made by *Pragsoluções*

(Jau, SP, Brazil - www.pragsolucoes.com.br). During the experimental period, body mass was monitored weekly. In addition, at three months of age, the systolic blood pressure of the offspring was measured weekly, using the noninvasive method of tail-cuff plethysmography (Insight, São Paulo, Brazil).

Oral glucose tolerance test (OGTT)

Oral glucose tolerance tests (OGTT) were performed in both dams (before mating and after weaning) and pups (at four months of age). To perform the OGTT, the animals underwent 12 h fasting and received a hypertonic glucose solution (2 g/kg body mass) by orogastric gavage. Blood was collected from the tail vein before glucose administration (time 0) and at 15, 30, 60, and 120 min after the glucose load, and blood glucose was measured using a glucometer (Accu-Chek, Roche, São Paulo, SP, Brazil). The area under the curve (AUC) was measured to assess glucose tolerance.

Sacrifice

After 12 hours of fasting, rats in the C/C, C/HF, HF/C, and HF/HF groups were killed by CO_2 inhalation, at four months of age. Blood samples were collected from the heart (right atrium) by cardiac puncture, for the serum analysis. The genital fat pad and testes were dissected, weighed, fixed (in formaldehyde and/or frozen), and prepared for histomorphometry and western blotting analyses. The gonadosomatic index was calculated by using the following formula: (testicular mass / body mass) × 100 (Uno et al., 2014).

Serum biochemistry and hormone levels

Serum was separated by centrifugation (3000 rpm, for 8 min). The concentrations of total cholesterol (TC), HDL-c, and triacylglycerol (TAG) were quantified using a colorimetric assay (BioSystems, Cat 11506, Barcelona, Spain), whereas the analyses for insulin and testosterone were performed by using available enzyme-linked immunosorbent assay (ELISA) kits: the rat/mouse insulin kit (Millipore, Cat. EZRMI-13K, St Charles, MO, USA) and the general testosterone kit (Enzo, Cat. ADI-900-065, New York, USA), respectively.

Sperm analyzes

The sperm was collected from the tail of the epididymis, and a spermatic solution was prepared to determine the concentration and motility of sperm in a Neubauer chamber. Sperm viability was evaluated by the hypo-osmotic swelling test, and 200 spermatozoids were evaluated per rat (Ribeiro et al., 2014).

Testes histomorphometry

The testes were fixed in Bouin's solution for 24 h,

Table 1. Composition of experimental diets according to the American Institute of Nutrition formulations AIN-93G and AIN-93M (Reeves et al., 1993).

Ingredients (g/Kg)	AIN-93 G		AIN-93 M	
	C	HF	C	HF
Corn starch	539.486	299.486	465.700	192.600
Casein	190.00	230.00	140.00	175.00
Sucrose	100.00	100.00	100.00	100.00
Soybean oil	70.00	70.00	40.00	40.00
Lard	0.00	200.00	0.00	238.00
Fiber	50.00	50.00	50.00	50.00
L-cistin	3.00	3.00	1.80	1.80
Colin	2.50	2.50	2.50	2.50
Antioxidant	0.014	0.014	0.008	0.060
Minerals mixed	35.00	35.00	35.00	35.00
Vitamins mixed	10.00	10.00	10.00	10.00
TOTAL (g)	1000.0	1000.0	1000.0	1000.0
Energy (Kcal/Kg)	3960.00	4960.00	3190.00	4380.00
Carbohydrate (%)	64.00	32.00	76.00	36.00
Protein (%)	19.00	19.00	14.00	14.00
Lipid (%)	17.00	49.00	10.00	50.00

C, control diet; HF, high-fat diet.

and in formaldehyde for 48 h at room temperature, after which they were embedded in paraffin. Subsequently, the material was sectioned at a thickness of 5 μm and stained with hematoxylin and eosin. Digital images were obtained using an Olympus BX51 light microscope with a coupled digital camera (Olympus DP70, Tokyo, Japan). The histomorphometric analysis was performed using the Image J software.

The diameter of the seminiferous tubule and the height of the seminiferous epithelium (25 fields/animal) were determined using the 'straight line' tool. The diameter of the seminiferous tubule was measured using a straight line that passed through the center of the tubule. For this analysis, tubules with an irregular shape were excluded (Ribeiro et al., 2013). For the measurement of the seminiferous epithelium height, we used the same tool and determined the distance from the tunica propria to the inner germinal cell, excluding the spermatozoids (Ribeiro et al., 2013). These analyzes were performed with an objective of 10× (seminiferous tubule diameter) and a 20× (seminiferous epithelium height).

The volume densities of the tubular (seminiferous epithelium, lumen, and tunica propria) and intertubular (interstitial cells, vessels, and nerves) compartments were determined using the 'cell counter' and 'grid' tools. The test grid, containing 100 points, was superimposed over the testicular photomicrographs, and each structure that was touched by a point was counted. These analyzes were performed using a 40× objective (25 fields/animal), and the results were expressed as percentages (De Souza et al., 2012).

The Sertoli cells were quantified by direct counting ('cell counter' tool). This counting was made randomly, disregarding the stage of the seminiferous epithelium cycle. However, only Sertoli cells with a visible nuclear profile were counted (Kotsampasi et al., 2009). For this, we used an immersion oil objective (60×) to evaluate 30 sections of the seminiferous tubules from each animal.

Western blotting

Frozen samples of the testes (100 mg) were homogenized in RIPA lysis buffer containing protease inhibitors. Lysates were centrifuged for 20 min at 4°C, and the supernatants were collected. Equal amounts of total protein were resuspended in sodium dodecyl sulfate (SDS)-containing sample buffer, heated for 5 min at 100°C, and separated by SDS-polyacrylamide gel electrophoresis. After electrophoresis, proteins were electroblotted on a nitrocellulose membrane (GE Healthcare Life Sciences, Little Chalfont, UK). The membrane was blocked, probed with the following primary antibodies (Santa Cruz Biotechnology, Heidelberg, Germany): rabbit anti-STAR - 1:200 (30 kDa, SC-25806) and mouse anti-β-actin - 1:200 (43 kDa, SC-81178), washed, and incubated with anti-rabbit or anti-mouse secondary antibodies, respectively. Protein expression levels were determined using an ECL kit (GE Healthcare Life Sciences, Uppsala, Sweden). Signals were visualized by autoradiography, and the quantification of the bands was carried out by densitometry using the Image J software.

Data analysis

The data were tested for their compatibility with a normal distribution, and reported as the mean ± standard deviation (SD). Differences between groups were assessed using the unpaired Student's t-test and a one-way analysis of variance (ANOVA), with a subsequent *post hoc* Bonferroni test. Differences were considered statistically significant if P≤0.05 (Prism 5.03, GraphPad Software Inc., San Diego, CA, USA).

Table 2. Biometry, food intake, gonad measurement, serum biochemistry, and hormone levels in the experimental groups.

Data	C/C	C/HF	HF/C	HF/HF
Body mass (g)	423.90±48.85	415.90±58.92	421.10±55.58	409.10±58.16
Epididymal fat mass (g)	6.47±2.87	6.35±1.26	4.45±0.20	6.65±2.68
Food intake - g/day/animal (g)	17.35±4.30	15.05±4.56	15.77±5.91	14.78±6.38
Systolic blood pressure (mmHg)	167.00±12.36	160.10±25.00	182.80±14.13	163.60±28.44
Testis mass (g)	1.72±0.10	1.74±0.14	1.76±0.15	1.74±0.18
Gonadosomatic index (%)	0.39±0.03	0.41±0.04	0.42±0.04	0.43±0.03
OGTT (AUC - a.u.)	268986±16385.00	282388±12534.00	251442±19400.00	250158±32170.00
Insulin (ng/mL)	1.11±0.86	1.03±0.60	2.04±0.77	1.69±0.53
Total cholesterol (mg/dL)	54.00±9.63	51.14±4.38	63.75±17.04	53.71±9.21
HDL-c (mg/dL)	41.43±6.24	33.29±2.87[a]	41.57±5.80[b]	35.80±3.49
Triacylglycerols (mg/dL)	92.50±24.00	83.86±13.95	146.80±37.03[a,b]	87.43±20.82[c]
Testosterone (ng/ml)	11.39±2.13	7.85±1.30[a]	9.34±1.69	10.43±0.85

C/C, HF/HF (pups that had the same diet as their dams after weaning), C/HF, HF/C (pups that after weaning had a different diet from that of their dams). Data are expressed as the mean ± SD. Significance was assessed using the analysis of variance (ANOVA) and a subsequent *post hoc* Bonferroni test. [a]: indicates a statistical difference in the C/C group; [b]: indicates a statistical difference in the C/HF group; [c]: indicates a statistical difference in the HF/C group, when P≤0.05.

Results

Maternal data

During gestation and lactation, the gain in body mass for HF dams did not differ from that of the C mothers (P=0.2388). Likewise, both groups were characterized by similar glucose curve AUC values, measured at mating and after weaning (P=0.6276 and P=0.0534, respectively). However, the food intake of C dams was higher than that of HF dams at the same time point (P=0.0163).

### Offspring data

Biometry, food intake, and gonad measurement

Body mass and food intake were similar in the C/C, C/HF, HF/C, and HF/HF groups (P=0.9339; P=0.5747, respectively). The systolic blood pressure of rats in the groups that received the HF diet prenatally and/or postnatally did not differ from that of C/C group (P=0.3554) (Table 2).

Moreover, table 2 shows that no differences were observed in the genital fat pad, testicular mass, and gonadosomatic index, in the different groups (P=0.3144; P=0.9001; P=0.0921, respectively).

Biochemistry and hormone levels

In addition, glucose and insulin levels were similar among the experimental groups (P=0.0576; P=0.0503; P=0.0505, respectively). The concentrations of total cholesterol were similar across all offspring groups (P=0.3616). However, the concentration of HDL-c in the C/HF group was lower than those in the C/C and HF/C
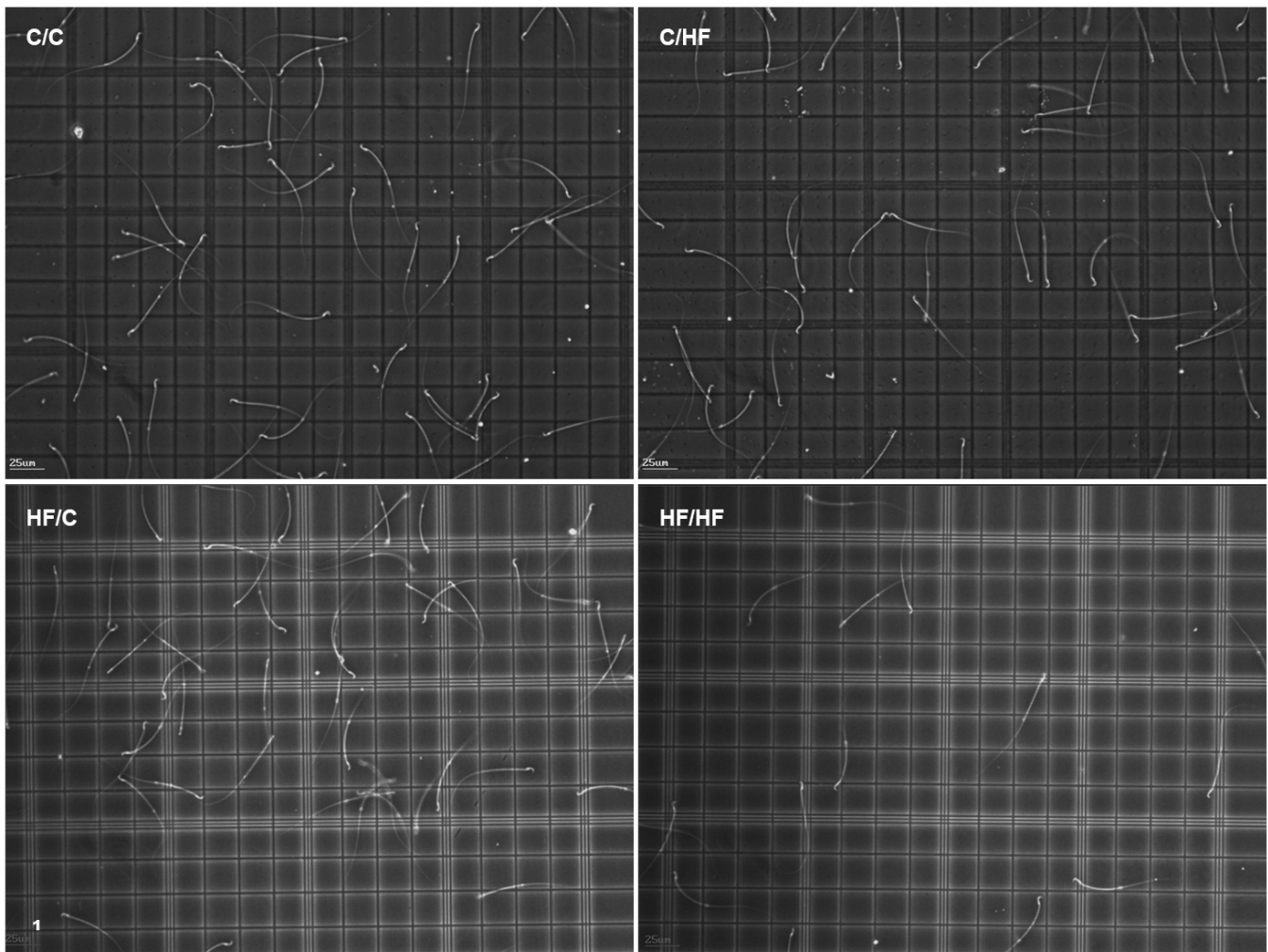
Fig. 1. Sperm concentration. C/C (control/control), C/HF (control/high-fat), HF/C (high-fat/control) and HF/HF (high-fat/high-fat). Data are expressed as the mean ± SD. Significant differences were assessed using the analysis of variance (ANOVA) and a subsequent post hoc Bonferroni test. C/C, C/HF and HF/C, normal production; HF/HF, lower sperm concentration. The figure contains the scale bar of 25 μm generated by the sperm class analyzer (SCA) software. Phase-contrast optics. x 100.

groups (P=0.0100). Furthermore, the TAG level was higher in HF/C rats than in other groups (P=0.0005). In addition, the testosterone level in the C/HF group was lower than that in the C/C group (P=0.0218) (Table 2).

Sperm parameters

The sperm concentration in the HF/HF group was lower than that in the HF/C group by 73% (P=0.0072) (Fig. 1). The viability of sperm in all groups that were exposed in any way to the HF diet was lower than that in the C/C group (C/HF: 28%; HF/C: 49%; HF/HF: 57%). Furthermore, in HF/HF rats, the sperm viability was 39% lower than that in the C/HF group (P<0.0001). Sperm motility values did not differ significantly between the groups (P=0.5217) (Table 3).

Testicular parameters

The volumetric density of the tubular compartment in the HF/HF group was 19% lower than the one measured in other groups (P<0.0001). In the HF/HF group the volumetric density of the intertubular compartment, was increased by 276%, 280%, and 296% compared to the values observed in the C/C, C/HF, and HF/C groups, respectively (P<0.0001) (Table 3).

The diameter of the seminiferous tubule in the HF/C group was wider than in the C/C, C/HF, and HF/HF groups, by 13%, 16%, and 11%, respectively (P <0.0001; Fig. 2). The seminiferous epithelium height in the C/HF, HF/C, and HF/HF groups was 8%, 13%, and 31% lower than that in the C/C group. In addition, the HF/HF group exhibited the lowest seminiferous
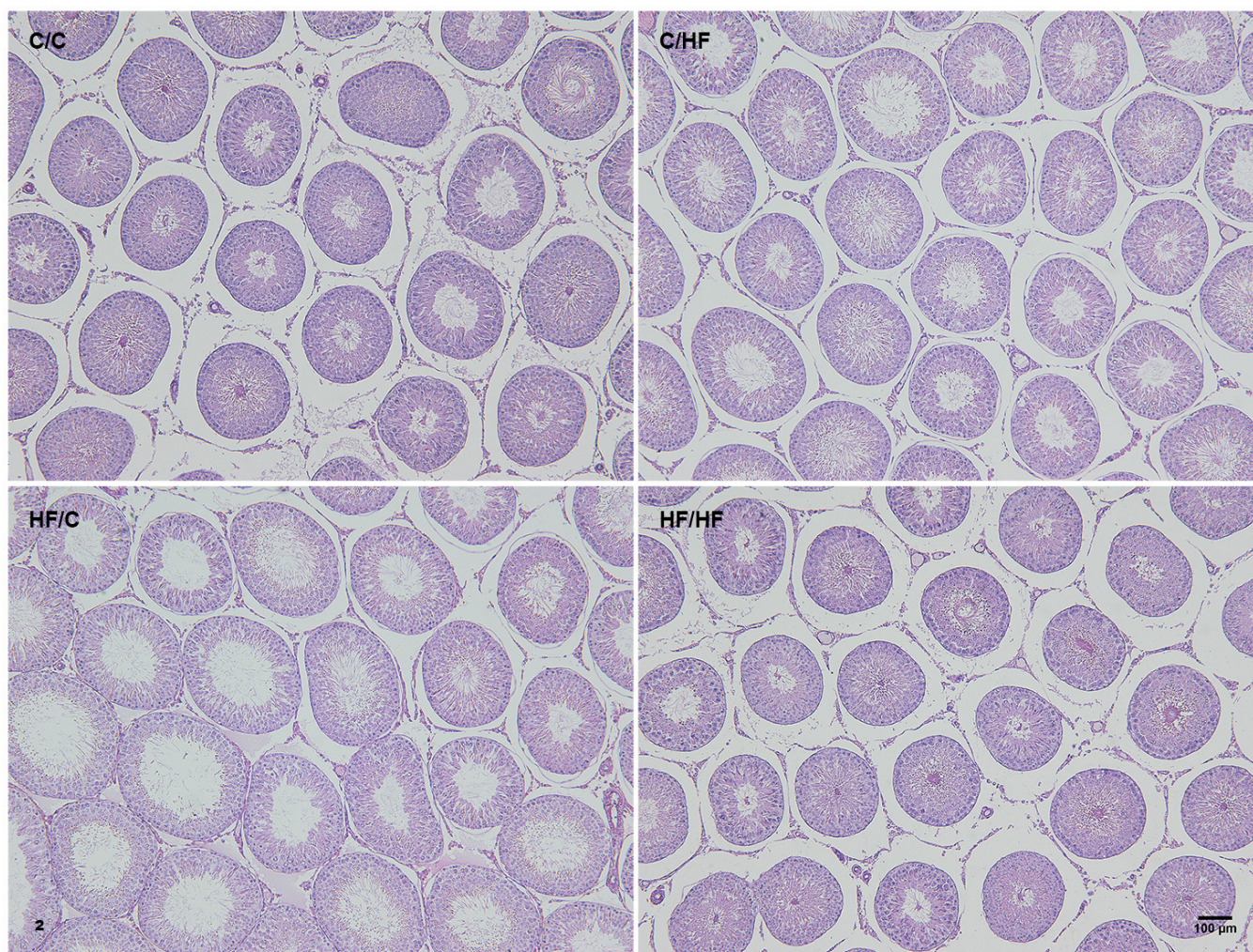
Fig. 2. Seminiferous tubule diameter. C/C (control/control), C/HF (control/high-fat), HF/C (high-fat/control) and HF/HF (high-fat/high-fat). Data are expressed as the mean ± SD. Significant differences were assessed using the analysis of variance (ANOVA) and a subsequent post hoc Bonferroni test. HF/C, testes with the largest seminiferous tubule diameter; the other groups preserved the testis histoarchitecture. H-E staining. x 100.

Table 3. Testicular parameters of the experimental groups.

Data	C/C	C/HF	HF/C	HF/HF
Sperm concentration (sptz/mL)	$1.71\times10^6\pm4.05\times10^5$	$1.29\times10^6\pm6.70\times10^5$	$2.10\times10^6\pm5.61\times10^5$	$5.70\times10^5\pm2.46\times10^{5c}$
Sperm viability (%)	19.57±2.94	14.00±2.37[a]	9.92±1.93[a]	8.50±2.63[a,b]
Sperm motility (%)	47.36±19.09	40.40±14.49	52.35±13.54	52.68±3.68
Tubular compartment Vv (%)	93.97±0.96	94.05±0.93	94.20±0.85	75.91±2.24[a,b,c]
Intertubular compartment Vv (%)	6.14±0.84	6.08±0.94	5.83±0.79	23.09±2.24[a,b,c]
Seminiferous tubule diameter (μm)	262.90±9.83	256.80±13.19	297.60±11.91[a,b]	267.90±8.31[c]
Seminiferous epithelium height (μm)	60.52±3.18	55.68±3.46[a]	52.70±2.37[a]	41.87±2.69[a,b,c]
Sertoli cells no./cross section	21.86±0.98	20.68±0.15	20.86±1.16	20.56±1.07

C/C, HF/HF (pups that had the same diet as their dams after weaning), C/HF, HF/C (pups that after weaning had a different diet from that of their dams). Data are expressed as the mean ± SD. Significance was assessed using the analysis of variance (ANOVA) and a subsequent post hoc Bonferroni test. [a]: indicates a statistical difference in the C/C group; [b]: indicates a statistical difference in the C/HF group; [c]: indicates a statistical difference in the HF/C group, when P≤0.05.
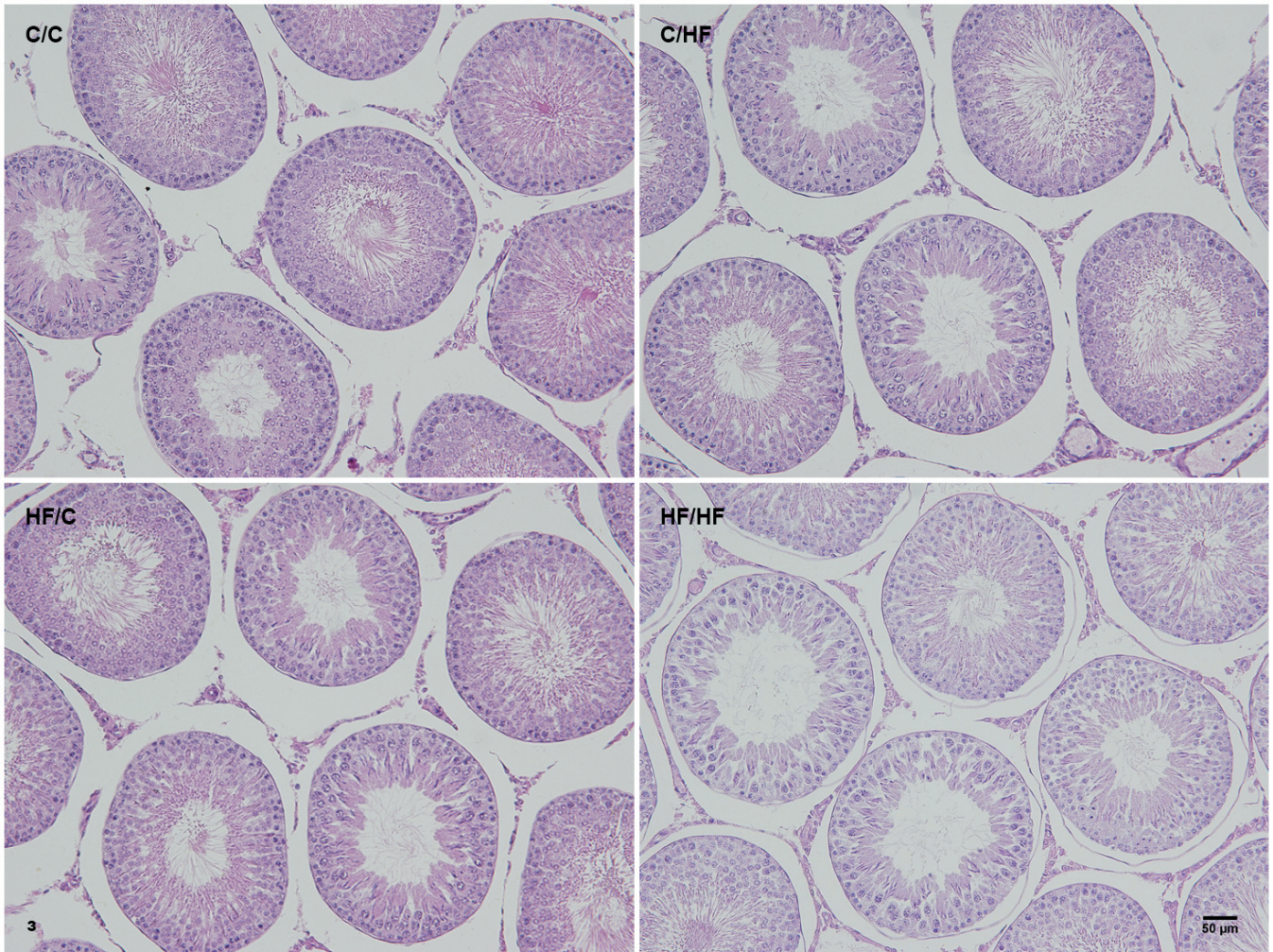
Fig. 3. Seminiferous epithelium height. C/C (control/control), C/HF (control/high-fat), HF/C (high-fat/control) and HF/HF (high-fat/high-fat). Data are expressed as the mean ± SD. Significant differences were assessed using the analysis of variance (ANOVA) and a subsequent post hoc Bonferroni test. C/C, preserved testis histoarchitecture; C/HF, a decreased seminiferous epithelium height; HF/C, a decreased seminiferous epithelium height; HF/HF, the lowest seminiferous epithelium height. H-E staining. x 200.

epithelium height, which was 25% and 21% lower than those in the C/HF and HF/C groups, respectively (P<0.0001; Fig. 3). The number of Sertoli cells in a cross section of the seminiferous tubule was similar among all groups (P=0.0509) (Table 3).

Moreover, the expression level of the STAR protein in C/HF rats was lower than in that in C/C animals (P=0.0215; Fig. 4).

Discussion

Testicular development and spermatogenesis (proliferation phase) begin during the intrauterine period (Dolci et al., 2015). The nutritional status during the critical period of ontogenesis is pivotal for the maturation of offspring organ systems. Our findings suggest that the administration of a HF diet during gestation and lactation does not affect the maternal metabolism. It has been previously noted that this type of diet is not closely correlated with maternal obesity (Gregorio et al., 2010). Similarly, it was observed that the HF diet was not able to alter the body mass and the testicular mass of the offspring. Since food intake was similar among experimental groups, it is believed that the increase in energy following the administration of a HF diet leads to animals that achieve satiety more easily.

Fig. 4. STAR protein expression levels (mean ± SD) and representative protein bands, as determined by western blot. C/C (control/control), C/HF (control/high-fat), HF/C (high-fat/control) and HF/HF (high-fat/high-fat). Significant differences were assessed using the analysis of variance (ANOVA), and a subsequent post hoc Bonferroni test. [a] indicates a statistical difference for the C/C group, when P≤0.05.

Notably, the HF diet reduced the HDL-c plasma levels in the C/HF group and increased the TAG serum levels in the offspring of mothers who had received a HF diet throughout gestation and lactation. Obesogenic diets increase the plasma levels of TC and LDL-c, and decrease the level of HDL-c, a lipoprotein that is fundamentally important in the reverse cholesterol transport (Ramirez and Hu, 2015). It is known that dyslipidemia is linked to infertility (Schisterman et al., 2014). Moreover, experimental studies have shown that hyperlipidemic mice fed a HF diet exhibit histopathological changes in their testes (Zhang et al., 2012).

In addition to hypertriglyceridemia, a prenatal HF diet causes structural changes in the testes, that could compromise sperm production. In animals that had received a prenatal HF diet, the sperm viability and seminiferous epithelium height were decreased, compared to the corresponding parameters in the C/C group. These findings demonstrate the deleterious, but limited effects, of the consumption of a HF diet during gestation and lactation. Experimental (Louei Monfared, 2013) and clinical studies (Ergun et al., 2007) have already shown a negative correlation between TAG levels, on the one hand, and sperm quality and morphometric parameters on the other.

Despite these findings, the mechanism underlying the direct association between serum TAG levels and testicular characteristics remains unclear. The hormone-sensitive lipase (HSL) is responsible for the conversion of cholesterol esters to free cholesterol, as well as for the hydrolysis of TAG leading to the release of fatty acids, which is important for energy production. HSL is ubiquitously expressed in the male reproductive system and may play an important role in the regulation of the pathophysiological processes in the testes (Wang and Xu, 2015). Previous studies have shown that male mice, in which the *Lipe* gene, encoding for HSL, was knocked out, were infertile and presented unique morphological abnormalities such as round, elongating spermatids, deficient spermatogenesis, and azoospermia (Wang et al., 2004, 2014). Although we did not measure HSL levels, the possible reduction in the amount of this enzyme may have negatively influenced testicular morphology and sperm viability.

A HF diet influences the lipid composition of the plasma membrane in animal cells (Perona, 2017) and the excess cholesterol decreases the fluidity of the membrane (De Craene et al., 2017). In this study, we observed that the HF diet, regardless of the period of administration, promoted a reduction in sperm viability, which may suggest disruptions in the functional integrity of the sperm plasma membrane (Ribeiro et al., 2014). The concentration of sperm decreased in the group that had received a HF diet throughout their lives, compared to that in the HF/C group, showing that the consumption of a C diet after weaning minimized the damages caused by the intake of a HF diet during the prenatal period. Sperm motility was not altered by this fetal
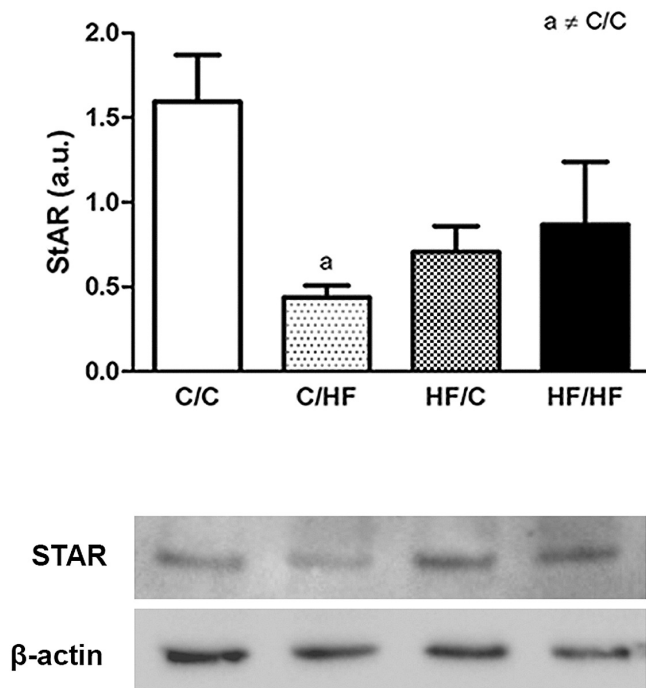
programming model. In support of these results, we observed that the volumetric density of the tubular compartment was reduced in the HF/HF group. The tubular compartment represents the larger part of the testis, occupying 95% of the testicular parenchyma (Morais et al., 2014) and is fundamental for the production of spermatozoa.

Morphometric parameters, such as the diameter of the seminiferous tubule and the height of the seminiferous epithelium are also indicators of the spermatogenic activity (Ribeiro et al., 2014). The diameter of the seminiferous tubules was larger in rats that were exposed to a HF diet during the prenatal period. The high cholesterol levels in the diet can increase lipid peroxidation levels in various tissues, compromising cell integrity (Rani et al., 2016). Therefore, we believe that the lipids passing through the placenta may have caused the sloughing of the seminiferous epithelium, that consequently increased the amount of luminal cellular debris. This condition may have caused the obstruction of the efferent ducts, and impaired the passage of fluid from the testes to the epididymis, thus widening the diameter of the seminiferous tubule (Moffit et al., 2007).

The group that was exposed to a prenatal HF diet also exhibited a reduction in the height of the seminiferous epithelium. Similarly, this parameter was lower in the group that had been fed a HF diet postnatally. The largest decrease was observed in the HF/HF group, which had received a HF diet throughout the life of the rats, confirming the negative influence of a lipid-rich diet on spermatogenesis. Excess cholesterol promotes the rigidity of the plasma membrane and alters its physico-chemical properties (Perona, 2017). However, no data exist on fetal programming using a high cholesterol diet, and its effects on the testicular morphology of adult animals are unknown. It has been reported that maternal obesity alters the development of gonocytes, and decreases sex steroid levels in rat pups, during the first days of life (0.5 to 14.5 days postpartum) (Christante et al., 2013). The gonocytes represent the precursors of male germ cells (spermatogonia), and therefore a reduction in their number may impair sperm production in adult offspring.

With respect to the reduction of the height of the seminiferous epithelium, our work and that of others (Reame et al., 2014) has demonstrated that the administration of a HF diet during the postnatal period negatively affects serum testosterone levels, when compared to those in the C/C group. Testosterone, a hormone produced by Leydig cells, regulates the testicular maturation and differentiation of cells of the spermatogenic lineage, which form the seminiferous epithelium, and are responsible for the production of spermatozoa (Martin, 2016). Although we have not analyzed Leydig cells, it is known that they complete their differentiation at puberty (Teerds and Huhtaniemi, 2015). In this regard, it is possible that a HF diet directly delays the maturation of Leydig cells, while this was not observed when the exposure to excess dietary cholesterol occurred only during the prenatal period.

In agreement with our serum testosterone levels results and data published by Li et al. (2013), the expression of the STAR protein was reduced in the C/HF group. This may be potentially due to the fact that the group that had received the high-fat diet throughout life (HF/HF) had undergone an adaptation process. The predictive adaptive response implies that exposure to insults during the prenatal period causes metabolic adaptations in the offspring, that ensure their survival in similar conditions during the postnatal period (Gallou-Kabani et al., 2007; Bateson et al., 2014).

In the interstitial cells of the testis, the STAR protein is responsible for initiating the conversion of cholesterol to testosterone, and facilitating the transport of free cholesterol to the inner mitochondrial membrane. Subsequently, testosterone is synthesized in a series of steroidogenic steps, catalyzed by different proteins (Manna et al., 2016). Sebokova et al. (1988) observed that dietary lipids can affect the organization of the plasma membrane in the testis. Specifically, when the availability of LH receptors, located in Leydig cells, is altered, testosterone production is compromised (Sebokova et al., 1988).

Sertoli cells are the most resistant structures in the testes and the last ones to be modified when exposed to insults. It is known that alterations in the number of Sertoli cells are rarely observed (Stumpp et al., 2008). Our study is the first to evaluate the effect of fetal programming using a HF diet on the number of Sertoli cells. The number of Sertoli cells did not differ among the tested groups, that may suggest that the exposure time to the HF diet was insufficient to cause changes in this parameter.

In conclusion, a HF diet, regardless of its administration period, promotes a limited number of metabolic changes. However, it alters sperm parameters, namely by reducing sperm concentration and viability. Several important morphological and morphometrical changes were also observed in the testes, where the diameter of the seminiferous tubule widened, whereas the volumetric density of the tubular compartment and the height of the seminiferous epithelium decreased. These data suggest that the consumption of a HF diet may lead to disturbances in spermatogenesis, and impair fertility during adulthood.

References

Barker D.J.P. and Osmond C. (1986). Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. Lancet

1, 1077-1081.

Bateson P., Gluckman P. and Hanson M. (2014). The biology of developmental plasticity and the Predictive Adaptive Response hypothesis. J. Physiol. 592, 2357-2368.

Campos-Silva P., Furriel A., Costa W.S., Sampaio F.J. and Gregorio B.M. (2015). Metabolic and testicular effects of the long-term administration of different high-fat diets in adult rats. Int. Braz. J. Urol. 41, 569-575.

Chavarro J.E., Minguez-Alarcon L., Mendiola J., Cutillas-Tolin A., Lopez-Espin J.J. and Torres-Cantero A.M. (2014). Trans fatty acid intake is inversely related to total sperm count in young healthy men. Hum. Reprod. 29, 429-440.

Christante C.M., Taboga S.R., Pinto-Fochi M.E. and Goes R.M. (2013). Maternal obesity disturbs the postnatal development of gonocytes in the rat without impairment of testis structure at prepubertal age. Reproduction 146, 549-558.

De Craene J.O., Bertazzi D.L., Bär S. and Friant S. (2017). Phosphoinositides, major actors in membrane trafficking and lipid signaling pathways. Int. J. Mol. Sci. 18, 634.

De Souza D.B., Silva D., Cortez C.M., Costa W.S. and Sampaio F.J. (2012). Effects of chronic stress on penile corpus cavernosum of rats. J. Androl. 33, 735-739.

Dolci S., Campolo F. and De Felici M. (2015). Gonadal development and germ cell tumors in mouse and humans. Semin. Cell. Dev. Biol. 45, 114-123.

Ergun A., Kose S.K., Aydos K., Ata A. and Avci A. (2007). Correlation of seminal parameters with serum lipid profile and sex hormones. Arch. Androl. 53, 21-23.

Gallou-Kabani C., Vigé A., Gross M.S., Boileau C., Rabes J.P., Fruchart-Najib J., Jais J.P. and Junien C. (2007). Resistance to high-fat diet in the female progeny of obese mice fed a control diet during the periconceptual, gestation, and lactation periods. Am. J. Physiol. Endocrinol. Metab. 292, E1095-E1100.

Gregorio B.M., Souza-Mello V., Carvalho J.J., Mandarim-de-Lacerda C.A. and Aguila M.B. (2010). Maternal high-fat intake predisposes nonalcoholic fatty liver disease in C57BL/6 offspring. Am. J. Obstet. Gynecol. 203, 495.e1-8.

Kotsampasi B., Balaskas C., Papadomichelakis G. and Chadio S.E. (2009). Reduced Sertoli cell number and altered pituitary responsiveness in male lambs undernourished in utero. Anim. Reprod. Sci. 114, 135-147.

Langley-Evans S.C., Gardner D.S. and Jackson A.A. (1996). Maternal protein restriction influences the programming of the rat hypothalamic-pituitary-adrenal axis. J. Nutr. 126, 1578-1585.

Li Y., Liu L., Wang B., Xiong J., Li Q., Wang J. and Chen D. (2013). Impairment of reproductive function in a male rat model of non-alcoholic fatty liver disease and beneficial effect of N-3 fatty acid supplementation. Toxicol. Lett. 222, 224-232.

Louei Monfared A. (2013). Correlation of serum lipid profile with histological and seminal parameters of testis in the goat. Int. J. Fertil. Steril. 7, 122-129.

Manna P.R., Stetson C.L., Slominski A.T. and Pruitt K. (2016). Role of the steroidogenic acute regulatory protein in health and disease. Endocrine 51, 7-21.

Martin L.J. (2016). Cell interactions and genetic regulation that contribute to testicular Leydig cell development and differentiation. Mol. Reprod. Dev. 83, 470-487.

Moffit J.S., Bryant B.H., Hall S.J. and Boekelheide K. (2007). Dose-dependent effects of sertoli cell toxicants 2,5-hexanedione, carbendazim, and mono-(2-ethylhexyl) phthalate in adult rat testis. Toxicol. Pathol. 35, 719-727.

Morais A.C., Balarini M.K., Lopes E.O., Menezes T.P., Quintela F.M., Morais D.B., Gomes Mde.L. and Matta S.L. (2014). The tubular compartment and the spermatogenic dynamics of the wild rodent *Oxymycterus nasutus* (Rodentia: Cricetidae). Anim. Reprod. Sci. 149, 249-258.

Perona J.S. (2017). Membrane lipid alterations in the metabolic syndrome and the role of dietary oils. Biochim. Biophys. Acta. 1859, 1690-1703.

Ramirez A. and Hu P.P. (2015). Low high-density lipoprotein and risk of myocardial infarction. Clin. Med. Insights. Cardiol. 9, 113-117.

Rani V., Deep G., Singh R.K., Palle K. and Yadav U.C. (2016). Oxidative stress and metabolic disorders: Pathogenesis and therapeutic strategies. Life. Sci. 148, 183-193.

Reame V., Pytlowanciv E.Z., Ribeiro D.L., Pissolato T.F., Taboga S.R., Goes R.M. and Pinto-Fochi M.E. (2014). Obesogenic environment by excess of dietary fats in different phases of development reduces spermatic efficiency of Wistar rats at adulthood: correlations with metabolic status. Biol. Reprod. 91, 151.

Reeves P.G., Nielsen F.H. and Fahey G.C. Jr. (1993). AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J. Nutr. 123, 1939-1951.

Ribeiro C.T., De Souza D.B., Medeiros J.L. Jr., Costa W.S., Pereira-Sampaio M.A. and Sampaio F.J. (2013). Pneumoperitoneum induces morphological alterations in the rat testicle. Acta. Cirurg. Bras. 28, 419-422.

Ribeiro C.T., Milhomem R., De Souza D.B., Costa W.S., Sampaio F.J. and Pereira-Sampaio M.A. (2014). Effect of antioxidants on outcome of testicular torsion in rats of different ages. J. Urol. 191, 1578-1584.

Rodriguez-Gonzalez G.L., Reyes-Castro L.A., Vega C.C., Boeck L., Ibanez C., Nathanielsz P.W., Larrea F. and Zambrano E. (2014). Accelerated aging of reproductive capacity in male rat offspring of protein-restricted mothers is associated with increased testicular and sperm oxidative stress. Age (Dordr) 36, 9721.

Schisterman E.F., Mumford S.L., Chen Z., Browne R.W., Boyd Barr D., Kim S. and Buck Louis G.M. (2014). Lipid concentrations and semen quality: the LIFE study. Andrology 2, 408-415.

Sebokova E., Garg M.L. and Clandinin M.T. (1988). Modulation of receptor-mediated gonadotropin action in rat testes by dietary fat. Am. J. Physiol. 254, E708-E712.

Stumpp T., Freymuller E. and Miraglia S.M. (2008). Sertoli cell morphological alterations in albino rats treated with etoposide during prepubertal phase. Microsc. Microanal. 14, 225-235.

Teerds K.J. and Huhtaniemi I.T. (2015). Morphological and functional maturation of Leydig cells: from rodent models to primates. Hum. Reprod. Update. 21, 310-328.

Uno T., Kato T., Seki Y., Kawakami E. and Hayama S. (2014). Methods of evaluating the spermatogenic ability of male raccoons (Procyon lotor). J. Reprod. Dev. 60, 421-425.

Wang W.Y. and Xu G.H. (2015). The integration and regulation of hormone-sensitive lipase in reproductive system. Sheng. Li. Ke. Xue. Jin. Zhan. 46, 1-5 (in chinese).

Wang S.P., Chung S., Soni K., Bourdages H., Hermo L., Trasler J. and Mitchell G.A. (2004). Expression of human hormone-sensitive lipase

(HSL) in postmeiotic germ cells confers normal fertility to HSL-deficient mice. Endocrinology 145, 5688-5693.

Wang S.P., Wu J.W., Bourdages H., Lefebvre J.F., Casavant S., Leavitt B.R., Labuda D., Trasler J., Smith C.E., Hermo L. and Mitchell G.A. (2014). The catalytic function of hormone-sensitive lipase is essential for fertility in male mice. Endocrinology. 155, 3047-3053.

Zhang K., Lv Z., Jia X. and Huang D. (2012). Melatonin prevents testicular damage in hyperlipidaemic mice. Andrologia 44, 230-236.