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COMPARISON OF THE SPERMATOGENIC PROCESS IN THREE DIFFERENT MICE STRAINS: SWISS, BALB/C AND C57BL/6

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Abstract

Many studies have been trying to establish standard protocols for animal experimentation, especially for animal species or strains, to master research variables with high precision. The main mouse strains used in the field of the biology of reproduction are Swiss, Balb/c, and C57BL/6. Since some of the strains show reproduction limitations, such as the size of the litter, the present study aimed to compare their spermatogenic processes to verify differences regarding the testicular parenchyma and germ cell populations, which could explain low offspring production. In addition, the present study provides additional information concerning the testicular parenchyma of such strains, which consequently would help researchers to choose the most suitable strain for reproductive studies. Six adult male mice were used for each of the strains. After euthanasia, the testes were weighed, fixated with Karnovsky fixative, embedded in methacrylate, sectioned, and stained with toluidine blue/sodium borate 1%. Morphometrical analyses from the testicular parenchyma (seminiferous tubules and interstitium) were made using the software ImageJ. Germ and Sertoli cells populations were counted in seminiferous tubules cross-sections at stage I of the seminiferous epithelium cycle. The lowest body and testicular weights were observed in C57BL/6 animals, followed by Balb/c and Swiss, however, the relative testes, parenchyma, and albuginea weights were significantly lower only in C57BL/6. Despite the seminiferous tubules and seminiferous epithelium proportions were lower in Swiss animals, their relative amount related to the body weight was the same among strains. The total number of germ cells was higher in Swiss animals, reflecting higher spermatogenic yield and daily sperm production. Due to the lower relative number of Sertoli cells, the Swiss animals showed the highest Sertoli cell index and support capacity. On the other hand, the lowest pathological indexes regarding the germ cells were observed in Balb/c animals, followed by Swiss and C57BL/6. In the interstitium, the proportion of blood vessels was lower in Swiss mice, while the lymphatic cell proportion was lower in C57BL/6 animals. Moreover, the highest proportions of Leydig cells and macrophages were noticed in Swiss mice, which may indicate increased testosterone levels. Altogether, such observations must be taken into account when choosing any of the studied strains for reproduction studies.

Keywords: Germ Cells. Male Reproduction. Morphometry. Spermatogenesis. Testis.

1. Introduction

The use of animal models for experimentation was responsible to improve methods of prevention, diagnosis, and treatment (Green 1986; Fernandes et al. 2001; Almeida et al. 2008). Initially, anatomy and physiology studies involved dissection for analysis of organs and system functions. Over time, the use of non-human animals in research has significantly increased, allowing scientific advances such as the description of vital mechanisms, especially in medicine and physiology (Simmons 2004). In order to, many studies have been trying to establish standard protocols, from chemical reagents to animal species or strains, to master the research variables with high precision, avoiding misinterpretation of the results, and thus increasing reliability (Spinelli et al. 2012; Oliveira 2014).

One of the fundamental issues of the scientific method is the reproducibility of the results. Therefore, there is a need to establish the concept of the animal model, which would be the animal that best responds to the experimental approach as well as its reliable reproduction so that any researcher could have access to the same results (Maia et al. 2006). Therefore, the animal species must be capable of providing the appropriate answer to that specific experiment (Braga 2017).

The main mouse strains used in several study fields, including biology of reproduction, are C57BL/6, Swiss, and Balb/c. The C57BL/6 strain shows specific characteristics that are a crucial point in the choice of the strain, as it presents hematopoietic stem cells with delayed senescence in comparison to Balb/c, its low tumor susceptibility, low bone density, high susceptibility to the development of obesity, type 2 diabetes and atherosclerosis induction (Johnston et al. 2006; Watanabe et al. 2017). Balb/c mice, also classified as inbred, show genetic uniformity, making the response between animals similar (Magalhães 2012). However, both strains have reproductive limitations, generating a low number of animals per offspring when compared to other strains, as Swiss mice for instance. The Swiss strain is heterogeneous, representing natural populations, having excellent reproductive performance with high fertility, prolificity, and productivity (Magalhães 2012).

Even though they are widely used for studies in the field of reproductive biology, few of them considered the testicular changes inherent to such strains. Hence, in the present study, we aimed to compare the testicular parenchyma of Swiss, C57BL/6, and Balb/c mice, describing in detail their spermatogenic process using morphometric and stereological tools, to provide important data that would help researchers to choose the best strain in the future reproduction studies.

2. Material and Methods

Animals

Adult male mice (70 days old) from the Swiss (n = 5), C57BL/6 (n = 5), and Balb/c^{-} (n = 5) strains were housed in cages at 22±2ºC, with a 12h:12h day: night photoperiod and access to water and food *ad libitum*. The experimental protocol was approved and carried out at the Experimental Bioterium of the Federal University of Viçosa (UFV), following the rules and protocols of the UFV Ethics Committee (protocol No. 706/2017).

Light microscopy evaluation

Animals were weighed and the euthanasia occurred after administration of thiopental (i.p. 30 mg/kg). The organs were perfused with saline solution (NaCl 0.9%) through the ascending aorta, for 2 minutes. The testicles were removed, dissected, weighed, and fixed in Karnovsky solution (glutaraldehyde 4% + paraformaldehyde 2.5% in 0.01M sodium cacodylate buffer, pH 7.4) (Karnovsky 1965) for 24h. The organs were dehydrated in ethanol and embedded in methacrylate (Historesin, Leica). Semi-serial sections (3µm thick) were prepared in a microtome (RM 2255, Leica), respecting the minimum interval of 39µm between sections. Histological slides were stained with toluidine blue/sodium borate 1%. For morphometrical and stereological analysis, digital images were taken in a light field photomicroscope (Olympus BX -53) equipped with a digital camera (Olympus AX 70 TRF). All images were analyzed using the Image J® program (National Institute of Health, USA).

Biometric and testicular evaluations

The testis were weighed on a precision scale (0.0001g). The testicular parenchyma weight was calculated by the testicular weight minus the albuginea weight. Based on the body and testicular weights, the gonadosomatic index (GSI, %) was calculated from the division of the total testicular weight by body weight, multiplied by 100 (Amann 1970). The parenchymosomatic index (PSI) was determined by dividing the weight of the testicular parenchyma by body weight, multiplied by 100.

Testicular morphometry and stereology: Seminiferous tubules

The volumetric proportion (%) of seminiferous tubules was estimated from the count of 266 points in 10 random fields of the histological slides, totaling 2,660 points for each animal. The volume (mL) of each testicular component was estimated considering the percentage occupied by each constituent multiplied by the volume of the testicular parenchyma. Since the density of the testis of mammals is around 1 (Johnson et al. 1981) the mass of the testis was considered equal to its volume. The tubulesomatic index (TSI) was calculated after dividing the volume of the seminiferous tubule by the body weight, and then multiplied by 100 (Amann 1970). The epitheliumsomatic index (ESI) was obtained after dividing the volume of the seminiferous epithelium divided by the body weight, multiplied by 100. The average tubular diameter per animal was obtained by randomly measuring 20 seminiferous tubules cross-sections, as circular as possible, without considering the seminiferous epithelium stage (França and Cardoso 1998). The same sections were used to measure the tubular diameter and the height of the seminiferous epithelium. The tubular (STAr), luminal (LAr) and epithelial (EAr) areas were calculated according to the formulas: $STAr = \pi TR^2$ (TR =tubular radius); $LAr = \pi LR^2$ (LR= luminal radius); EAr = STAr - LAr. The epithelium-tubule ratio (ETR) was calculated by the ratio EAr/STAr.

The total length of seminiferous tubules (STL) was estimated based on their volume within the testicular parenchyma, and on the mean tubule diameter (Attal 1963; Dorst 1974). The total tubule length per gram of testis was calculated dividing the STL by the testis' weight.

Testicular morphometry and stereology: Germ and Sertoli cells count

To evaluate the populations of germ cell types located in the seminiferous epithelium in stage I of the seminiferous epithelium cycle (Swierstra 1968; Amann and Schanbacher 1983), twenty tubule cross-sections were used for each animal to count the following cell types: type A spermatogonia (SGA); spermatocytes I at preleptotene/leptotene (PL/L), spermatocytes I at pachytene (P), round spermatids (RS), and Sertoli cells (SC). The obtained results were corrected for the nuclear/nucleolar diameter and the thickness of the histological section according to Amann and Almquist (1962). The diameters of 30 nuclei/nucleoli of the mentioned cell types were measured for each animal. Furthermore, to evaluate the efficiency of the spermatogenic process and the support capacity of the Sertoli cells, the following ratios were calculated: efficiency of the mitotic process ((PL/L)/SGA); spermatogenesis yield (RS/SGA), meiotic index (RS/P), Sertoli cell index (RS/S), total support capacity of the Sertoli cell (SGA+PL/L+P+RS/S).

The number of Sertoli cells was estimated from the corrected number of Sertoli nucleoli and the total length of seminiferous tubules per testis (Hochereau-de-Reviers and Lincoln 1978). From this calculation, the number of Sertoli cells per gram of testis was estimated.

The daily sperm production (DSP) was estimated using the seminiferous tubule volume, the number of round spermatids, the seminiferous tubule cross-sectional area in stage 1, the duration of the cycle of the seminiferous epithelium, and the thickness of the histological section according to Amann (1970). The relative frequency of the stages of the seminiferous epithelium cycle (SEC) was determined by counting 200 random tubules cross-sections per animal. SEC stages were characterized by the method of tubular morphology (Berndtson 1977).

Testicular morphometry and stereology: Histopathology

In an attempt to estimate the proportions of normal and pathological seminiferous tubules, 200 tubules were randomly analyzed per animal. Tubules with pathologies were considered to be those that showed any alteration according to Russell et al. (1990). Changes in seminiferous tubules were classified according to the modified score by Dias et al. (2019) as described below:

Level 1: intact tubule, with germ cells arranged in their normal location with few vacuoles;
Level 2: vacuoles at the base of the epithelium;
Level 3: vacuoles at the apex of the epithelium;
Level 4: vacuoles at the base and apex of the epithelium;
Level 5: sperm cells inside the lumen and presence of cells in the process of degeneration;
Level 6: tubules showing only basal cells;
Level 7: tubules showing only Sertoli cells;
Level 8: empty tubules, characterizing an irreversible state of degeneration.

Testicular morphometry and stereology: Interstitium

In order to calculate the volumetric proportion occupied by the interstitium, 1000 intersection points of a square grid placed on digital images of the testicular parenchyma (100X magnification) were counted, per animal. The number of points coincident with the interstitium was divided by 1000 and multiplied by 100. Furthermore, the volumetric proportions of the following interstitium components were evaluated: Leydig cells, macrophages, blood vessels, connective tissue and lymphatic space. To do so, a square grid was placed on digital images of the interstitium, at 400X magnification. A total of 1000 intersection points were counted for each animal, considering those matching the components previously mentioned.

The volume of each component (mL) was estimated by multiplying the percentage it occupies within the testis multiplied by the parenchyma weight, divided by 100. The calculation of the proportion occupied by the elements of the interstitium was possible by multiplying the volumetric proportion of the interstitium by the volumetric proportion of the element, divided by 100.

Testicular morphometry and stereology: Leydig cells

The mean nuclear diameter of 30 Leydig cells (LC), per animal, was measured at 400X magnification. The roundest nuclei were used, allowing to calculate the nuclear volume by the sphere formula: $4/3\pi R^3$ (R= nuclear diameter/2). The cytoplasmic volume was calculated using the formula: *% cytoplasm x nuclear volume/% nucleus*. The whole LC volume was the sum of nuclear and cytoplasmic volumes.

If the volume of the interstitium along with the volume of one Leydig cell are known, as well as the proportion occupied by the Leydig cells within the interstitium, thus the total volume occupied by the Leydig cells could be inferred. The following formulas were used to calculate the volume occupied by the LC within the testis: 1. *LC volume (per testis) = LC proportion in the testis x testicular parenchyma weight / 100*; 2. *LC volume (per gram of testis) = LC volume (per testis) / testicular parenchyma weight*.

Therefore, the number of Leydig cells per testis could be calculated by dividing the total volume of the Leydig cell per testis by the volume of one Leydig cell. On the other hand, to calculate the number of Leydig cells per gram of testis, the volume that the Leydig cell occupies per gram of testis must be divided by the volume of one Leydig cell.

Statistics

The studied parameters were compared by using analysis of variance (ANOVA) followed by the Student Newman-Keuls test. Pearson's correlation was used to assess the relationship between two variables. The software Statistica for Windows3.11 was used, with a significance level of $p < 0.05$.

The Principal Component Analysis (PCA) was performed to identify possible clusters, to eliminate redundancies and to define the most important variables in the separation of groups. Data were transformed (varying) for standardization due to different magnitudes of scale and level of importance of each variable that was determined by the values of the auto vectors (MCgarigal et al. 2000), with substantial correlation

values shown for each attribute in principal components (PC). The level of importance of each PC was determined by the Broken-stick method, where the values exceeded the expected were kept for interpretation. PCA was performed using the software Fitopac 2.1.2.85 (Shepherd 2010). All analyzes were performed using the Genes software (Cruz 2008).

3. Results and Discussion

Morphometry

The lowest body weight was observed in C57BL/6 animals, followed by Balb/c and Swiss. Furthermore, the testes weight, the GSI, the parenchyma weight and the albuginea weight were significantly lower in the C57BL/6 animals. Nevertheless, the PSI did not vary among groups (Figure 1). The GSI can be used as an indication of alterations in the testicular parenchyma composition, since it expresses the body's investment in gonads (Berndtson 1977). The testicular parenchyma contains the seminiferous tubules and the interstitium compartments, which in turn are responsible for the organ's functions. Alterations in such compartments could imply modifications of the spermatogenic process as a whole.

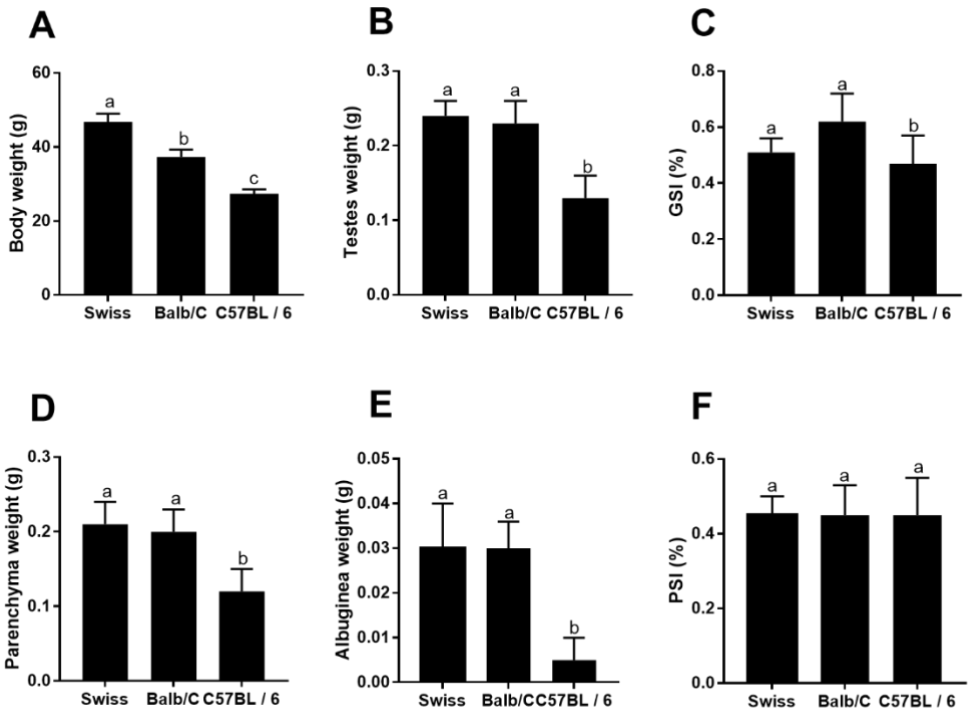

Figure 1. Body and testicular analysis of adult male Swiss, C57BL/6 and BALB/C mice. A – body weight difference among strains; B – testis weight is lower in C57BL/6 mice; C – the relative testis weight (GSI) is lower in C57BL/6 mice; D – parenchyma weight is lower in C57BL/6 mice; E – albuginea weight is lower in C57BL/6 due to smaller testes in the strain; F – the relative parenchyma weight (PSI) was not different among groups. GSI: gonadosomatic index. PSI: parenchymosomatic index. Values are mean ± SD. Different superscripts = $p<0.05$ (ANOVA and Student Newman-Keuls test).

Seminiferous tubules

Swiss mice showed lower seminiferous tubules proportions compared to the other strains, however the lower tubular volume was observed in C57BL/6, due to the diminished size of the testes in such strain. The seminiferous epithelium followed the same pattern (Table 1).

In addition to the lower epithelium height, the lower proportions of seminiferous tubules, seminiferous epithelium and tunica propria observed in Swiss and C57BL/6 animals (Table 1) would indicate diminished germ and Sertoli cell's populations, leading to lower spermatogenic yield, since the seminiferous tubules are the sites where spermatogenesis takes place (Russell et al. 1990; França and Russell 1998).

Nonetheless, the Swiss mice showed the highest germ cell's population, which explains the highest daily sperm production and spermatogenic yield in the strain (Table 2). Moreover, the highest germ cell's numbers along with the lower relative SC number per testis increased the SC cell capacity when compared with Balb/c and C57BL/6 animals (Table 2). Although no differences were observed in the tubule and epithelial areas among strains, the highest luminal area was observed in C57BL/6 mice, as well as the epithelial/tubule ratio. Such relation aims to normalize the amount of epithelium within the tubule, and by doing so, it is possible to indicate which group would display higher amounts of epithelium, where germ and Sertoli cells are located. Despite the differences observed for the overall seminiferous tubule proportions, the tubulesomatic and epithelium somatic indexes were not different between strains. Those indexes are used to provide information regarding the amount of seminiferous tubule and epithelium are allocated in body weight (Dias et al. 2019).

The number of spermatogonia, primary spermatocytes and Sertoli cells (SC) were not different between strains per tubule cross section. The most important variation occurred in the round spermatid's population, which was lower in C57BL/6 and Balb/c strains, reflecting directly on the total germ cell population, the spermatogenic yield and daily sperm production. In addition, the Sertoli cell's index and support capacity were lower in the same strains (Table 2). From all strains, C57BL/6 animals showed the higher proportions of seminiferous tubules pathologies (Figure 2), which could also interfere in the spermatogenesis process, reducing the germ cell population and the fertility rate of the strain as previously showed (Russell et al. 1990).

Table 1. Seminiferous tubules morphometry and stereology.

Parameters	Swiss	Balb/c	C57BL/6
Seminiferous tubules (%)	90.37 ± 1.78^b	91.79 ± 1.58^a	92.63 ± 0.71^a
Seminiferous epithelium (%)	81.19 ± 2.03^b	85.22 ± 1.16^a	81.44 ± 1.12^b
Tunica propria (%)	1.75 ± 0.30^b	2.64 ± 0.34^a	2.39 ± 0.51^a
Lumen (%)	7.44 ± 1.44^a	3.93 ± 0.69^b	8.80 ± 1.52^a
Seminiferous tubules (mL)	0.19 ± 0.02^a	0.19 ± 0.03^a	0.12 ± 0.03^b
Seminiferous epithelium (mL)	0.17 ± 0.02^a	0.17 ± 0.03^a	0.10 ± 0.03^b
Tunica propria (mL)	0.0037 ± 0.0008^b	0.0054 ± 0.0013^a	0.0029 ± 0.0007^b
Lumen (mL)	0.0156 ± 0.0028^a	0.0080 ± 0.0017^b	0.0109 ± 0.0035^b
Tubule morphometry			
Tubule diameter (µm)	184.36 ± 19.00^a	206.54 ± 11.35^a	201.57 ± 10.56^a
Epithelium height (µm)	68.99 ± 6.00^b	84.38 ± 2.32^a	72.36 ± 1.15^b
Lumen diameter (µm)	46.38 ± 11.32^b	37.79 ± 6.87^b	56.85 ± 11.51^a
Tubular area (µm^2)	26908.64 ± 5496.99^a	33569.15 ± 3740.24^a	31966.56 ± 3313.31^a
Epithelial area (µm^2)	25139.74 ± 4797.66^a	32418.60 ± 3306.92^a	29346.23 ± 2363.31^a
Luminal area (µm^2)	1768.91 ± 934.06^a	1150.55 ± 439.89^a	2620.34 ± 971.82^b
ESI (x10^{-3} %)	0.04 ± 0.005^a	0.05 ± 0.01^a	0.04 ± 0.008^a
TSI (%)	0.40 ± 0.05^a	0.50 ± 0.08^a	0.41 ± 0.09^a
STL/testis (m)	1768.9 ± 934.06^a	1150.55 ± 439.89^a	2620.3 ± 971.82^b
STL/g of testis (m)	25140.0 ± 4797.66^b	32418.60 ± 3306.92^a	29346.0 ± 2363.31^b
ETR	0.94 ± 0.02^b	0.97 ± 0.01^a	0.92 ± 0.02^b

ESI = epitheliumsomatic index, TSI = tubulesomatic index, ETR = epithelium-tubule ratio, STL= seminiferous tubules length. Values are mean ± SD. Different superscripts = $p<0.05$ (ANOVA and Student Newman-Keuls test).

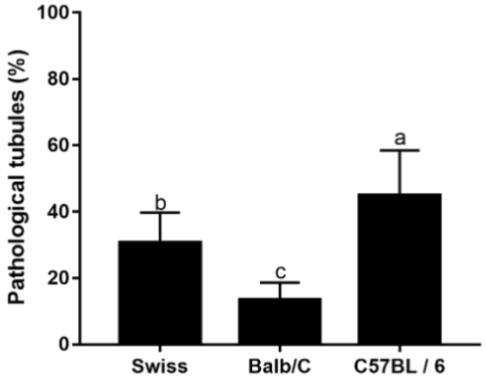

Figure 2. Proportion of pathological seminiferous tubules. Different letters ($p<0.05$).

Table 2. Germ and Sertoli cell's counts (per tubule cross section) and spermatogenic indexes.

Parameters	Swiss	Balb/c	C57BL/6
Spermatogonia	0.86 ± 0.19^{a}	1.09 ± 0.10^{a}	0.92 ± 0.38^{a}
PL/L	21.06 ± 2.47^{a}	20.49 ± 1.52^{a}	20.01 ± 2.76^{a}
P	20.18 ± 1.68^{a}	18.13 ± 1.08^{a}	20.32 ± 2.73^{a}
Round spermatid	60.28 ± 2.90^{a}	47.69 ± 6.93^{b}	49.35 ± 5.78^{b}
Germ Cells	102.37 ± 6.74^{a}	90.62 ± 9.87^{b}	87.41 ± 2.88^{b}
Sertoli cell	5.02 ± 0.42^{a}	5.27 ± 0.30^{a}	6.16 ± 1.20^{a}
Mitotic index	25.79 ± 6.58^{a}	18.92 ± 2.44^{a}	23.50 ± 7.92^{a}
Meiotic index	3.03 ± 0.14^{a}	2.64 ± 0.44^{a}	2.44 ± 0.28^{a}
SC index	12.04 ± 0.53^{a}	9.02 ± 0.86^{b}	8.22 ± 2.00^{b}
SC support capacity	20.41 ± 0.51^{a}	15.04 ± 2.70^{b}	16.57 ± 0.19^{b}
SC/testis ($x10^6$)	49.44 ± 11.38^{c}	53.65 ± 8.57^{b}	85.425 ± 10.55^{a}
SC/g testis ($x10^6$)	205.92 ± 42.19^{b}	379.63 ± 104.07^{a}	422.53 ± 59.22^{a}
Spermatogenic yield	73.77 ± 16.35^{a}	44.23 ± 6.30^{b}	58.80 ± 23.22^{b}
DSP/testis ($x10^6$)	22.71 ± 6.71^{a}	10.16 ± 2.98^{b}	13.15 ± 2.83^{b}
DSP/g testis ($x10^6$)	5.71 ± 1.96^{a}	1.36 ± 0.49^{b}	1.68 ± 0.40^{b}

PL/L = primary spermatocyte in pre leptotene/leptotene, P = primary spermatocyte in pachytene, SC= Sertoli cell, DSP= daily sperm production Values are mean ± SD. Different superscripts = $p<0.05$ (ANOVA and Student Newman-Keuls test).

Interstitium

The interstitium comprises the endocrine compartment that contains Leydig cells, the site of testosterone biosynthesis; macrophages, the endothelium, which is the distal aspect of the testicular circulation, and the lymphatic space that surrounds the cellular components of the interstitium (Hales 2002). They play an important role in keeping the organ's homeostasis, maintaining cells such as the macrophages and the Leydig cells that act together as important players in the production and secretion of several cytokines and testosterone. Testosterone and its derivatives are essential to maintain the spermatogenic process, culminating in germ cells proliferation (Smith and Walker 2014).

Macrophages and Leydig cells are closely related to each other (Hales 2002). There are many locally produced factors that are important to the control of spermatogenesis, and testicular macrophages are potential sources for several growth and differentiation factors. Their close developmental and physical association with Leydig cells would allow them to provide some of the regulatory factors that govern Leydig cell proliferation and differentiation (Hales et al. 1999). In this regard, the present results showed that the highest volumes and proportions of Leydig cells and macrophages were observed in Swiss animals (Table 3), as well as the amount of Leydig cell's cytoplasm, which may be related to increased testosterone levels and to the higher number of spermatids, thus the daily sperm production (Souza et al. 2018). Furthermore, the

stereological and morphometrical results for Leydig cells in Balb/c and C57BL/6 mice indicated that even with similar LSI and Leydig cell's numbers (Table 4) the spermatogenic process was somehow less effective in such strains.

Principal Component Analysis

The total data variation was 58.20%, with the most important attributes in distinguishing the group having correlation values > 0.6 (Figure 2). In Axis 1 (horizontal axis), for body weight (horizontal axis) the most relevant attributes and their respective correlation values were: albuginea weight (0.4071), GSI (0.4380), lumen diameter (-0.5900), epithelium (%) (0.4272), lumen (%) (-0.6626), TSI (0.4504), ESI (0.4901), PSI (0.4259), luminal area (-0.6693), ETR (0.4609), lymphatic space (%) (0.6297), Sertoli/testis (0.5855) and Sertoli/g testis (0.5189). The separation of the Swiss strains for the C57BL/6 and Balb/c is shown in Figure 3.

In axis 2 (vertical axis), the treatments were separated mainly by body weight (0.4459), tubule diameter (-0.4847), lumen diameter (-0.4839), tubules (%) (- 0.4888), tunica propria (%) (- 0.4909), tubule area (-0.4991), luminal area (-0.5370), epithelial area (-0.4787), Leydig volume/testis (0.4002), Sertoli (-0.5114), Spermatogenic yield (0.6629), Spermatogenic yield/g (0.4537) and DSP/testis (0.6031). The separation of the C57BL/6 and Balb/c strains from each other is evident. Such difference is giving according to the ESI, PSI, LSI, and to the number of Sertoli and Leydig cells and testicular biometric parameters.

Table 3. Morphometry and stereology of the interstitium components.

Volumetric proportions	Swiss	Balb/c	C57BL/6
Interstitium (%)	9.63 ± 1.78^a	8.07 ± 0.76^a	7.37 ± 0.71^a
Blood vessel	0.29 ± 0.38^b	0.70 ± 0.14^a	0.70 ± 0.22^a
Lymphatic space	0.50 ± 0.32^a	1.18 ± 0.58^a	0.28 ± 0.41^b
Leydig cell	7.75 ± 0.18^a	5.73 ± 1.18^b	5.93 ± 0.33^b
Leydig cell nucleus	1.02 ± 1.18^a	0.86 ± 0.32^a	0.95 ± 0.17^a
Leydig cell cytoplasm	6.73 ± 1.48^a	4.87 ± 0.88^b	4.98 ± 0.44^b
Connective tissue	0.22 ± 0.43^a	0.43 ± 0.26^a	0.35 ± 0.20^a
Macrophage	0.87 ± 0.43^a	0.03 ± 0.0007^b	0.11 ± 0.06^b
Interstitium	0.020 ± 0.003^a	0.016 ± 0.002^b	0.009 ± 0.002^c
Blood vessel	0.0005 ± 0.0007^b	0.0013 ± 0.0004^a	0.0009 ± 0.0003^a
Lymphatic space	0.00105 ± 0.0007 ^{b}	0.00243 ± 0.0012^a	0.00037 ± 0.0006^b
Leydig cell	0.01619 ± 0.0027^a	0.01111 ± 0.0019^b	0.00741 ± 0.0019^c
Connective tissue	0.00046 ± 0.0004^a	0.00077 ± 0.0004^a	0.00045 ± 0.0003^a
Macrophage	0.001875 ± 0.00116^a	0.000003 ± 0.000006^b	0.000144 ± 0.00009^b

Values are mean ± SD. Different superscripts = p<0.05 (ANOVA and Student Newman-Keuls test).

Table 4. Leydig cell's morphometry.

Parameters	Swiss	Balb/c	C57BL/6
Nucleus diameter (µm)	6.48 ± 0.46^a	6.56 ± 0.33^a	5.73 ± 0.22^a
Nucleus volume (µm^3)	144.15 ± 30.72^a	148.74 ± 22.49^a	98.58 ± 11.54^b
Cytoplasmic volume	992.24 ± 309.23^a	882.29 ± 204.95^a	539.53 ± 159.98^b
Leydig cell volume	1136.4 ± 334.37^a	1031.03 ± 223.34^a	638.12 ± 0.0002^b
LC number/t (x10^6)	14.31 ± 3.21^b	15.82 ± 3.53^a	12.68 ± 6.28^b
LC number/gt (x10^6)	62.48 ± 13.93^a	52.40 ± 23.19^a	67.70 ± 32.64^a
LSI (%)	0.03462 ± 0.0006^a	0.03 ± 0.01^a	0.0269 ± 0.0006^a

LC = Leydig cell, LSI = leydigsomatic index. Values are mean ± SD. Different superscripts = p<0.05 (ANOVA and Student Newman-Keuls test).

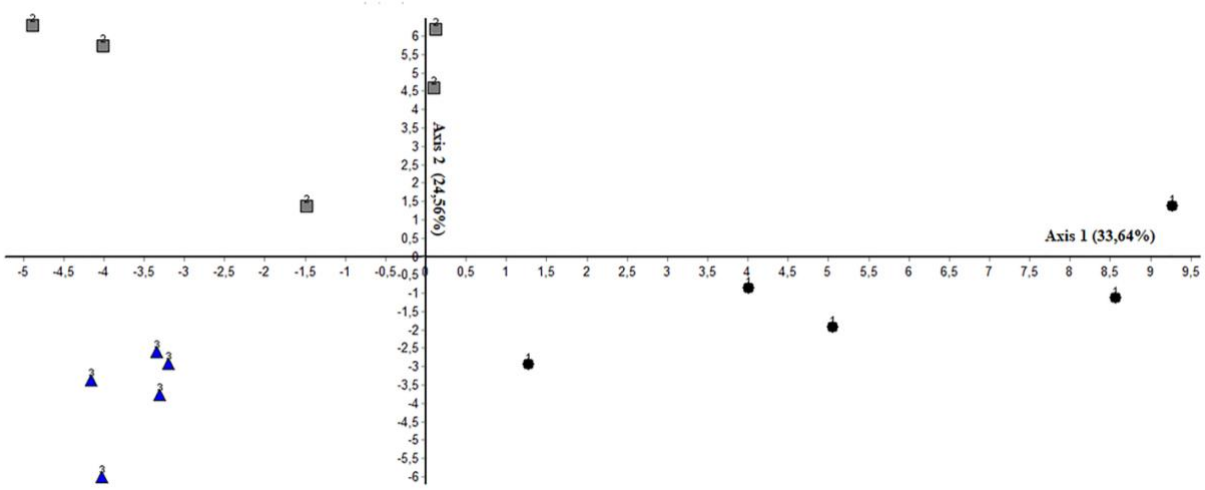

Figure 3. Principal Component Analysis (PCA). (Circle) Swiss, (Square) Balb/c e (Triangle) C57BL/6.

4. Conclusions

The results showed that the most effective spermatogenic process is seen in the Swiss animals with the highest yield, daily sperm production and germ cells counting. Moreover, the volumetric proportions of Leydig cells and macrophages were higher than the observed for Balb/c and C57BL/6 strains, which may indicate higher testosterone production. In addition, C57BL/6 animals showed the lowest germ cell counting and the highest proportions of pathological tubules, which must be considered when choosing such strain to reproduction studies. Considering the PCA analysis and the variables used, all strains are separated and different from one another.

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