Absence of effect of scrotal length and width over litter size in Wistar rats

D.C.P. Marçal[1], L.G. Gaya[2,4], A.P. Madureira[3], G. Tarôco[1], D.R. Rezende[3], I.C. Santos[3]

[1]Animal Science Undergraduate Program, Federal University of São João del-Rei, MG, Brazil.
[2]Animal Science Department, Federal University of São João del-Rei, São João del-Rei, MG, Brazil.
[3]Biosystems Engineering Departament, Federal University of São João del-Rei, São João del-Rei, MG, Brazil.

Abstract

The increase of the bioterium's production capacity and the quality of its products are a necessity due to the biological experimentation requirements. The identification of efficient selection criteria for the rodents could contribute to production optimization. An important trait for Wistar rats is litter size, over which there may be interference of sires' scrotal measurements. Thus, the aim of this study was to evaluate the effect of scrotal length and width over litter size in Wistar rats. The measurements of the scrotal length and width of 27 sires at 60 days of age were made aiming at evaluating such effects over litter size at birth, by means of a nonparametric evaluation of the data. The correlations among these variables were also estimated. There was no significant effect of scrotal length and width, season at calving and dam age at calving over the variable litter size at birth ($P > 0.05$). The correlations obtained also did not indicate any association between litter size and the scrotal size of rats. We conclude that the selection of Wistar sires for scrotal size at 60 days of age is not an efficient strategy in order to improve litter size in the studied population. These results indicate that other factors may be involved in the variability in litter size at birth in this colony.

Keywords: animal laboratory science, fertility, reproduction, selection, testis.

Introduction

The Wistar line of *Rattus norvegicus* is the most consistently internationally used rodent by research centers which carry on animal experimentation (Festing, 1987) and their husbandry tools may be used as resources for the improvement of rodent production. Among them is Animal Breeding, which consists primarily of the application of genetic theories and statistical analysis of data collected in animals to evaluate the variability of the population and strategies of selection and mating (Pereira, 1999; Olesen *et al*., 2000). In heterogeneous strains, that is, where there is genetic variability, selection criteria that are related to traits of interest may be established, mainly involving the reproductive aspects of the animals.

Reproductive efficiency is fundamental in raising colonies for laboratory animals in order to maintain a constant production, and attention should be given to litter size at birth. It is clear, therefore, the need to investigate and detect the variability of reproductive traits in Wistar rats, as well as identify the factors associated with these traits, such as scrotal size, in an attempt to obtain an *in vivo* non-invasive perception of the volume of testicular parenchyma. This may be an indication of the testicular development and consequently of the profile of the semen and has been used in veterinary and human medicine (Amann, 2010; Spears *et al*., 2013).

This can make the establishment of breeding strategies in heterogeneous strains of laboratory rodents more efficient, and, therefore, the reproduction of individuals that tend to maximize the reproductive indices may be favored. Increasing these rates, we can reduce the number of sires maintained in the bioterium and optimize the production of animals, providing greater efficiency in the production system (White, 2000). In other species of animal husbandry interest (e.g. cattle, dogs and horses) reproductive ability, which can be evaluated through the scrotal size, is a major factor for improving production indices (Thompson *et al*., 1979; Olar *et al*., 1983; Nadarajah *et al*., 1988; Van Melis *et al*., 2010). Studies on this theme are rare in the literature about rats.

Thus, the aim of this study was to evaluate the effect of scrotal length and width over litter size at birth in a colony of heterogenic Wistar rats, with the purpose of evaluating the efficiency of scrotal size as selection criteria in the population studied aiming to increase its reproductive indexes.

Materials and Methods

This study was approved by the Ethics Committee on Animal Use of Federal University of São João del-Rei, MG, Brazil (protocol number 04/2009, approved on December 14th, 2009), under the criteria established by the Brazilian Federal Law number 11,794 (October 8th, 2008). Initially 35 sire Wistar Hannover rats (*Rattus norvegicus*) were used, obtained from the Reproduction Biology Center of Federal University of Juiz de Fora, MG, Brazil, and raised in the Central Bioterium Facility of the Federal University of São João del-Rei, MG, Brazil under controlled temperature environment (between 18 and 22°C) and 12 h light cycle. Water and r

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[4]Corresponding author: lggaya@yahoo.com.br
Phone/Fax: +55(32)3373-3975

laboratory rodent feed were offered *ad libitum* during the experimental period. The cages were cleaned and the bed and water were changed on alternate days. Animals were handled by the same handler during the entire experimental period. Between the months of March and December 2010, data regarding husbandry bookkeeping of the breeding colony were recorded, including animal identification, birth date, calving date, age at calving, litter size at birth and scrotal length and width. During the study period, each sire was kept with one dam, considering only the data from the first calving of each dam under mating. Measurements of scrotal width and length were made at 60 days of age. The variable analyzed was litter size at birth (LSB), which corresponded to the number of animals born per couple in the first calving. Records from couples who did not have offspring during the period of data collection were removed, so that out the 35 couples, only 27 were used for analysis.

The descriptive statistics (number of observations, mean, standard deviation, median and minimum and maximum values) of the variable litter size at birth was calculated through the Statistical Analysis System® software, version 9.2, 1999. Using the same software, we proceeded with the normality test of Kolmogorov-Smirnov for this variable, observing that there was no normal distribution of data (P < 0.01). Thus, non-parametric analysis of data was performed by the Kruskal-Wallis test, using the statistical significance level of 5%. This method is recommended for models with more than two classes of possible significant effects. The sources of variation tested for LS, individually, in the non-parametric models were:

- Scrotal length, measured in cm, using a measuring tape, turned into classes (LEN) according to the distribution of their frequencies. Class 1 was composed of scrotal lengths between 2 and 3 cm, class 2 consisted of animals that showed scrotal

length between 3.1 and 3.3 cm, and class 3 was composed of scrotal lengths between 3.4 and 3.7 cm;

- Scrotal width, measured in cm, using a measuring tape, turned into classes (WID) according to the distribution of their frequencies. Class 1 was composed of animals with scrotal width between 3 and 4 cm, class 2 comprised animals with scrotal width between 4.1 and 4.3 cm and class 3 corresponded to scrotal widths ranging from 4.4 to 4.8 cm;

- Dam age at calving (DAC), corresponding to the age of the dam at birth, in days, and in classes, assigning class 1 for dams who gave birth with ages below or equal to 80 days and class 2 for dams calving at an age above 80 days;

- Season at calving (SEC), corresponding to the season in which calving took place, assigning values 1 for fall, 2 for winter and 3 for spring. There were no calvings in the summer.

The descriptive statistics for litter size at birth, scrotal length and scrotal width were calculated (number of observations, mean, standard deviation, median and minimum and maximum values) using the Statistical software Statistical Analysis System®. Spearman correlations among litter size at birth, scrotal length and scrotal width were also calculated at a level of statistical significance of 5%.

Results

Table 1 presents the descriptive statistics of the variables litter size at birth (LSB) and its possible co-variables scrotal length and width. Table 2 presents the results for the analysis of variance for the variable size of litter at birth.

There was no statistical significance for the sources of variation LEN, WID, SEC and DAC on the variable LSB, since the P values were greater than 5% for all models tested (Table 2).

Table 1. Number of observations (n), mean (M), standard deviation (SD), median (MED) and minimum (MIN) and maximum (MAX) for the variables and possible co-variables studied.

Variable	n	M	SD	MED	MIN	MAX
LSB	27	11.39	3.14	12.00	2.00	16.00
LEN (cm)	27	3.11	0.52	3.20	2.00	3.70
WID (cm)	27	4.00	0.53	4.20	3.00	4.80

LSB= litter size at birth; LEN= scrotal length; WID= scrotal width.

Table 2. Variance analysis obtained by the Kruskal-Wallis Test for the variable litter size at birth.

	Sources of variation										
	LEN			WID			SEC			DAC	
Levels for sources of variation	1	2	3	1	2	3	1	2	3	1	2
n	10	11	6	11	8	8	13	8	6	19	8
Mean per level of source of variation	10.12	11.02	13.03	11.78	11.27	11.13	11.30	11.87	11.00	11.37	11.41
Value of P		0.9559			0.5053			0.9652			0.9855	

n= number of observations per class; LEN= class of scrotal length; WID= class of scrotal width; SEC= season at calving; DAC= dam age at calving.

Table 3 presents Spearman's phenotypic correlations between litter size at birth, scrotal length and width. Litter size was not significantly correlated with scrotal measurements (P > 0.05). However, we obtained a significant correlation between the scrotal measurements (P < 0.01), of 0.41.

Table 3. Spearman correlations among Litter Size at Birth (LSB), Scrotal Length (LEN) and Scrotal Width (WID).

	LSB	LEN
LEN	-0.07 (P = 0.721)	-
WID	-0.21 (P = 0.269)	0.41 (P = 0.026)

Discussion

Both dam age and season at calving were not identified in this study as source of variation that could significantly affect litter size at birth. Thus, the lack of seasonality on fertility of these animals was possibly because they are raised in a controlled environment and unaware of changes in temperature, humidity and food supply. There was no relationship between scrotal size of sires and reproductive efficiency of Wistar rats, represented by the initial size of the litter which differed from those previously reported in cattle, dogs and horses (Thompson *et al*., 1979; Olar *et al*., 1983; Nadarajah *et al*., 1988; Van Melis *et al*., 2010).The latter argue that testis size can be an important parameter to predict sperm production and reproductive potential of individuals, that is, the number of progenies obtained. We conclude that the selection criterium potentially efficient for some species of economic interest, as previously mentioned, cannot be extended to all other species. Hence, phenotypic selection of Wistar sires by scrotal size at 60 days of age is not an efficient strategy in order to increment litter size in the population studied. Therefore, the selection criteria and objectives to be used for the laboratory rodents should be previously researched and validated.

Amman (1986) reports differences among species for the relation between daily sperm production and testis weight, emphasizing the high sperm production efficiency of rats, for which there is an excess of sperm in the ejaculate and 90% of reduction of the sperm volume would not be able to affect fertility. Therefore, the selection of bigger testes, that presupposes a higher production and quality of semen and indirectly an improvement in fertility, is not valid for rats. This species has a high reproductive efficiency, even with a low sperm production and/or small testicular parenchyma, which could explain the absence of effects of the scrotal size over the litter size in our study. Additionally, although the scrotal width seems to be a reliable predictor of testicular weight in mice (Spears *et al*., 2013), Amann (2010) affirms that the testis size does not significantly influence the variation in daily sperm production and consequently could not contribute expressively to the fertility efficiency, which is corroborated by our results.

Although the effects evaluated in this study were not significant for litter size at birth, other effects not considered in this study may have caused changes in the fertility of rats. Dams and sire genotypes could potentially had caused variation in the fertility of couples, since it has been reported in literature the action of genes involved in fertility and reproductive ability, comprising several cellular functions and hormonal mechanisms (Amann, 1986). Therefore, further studies to identify these sources of variation, possibly involving genetic data, are recommended in an attempt to identify effective selection criteria for the increase in litter size at birth in this population.

Acknowledgments

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