GENETICS

Genetic Variability of Spermatozoon Production and Morphology in Laboratory Mice

L. V. Osadchuk and A. V. Osadchuk

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Strain-specific differences in the production of spermatozoa and incidence of atypical spermatogenesis were studied in males of 7 inbred strains: BALB/cLac, PT, CBA/Lac, DD, A/He, C57Bl/6J, and YT. The results attest to significant genetic variability of the counts of epididymal spermatozoa (differing by more than 1.5-2 times) and incidence of abnormal spermatozoon heads (differing by more than 4 times). However, the strain-specific variability of both signs was not coordinated. The selected set of inbred mouse strains can become a prospective genetic model for studies of physiological and hereditary nature of spermatogenesis variability.

Key Words: *spermatogenesis; inbred mouse strains; genetic variability*

The factors essential for male fertility are studied mainly on genetically or pharmacologically modified models or reproductive abnormalities and the studies are mainly aimed at detection of the causes of male sterility and its correction. Natural variability of male fertility in the natural animal populations and in humans received less attention [4,8]. This variability can be due to environmental factors or genetic polymorphism. The collection of inbred mouse strains presents an informative picture of natural hereditary variability of male fertility, because inbred strains differ significantly by various functional markers of spermatogenesis. The collection of inbred mice used in our study is characterized by significant variability of male reproductive function parameters not associated with serious disorders in fertility or with sterility and hence, it reflects the natural variability of male fertility in various populations.

Laboratory of Endocrinological Genetics, Institute of Cytology and Genetics, Siberian Division of the Russian Academy of Sciences, Novosibirsk, Russia. ***Address for correspondence:*** losadch@bionet.nsc.ru. L. V. Osadchuk

The aim of this study was to detect strain-specific differences in the main characteristics of male fertility, such as production of epididymal spermatozoa and incidence of their atypical forms in 7 inbred mouse strains.

MATERIALS AND METHODS

The study was carried out on 104 naive adult inbred males of strains BALB/cLac, PT, CBA/Lac, DD, A/He, C57Bl/6J, and YT aged 90-110 days. All animals were bred under standard vivarium conditions at Institute of Cytology and Genetics at natural light with free access to water and food. The progeny was separated from parents at the age of 1 month. Groups of age-matched males (4-6 animals) were formed from several litters and put into standard plastic cages. Four days before sacrifice the animals were put into individual cages in order to eliminate the effects of group life.

The males were weighed and decapitated in the morning (from 10.00 to 11.00). The testes and epididymides were isolated and weighed. Both epididymides

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were fragmented in 2 ml phosphate buffer and left for 30 min with periodical stirring, after which the suspension was filtered through a capron filter. The spermatozoa were stained by adding 0.4 ml 1% water solution of eosin and exposed for 30 min. The spermatozoa were counted visually in a Goryaev chamber at ×200. The incidence of morphological abnormalities of the spermatozoon head was evaluated [12] in smears of stained spermatozoon suspension at ×400 as described previously. The first 300 spermatozoa were examined.

The data were statistically processed by unifactorial analysis of dispersions using Statistica 6.0 software. Duncan test for multiple comparison was used for analysis of dispersions for comparing the groups.

RESULTS

One-way analysis of variance demonstrated significant impact of the genotype on body weights ($F(6,104)=45.39$; $p<0.001$) and weights of the testes ($F(6,104)=45.39$; $p<0.001$) and epididymides ($F(6,104)=10.59$; $p<0.001$). A/He males had the greatest body weight and differed significantly ($p<0.01$) by this parameter from males of other strains (Fig. 1). The PT and YT males had the least body weights ($p<0.01$). Coefficient of variations in the means for 7 strains (interstrain variation coefficient) was 7.1% for this sign. The weight of the testicles was maximum in DD mice and minimum in CBA/Lac mice; both strains differed significantly from other strains ($p<0.01$). The interstrain coefficient of variations in testicular weights was 14.4%. The DD mice had the largest epididymides, differing significantly ($p<0.01$) from animals of other strain by this parameter. The epididymides were the smallest in YT mice, the weights differing significantly from those in other strains except CBA/Lac mice. Other strains were intermediate. The inter-strain coefficient of variations for epididymis weight was 12.8%.

The counts of epididymal spermatozoa varied significantly in different strains, the interstrain coefficient

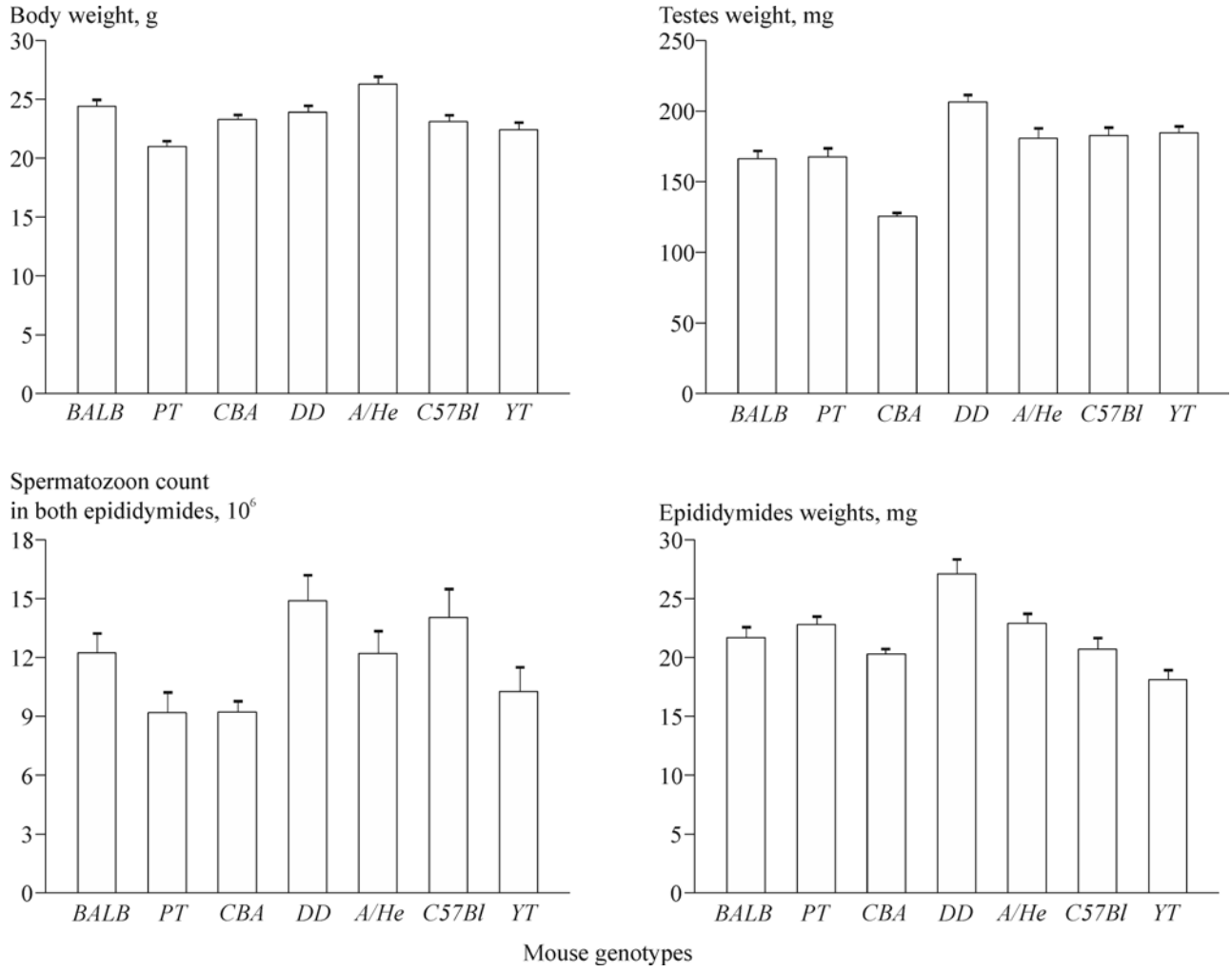

Fig. 1. Morphometric values and counts of epididymal spermatozoa in males of 7 inbred strains (number of animals in a group varied from 14 to 19)

of variations reaching 19.3% (Fig. 1). The strains can be divided into 2 groups by this parameter. The BALB/cLac, DD, A/He, and C57Bl/6J males are characterized by high counts of spermatozoa and do not differ much from each other (group 1). The PT, CBA/Lac, and YT males have lower counts of spermatozoa (group 2) in comparison with group 1 ($p<0.01$) and virtually do not differ from each other.

Morphologically atypical spermatozoon heads in mice are presented mainly by 3 types: amorphous head not corresponding to normal by size and shape; head without "beak" typical of mouse spermatozoa; and sharply narrowed "thread-like" head (without acrosome). Other heads with atypical size and shape are rather rare and can be united under the term "minor". The most incident of the head abnormalities are amorphous heads, their summary share varying from 50 to 75% in different strains.

Analysis of variance showed significant effect of the genotype for the summary percentage of spermatozoon atypical heads ($F(6,95)=34.77$; $p<0.001$). The interstrain coefficient of variations is 57% for this sign. Similar data were obtained for the above listed abnormalities: amorphous head ($F(6,95)=34.77$; $p<0.001$), head without beak ($F(6,104)=7.35$; $p<0.001$), and thread-like head ($F(6,104)=13.77$; $p<0.001$). The highest incidence of abnormal heads was recorded for BALB/cLac strain (21.14±1.32%); lesser incidence was observed in PT and C57Bl/6J, and still lesser values in CBA/Lac, DD, A/He, and YT strains. All the three groups of strains differed significantly from each other (Fig. 2). No interstrain correlations between the percentage of spermatozoon atypical heads and spermatozoon counts were detected.

Changes in the male fertility are usually evaluated by differences in such characteristics of the seminal fluid as the concentration and total count of spermatozoa in the ejaculate, volume of ejaculate, and spermatozoon mobility and morphology [1,9]. Some authors consider spermatozoon morphology as a prognostic

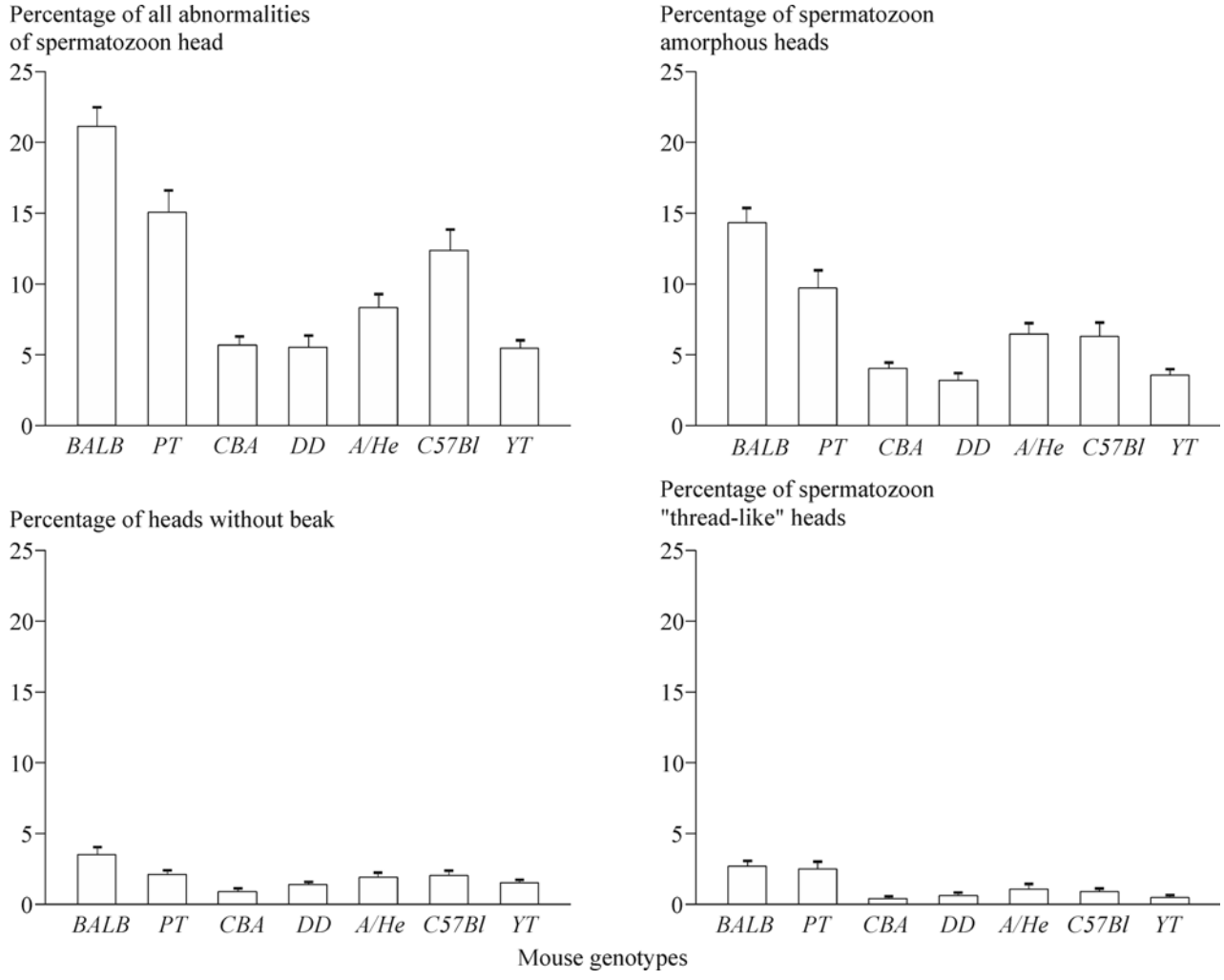

Fig. 2. Percentage of morphologically abnormal spermatozoon heads in male mice of 7 inbred strains (number of animals in a group varied from 12 to 16).

marker of male fertility, and it is used in toxicological studies or in manipulations on agricultural animals [4,9]. We found differences in the incidence of abnormal spermatozoon heads in 7 inbred mouse strains. This fact suggests deterioration of the quality of the semen in mice with high incidence of atypical forms (for example, in BALB/cLac and PT animals). This hypothesis is confirmed by the results of experiments on laboratory mice, demonstrating a reduction of the fertilizing activity of the semen with increase in the incidence of abnormal spermatozoon forms [5-7].

Male fertility and normal course of spermatogenesis depend on somatic testicular cells, Sertoli cells, producing factors essential for the developing sexual cells [2]. It is known that Sertoli cells proliferate and differentiate only until the beginning of sexual maturation; then they divide no longer and become "chaperones" for the developing sexual cells [10]. The future capacity of the testes to produce spermatozoa is determined from this time: the count of Sertoli cells determines the volume of the testes, count of sexual cells, and production of spermatozoa [10]. Hence, if the genetic differences in the production of spermatozoa in laboratory mice is determined by variability of Sertoli cell proliferative mechanism activity, these difference are forming as early as during the period of sexual maturing. This hypothesis can be verified on CBA/Lac and DD mouse strains, opposite by the counts of epididymal spermatozoa.

Peptide and steroid hormones (mainly follicle-stimulating hormone and testosterone) playing an important role in the spermatogenic epithelium functioning are essential for spermatogenesis development and maintenance in mammals [3,11]. Hence, differences between the strains in the functioning of the hypothalamic—pituitary—testicular axis can be one more causative factor leading to the differences in spermatozoon production and morphology in different strains.

Hence, study on 7 inbred mouse strains showed significant genetic variability of the epididymal spermatozoon count and morphology. This suggests the use of this set of strains for further genetic physiological analysis of spermatogenesis and fertility markers.

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