Modification of the Testicular Function in Laboratory Male Mice during Social Interactions: Effect of Female Presence

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The count of spermatozoa in both caudal epididymides, percentage of abnormal spermatozoon heads and of mobile spermatozoa, body weight, weights of the testes and caudal epididymides were evaluated in adult inbred males (PT and CBA/Lac) kept with females for 5 days. Male mice of the same genotypes and age separated from females served as controls. In males kept with females, the weights of the testes increased in PT male mice, the percentage of rapidly mobile spermatozoa increased in CBA/Lac mice, and body weights decreased in males of both genotypes. The morphometric and spermatogenic parameters in laboratory mice were modulated by the presence of a female, but the effect was determined by the male genotype.

Key Words: *testes; spermatogenesis; female effect; inbred mice*

Contacts with females cause physiological changes (primarily in the reproductive system) in males of many animal species. Production of androgens increases [5,4] and spermatogenesis is stimulated [12] in laboratory male mice and rats in the presence of a female or its smell (urine or bedding stained by females). The chemosensory signals from the female stimulate sexual behavior, modulate the aggressive behavior of male mice [11,8,12]. All these reactions seem to be aimed at facilitation of copulation and fertilization, thus improving the reproductive efficiency of the male.

The physiological mechanisms of the female chemosensory stimulus effects on the spermatogenesis remain unclear. Male fertility (and reproductive efficiency) directly depends on the quality of the semen determined by the concentration of spermatozoa in the ejaculate and their mobility and morphology [10,6]. It seems that the adaptive reactions formed in the males during contacts with females and stimulating the male reproductive efficiency involve the spermatogenic function. However, studies of the effects of a female on spermatogenesis are rare. A relevant study was carried out by Koyama *et al.* on male laboratory mice [11]. The effects of the female smell and male social rank on the quantity of spermatozoa in the caudal epididymides and their mobility were studied under conditions of established dominant and subordination relationships in a group of males. The female smell stimulated spermatogenesis in dominant animals but not in subordinates; the female chemosensory signals did not stimulate the mobility of spermatozoa under these conditions.

Studies of the genetic changeability in spermatogenesis parameters of a male in the presence of a female is an interesting problem, but no studies of this kind have been carried out. Inbred mice are a convenient model for these studies. We have previously found that males of only some genotypes develop stimulation of testicular steroidogenesis after a short (30 min) presence of a female. Of the three inbred mouse strains, testosterone production was stimulated in response to a female in BALB/c and CBA/Lac males, but not in PT males [4]. Hence, it seems that the female presence stimulates spermatogenesis depending on the male genotype.

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We studied the effects of lasting presence of a female on the spermatozoon number, mobility, and morphology in inbred males of CBA/Lac and PT strains contrast by hormonal response to females. In addition, we analyzed the morphometric values, such as body weight and weights of the testes and caudal epididymis.

MATERIALS AND METHODS

The study was carried out on 3-month-old male inbred CBA/Lac and PT mice (n=130). The animals were kept at standard vivarium conditions at 12:12 h light:dark regimen and 24°C in unisexual groups of 4-6 males. After 4-day isolation (in order to arrest the group effects) of the male a virgin DD female aged 90 days was put into the cage. Before caging with the males, the females were kept in unisexual groups of 4-6 animals.

Intact males of the same genotypes and age after 4-day isolation, which had no contacts with females, served as controls. The males were weighed before isolation and before decapitation. The animals were decapitated at 13:00-14:00, the testes and caudal epididymides were isolated and weighed. Both epididymides were directly plunged in 200 μl F-12(HAM):DMEM (1:1) with 3% bovine serum, finely fragmented, 800 μl of the same medium was added, and the suspension was processed on a shaker during 10 min. The resultant suspension was filtered through Nylon filters Falcon (pore diameter 70 μ) into plastic tubes. The percentage of mobile spermatozoa of category A (velocity >25 μ/sec) and category B (velocity of 2-25 μ/sec) was evaluated on a semen fertility analyzer SFA-500-2 (Biola). All manipulations were carried out at medium temperature of 37°C.

The spermatozoa in an aliquot of spermatozoon suspension stained with 1% eosin were counted visually in a Goryaev chamber under a light microscope at ×200. The results were converted per ml initial suspension, which corresponded to the spermatozoon count in both epididymides.

In order to count abnormal spermatozoon heads, suspension of stained spermatozoa was applied onto a slide and a smear was made. The smear was fixed by Canadian balm and covered with a slide. The first 300 spermatozoa were examined under a light microscope at ×400 as described previously [1].

The data were statistically processed by ANOVA analysis of dispersions and Statistica 6.0 software. Bifactorial analysis of dispersions was carried out. The main factors were the male genotype and the female presence. As body weights were measured in the same animals before female presentation and after it, body weight was analyzed as a repeated measures factor. Within the framework of analysis of dispersions, the groups were compared by Duncan's multiple comparison test. The data in the figure and in Table 1 are presented as the mean arithmetic and error of the mean (M±SEM).

RESULTS

Bifactorial analysis of dispersions (the main factors: genotype and female presence) showed the significance of the genotype ($F_{2,118}$=92.7, p<0.01) and female presence ($F_{1,48}$=10.006, p<0.05) for the male body weight. Body weights of CBA/Lac males were significantly higher than of PT mice (p<0.05, Duncan's test). After housing with the female, body weights of CBA/Lac males decreased from 28.54±0.74 to 27.09±0.68 g (p<0.05, Duncan's test). Body weights of PT mice also decreased after housing with the female (from 23.45±0.29 to 22.94±0.35 g), but this decrease was insignificant.

Bifactorial analysis of dispersions showed the impact of the genotype ($F_{1,98}$=111.51, p<0.001) and female presence ($F_{1.98}$=6.11, p<0.005) for testicular weight. The presence of a female increased significantly the testicular weight in PT males (p<0.05, Duncan's test). The increase of testicular weight in CBA/Lac males was insignificant.

Bifactorial analysis of dispersions showed no impact of the male genotype and female presence for the caudal epididymides weights (Table 1).

The effect of genotype on spermatozoon count in the caudal epididymides was detected ($F_{1.109}$=5.6412,

TABLE 1. Effect of Female Presence on Morphometric Parameters of Inbred PT and CBA/Lac Male Mice

Morphometric parameters	CDF/Lac		PT	
	control	female	control	female
Testicular weight, mg	112.84±0.37	120.53±4.64*	156.64±4.51	171.04±3.81*
Caudal epididymides weight, mg	11.32±0.26	11.15±0.33	10.57±0.37	11.41±0.71

Note. *p<0.05.

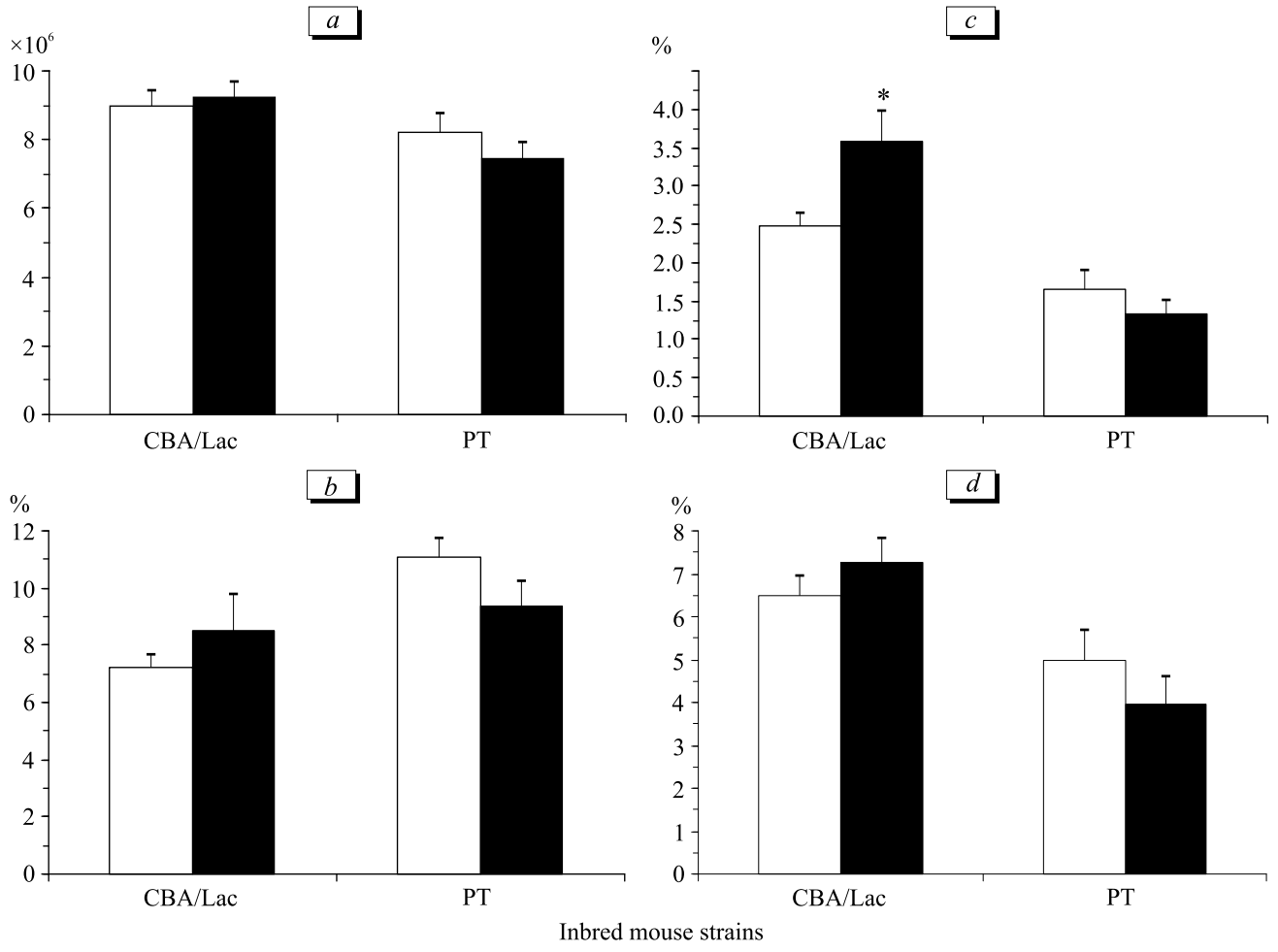

Fig. 1. Counts of spermatozoa in the caudal epididymis (*a*), percentage of abnormal spermatozoon heads (*b*), and of categories A (*c*) and B (*d*) mobile spermatozoa in intact males (light bars) and males after 5-day intercourse with females (dark bars). Number of animals in a group varied from 15 to 35. *$p<0.05$: female impact.

$p<0.05$; Fig. 1). However, female presence was inessential for spermatozoon count in the caudal epididymides in males of both strains.

The effect of genotype on the percentage of category A mobile spermatozoa ($F_{1.116}=27.855$, $p<0.01$) and factor interactions ($F_{1.116}=5.9708$, $p<0.05$) has been demonstrated. The percentage of category A mobile spermatozoa increased in the presence of the female only in CBA/Lac males ($p<0.05$, Duncan's test).

The effect of male genotype on the percentage of category B mobile spermatozoa was revealed ($F_{1.116}=14.055$, $p<0.01$). However, no significant effect of the female presence on the percentage of category B mobile spermatozoa or relationship between the male genotype and female presence were detected.

Only the genotype was essential for the percentage of abnormal spermatozoon heads ($F_{1.117}=7.7975$, $p<0.01$).

The results provided new data on the stimulatory effect of the female on the male testicular function. We showed that the female stimulatory effect depended on male genotype, because the presence of a female increased the percentage of mobile spermatozoa only in CBA/Lac, but not PT males. Interestingly, the behavioral reactions (mountings, naso-nasal and naso-anogenital contacts) of PT males to short-term (30 min) presence of receptive females were significantly less pronounced than the reactions of CBA/Lac males. Moreover, PT males developed a significant reduction of blood testosterone level after intercourse with the female, which could reflect the stress status of these animals [3].

The causes of this strain-associated changeability are not yet clear, but presumably, it is based on the polymorphism of genes coding for proteins involved in pheromone signal perception and transmission. For example, two large gene families, *V1R* and *V2R*, are known encoding pheromone receptors [13].

In our study the presence of a female had a genotype-dependent effect on the spermatozoon mobility but not on spermatozoon counts in the caudal epididymides or spermatozoon morphology. Presumably, the stimulatory effect of the female on these parameters

would manifest after a longer exposure. It is known that the female pheromones stimulate the production of gonadotropin releasing hormone [9], this, in turn, stimulating the synthesis of luteinizing and follicle-stimulating hormones regulating spermatogenesis [7]. However, the spermatogenic epithelium cycle in mice takes 28 days, and hence, the stimulatory effect of the female pheromones on these spermatogenesis parameters can manifest after this period.

Body weight loss in males of both genotypes after 5 days with the females is worthy of note. It is known that the metabolic processes in the males are stimulated in the presence of females, this promoting mobilization of resources for multiplication [2], and hence, body weight loss in our experiments could be caused by high energy expenditures of males.

Hence, the stimulatory effects of 5-day intercourse with females on such parameters of the reproductive system as the spermatozoon mobility and weight of the testes were detected in male laboratory mice, this effect depending on the male genotype.

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