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SHORT REPORTS

Regulation Mechanisms of Spermatogenesis in Red-Backed Mice at Different Population Phases

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Abstract—This paper gives morphological and functional analysis of the endocrine and germinal testicular sections in red-backed mice *Myodes glareolus* from a natural population at different population sizes. The decrease in the germinal testicular activity at the peak population is due to the reduced functional activity of the endocrine testicular section (Leydig and Sertoli cells), which leads to a decreased content of testosterone, androgen-binding protein, and inhibin that take part in the hormonal regulation of spermatogenesis. The trend towards suppression of spermatogenesis is noted to persist in animals at a low population size.

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It is known that the increased population density results in physiological stress that leads to a reduced reproductive ability of males, delayed sexual development of animals, inefficient mating, and increased mortality of young animals (Christian, 1974; Cergueira et al., 2006). Delayed sexual development in males can be due to delay and disorder in spermatogenesis. The testicle performs two major functions: the germinal function related to the development of sex cells and the endocrine function related to the production of steroid hormones. The development of spermatogenesis requires the active secretory activity of the endocrine testicular section represented by interstitial endocrinocytes (Leydig cells) that produce testosterone and nurse cells (Sertoli cells) that synthesize androgen-binding protein (ABP), inhibin, and other proteins. The normal course of spermatogenesis requires a high intratesticular concentration of testosterone, which is created thanks to ABP (Gaber et al., 1983; Russel, 1989; Parvinen, 1993). The major function of Leydig cells is to produce androgens for the paracrine regulation of spermatogenesis within the testicle as well as androgen and anabolic effects outside the testicle (Saez, 1994; Huleihel and Lunenfeld, 2004). Sertoli cells also have a paracrine effect on spermatogenesis (Sharpe et al., 2003; Mruk and Cheng, 2004), synthesizing a whole series of peptides that affect Leydig cells. Thus, inhibin strengthens the expression of receptors of luteinizing hormone (LH) on Leydig cells, thus activating steroid genesis. The hormonal control of spermatogenesis in mammals is implemented within the self-regulating system with the hypothalamic–pituitary–gonadal negative feedback (Steinberger, 1971; Holdcraft and Braun, 2004).

The normal individual blood testosterone content in mouselike rodents varies from 2.5 to 7.6 ng/mL; therefore, the correlation between the frequency of fertile couplings and blood testosterone content is not clearly fixed within the physiological fluctuations in testosterone levels (Osadchuk and Naumenko, 1983). A high variability is also noted for the content of LH that controls the production of testosterone by Leydig cells. Testosterone that performs the germinal function ensures competition between males, promoting their aggressive behavior (Salvator et al., 1997; McClothlin et al., 2007, 2008); it strengthens territoriality (Moore and Marler, 1987) and the attractiveness of a male for a female (Enstrom et al., 1997). That is why testosterone is used as an indicator of sexual behavior in small mammals from a natural population under various impacts, particularly, under stress (Hardy et al., 2005; Gerlinskaya, 2008; Novikov and Moshkin, 2009). The auto-regulation mechanisms that support the spatial and demographic population structure, which corresponds to living conditions, manifest themselves based on zoo-social behavior. The action of intrapopulation mechanisms is mediated mainly through the endocrine function of the gonads (Christian, 1971; Shilov, 1977; Naumenko, 1979). It is necessary to point out that there are almost no data on the interaction between the germinal and endocrine testicular sections at different population sizes. Apparently, this is due to fact that the problem of how spermatogenesis is affected by the paracrine factors produced by cells of the endocrine testicular section is still understudied and often debatable.

The goal of this study is to assess the germinal testicular function as well as the morphological and func-

Morphometric parameters of testicles at different population phases ($M \pm m$; $p < 0.05$)

Phase	Diameter of seminiferous tubules, μm	Area of nuclei, μm^2	
		Leydig cells	Sertoli cells
"Low"	169.8 ± 20.1* $n = 28$	31.8 ± 3.8** $n = 19$	49.7 ± 4.7** $n = 28$
"Growth"	176.4 ± 25.6 $n = 34$	35 ± 4 $n = 35$	57.8 ± 5.9 $n = 25$
"Peak"	151 ± 23.4*** $n = 24$	30.1 ± 2.1*** $n = 38$	51.4 ± 6.7*** $n = 24$

n is the number of animals.

* Significant distinctions between the "low" and "peak" phases.

** Significant distinctions between the "low" and "growth" phases.

*** Significant distinctions between the "peak" and "growth" phases.

tional state of Sertoli cells and interstitial endocrinocytes in red-backed mice from a natural population in order to reveal the regulation mechanisms of spermatogenesis at different population sizes.

The examination involved sexually mature red-backed mice *Clethrionomys glareolus* Schreber (1970) caught (June–August) in the Middle Urals (57°21′ N, 59°48′ E) using live traps (Karaseva et al., 2008) in the years of population peak (1992, 2001, 2004), growth (1991, 2003, 2006). Population cycle phases were determined with consideration for the demographic population structure and relative population size (Zhigal'skii and Kshnyasev, 2000). In the year of population growth, the relative abundance of red-backed mice was 3 individuals per 100 trap-days, and in the year of peak it was 12.5. Testicles of animals were fixed in 10% formalin; paraffin sections of testicles with a thickness of 5–7 μm were colored with hematoxylin-eosin. Histological preparations were subjected to morphological and morphometric analysis. The diameter of seminiferous tubules and the cell sizes in the endocrine testicular section were determined using the SIAMS Photolab software. The data were statistically processed by one-way variance analysis (Statistica 6 software package). The level of significance for statistical tests was taken to be 5%.

The performed morphometric analysis of seminiferous tubules showed that the germinal function of animals depended on the population size (table). If the population grows, the diameter of the seminiferous tubules corresponds to active spermatogenesis in almost all animals; at the population peak, most animals are noted to have a decreased diameter of seminiferous tubules, which indicates that the germinal testicular activity is reduced (Fig. 1a). The morphometric analysis of Leydig and Sertoli cells showed their functional dependence on the population density (table). The area of Leydig cell nuclei varies from 20 to 41 μm^2 (Fig. 1b). Normally, the sizes of perivascular Leydig cells are smaller than those of peritubular cells; moreover, the former have a less pronounced steroidogenesis (Fig. 2) The size of the nuclei varies from 27 to 41 during population growth and from 20 to 35 at its peak (moreover, the size corresponds to 41 μm^2 in 8% of animals); if the population is low, the size is from 20 to 37 μm^2 (it is 41 μm^2 in 10% of animals). At the population peak, the sizes of Sertoli cell nuclei are observed to decrease (table), which indicates the reduction of their functional activity. Seasonally breeding rodents are shown to have a decreased cytoplasm volume and cell size in Leydig and Sertoli cells in the period of testicle regression; if spermatogenesis is activated, the functional activity of the endocrine testicular section grows (Raitsina, 1985; Shevlyuk et al., 1999; Shevlyuk and Elina, 2008). The observed reduction of the germinal testicular function against the background of population peak is due to the disorder in the hormonal regulation of spermatogenesis (Fig. 3).

There are two loops regulating testosterone secretion (Connell, C.J. and Connell, G.M., 1977). Within a large loop, LH stimulates the production of testosterone by Leydig cells; if the feedback is negative, testicular testosterone inhibits the secretion of LH. A short loop (intratesticular) for regulation of the testosterone secretion is the inhibition of androgen production in Leydig cells by estradiol β-17 produced by

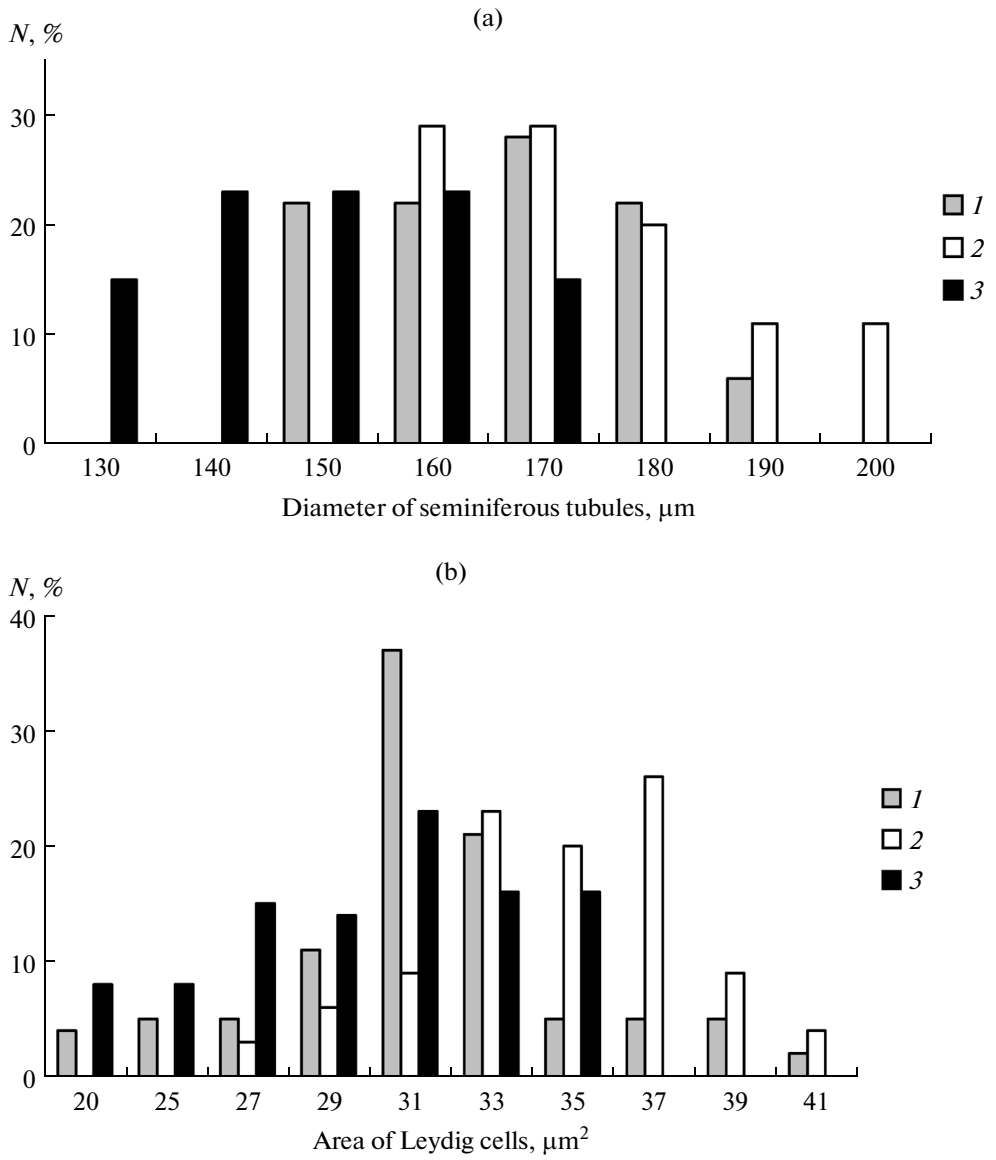

Fig. 1. Frequency of the occurrence of animals (*N*) according to the diameter of convoluted seminiferous tubules (a) and according to the diameter of Leydig cells (b) at the population phases "low" (*1*), "growth" (*2*), and "peak" (*3*).

Sertoli cells under the impact of the follicle-stimulating hormone (FSH). In addition, testosterone stimulates the secretion of ABP produced by Sertoli cells (Steele and Leung, 1992). The suppression of the androgen function of Leydig cells is due to the decreased content of LH. It was earlier noted that the population peak led to the increased functional activity of the adrenal gland and intensified synthesis of corticosteroids (Rogovin and Moshkin, 2007; Ermakova, 2008; Baitmirova et al., 2010) that inhibit the production of gonadotropic hormones. The role of the adrenal system in forming the structure of relations between animals was determined in a number of mammalian species (Sapolovsky et al., 2000; Creel et al., 2001; Goymann et al., 2001; Wielebnowski et al., 2002).

The reduced functional activity of Sertoli cells was ascertained to lead to decreased synthesis of ABP and inhibin. The shortage of ABP causes a decreased concentration of testosterone in the convoluted seminiferous tubules. The decrease in inhibin synthesis strengthens the production and release of FSH (Fig. 3). The reduced activity of the endocrine testicular section strengthens the degenerative processes in the seminiferous epithelium (Fig. 4). The degeneration of seminiferous cells is known to be accompanied by an increased concentration of FSH caused by the sup-

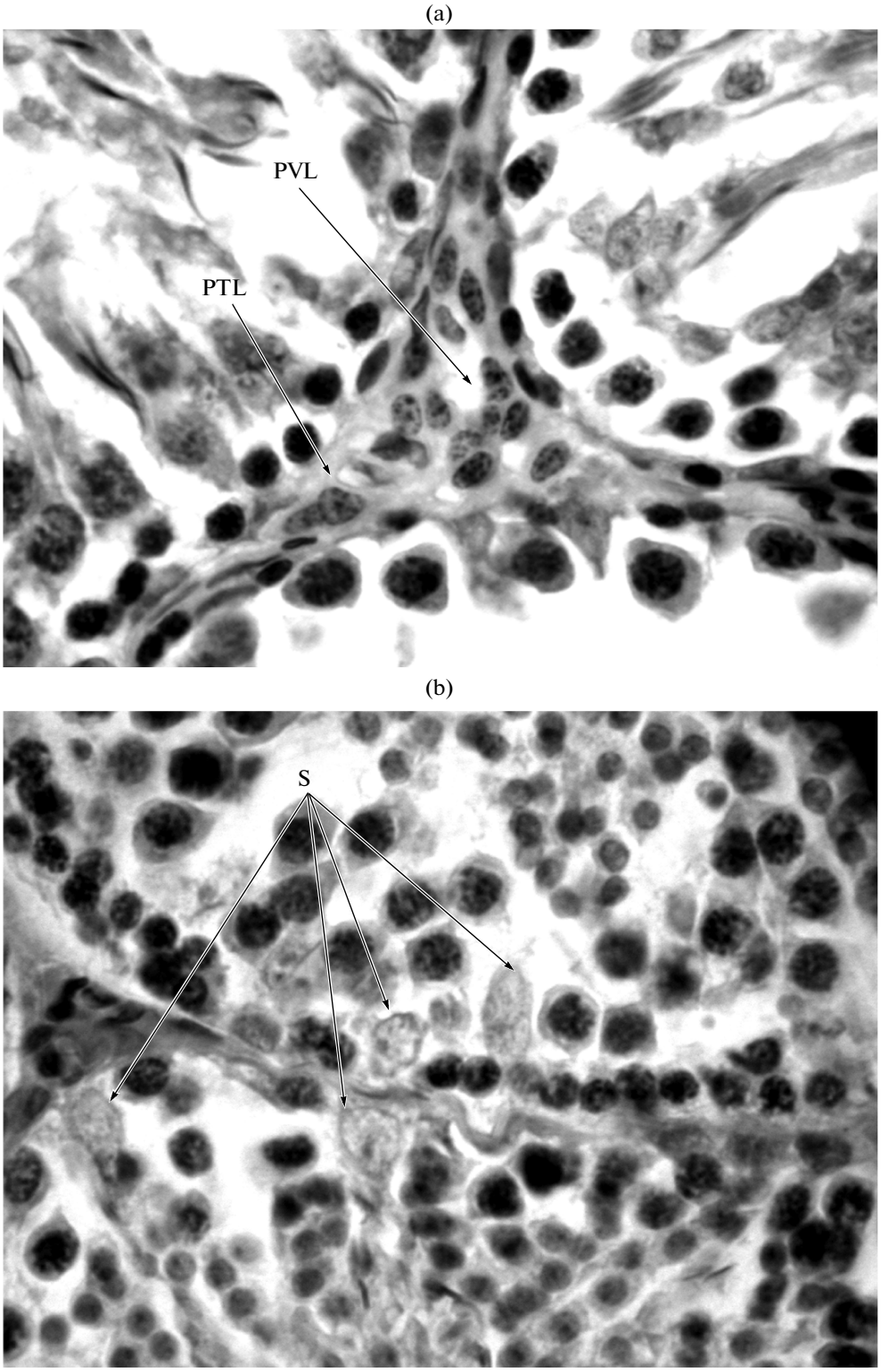

Fig. 2. Histological sections of the testicle of a red-backed mice. (a) Perivascular Leydig cells (PVL) and peritubular Leydig cells (PTL); (b) Sertoli cells (S).

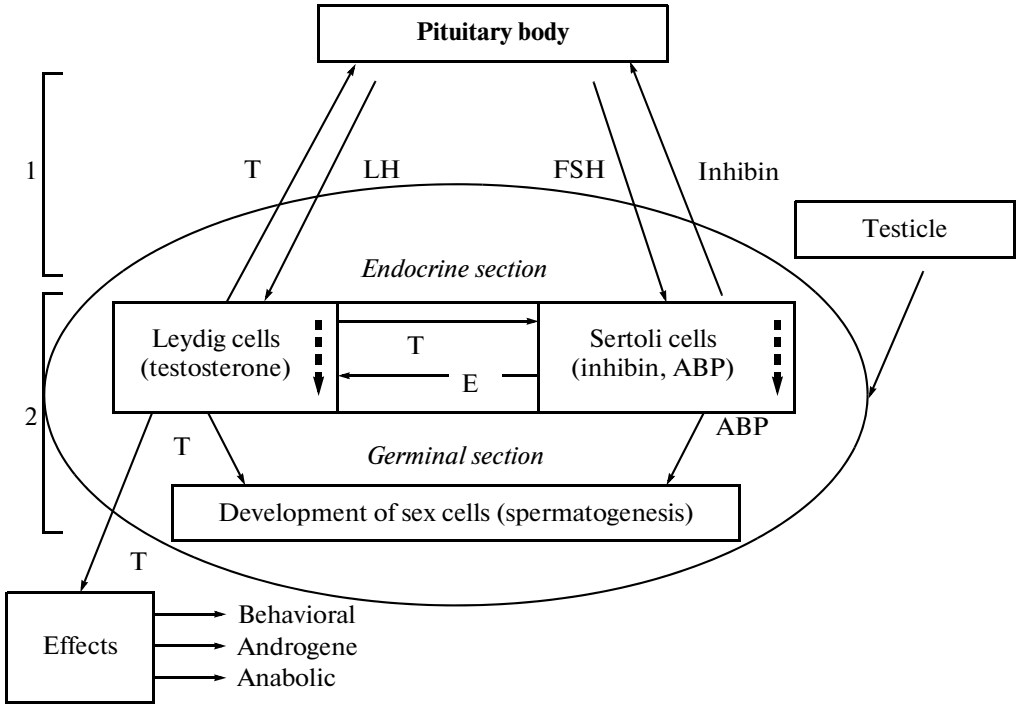

Fig. 3. Scheme that reflects the hormonal regulation of spermatogenesis. 1, large loop; 2, short intertesticular loop for hormonal regulation of spermatogenesis; T is testosterone; LH is luteinizing hormone; FSH is follicle-stimulating hormone; ABP is androgen-binding protein; E is estradiol. Point arrows show the functional activity of Leydig cells at the peak and at a low population size.

pression of inhibin secretion (Rich and de Krestser, 1977).

Consequently, the reduced functional activity of Sertoli cells leads to an increased concentration of FSH. If the population is small, there is also a reliably significant decrease in the functional activity of cells of the endocrine testicular section; however, the reduction of its germinal function is not so pronounced as at the population peak (table). Apparently, this is due to the effect of prenatal stress, when at the population peak pregnant females experience physiological stress from excessive compaction that affects the endocrine status of descendants (Weinstock, 1992; Von Holst, 1998; Kaiser et al., 2000; Kofman, 2002). It should be noted that at the "peak" population phase sexually immature animals are prevalent (up to 70%), and the remaining share is made up by overwintered animals, which have a high level of metabolism and rapidly grow old (Olenev and Grigorkina, 1998). It is known that hormonal unbalance occurs in rapidly aging animals, which leads to the suppression of spermatogenesis (Zakhidov, 2007). In our studies, the suppression of germinal testicular activity is observed at the population peak in most of animals (up to 70%), which is due to the reduced functional activity of endocrine section cells. Therefore, at the population peak animals finish breeding earlier (in July) under the conditions of physiological stress and against the background of accelerated aging, whereas at a lower population size, breeding can continue up to autumn (Zhigal'skii and Bernshtein, 1989).

Consequently, the germinal and endocrine testicular functions depend on the population phase. If the population grows, almost all animals (up to 95%) are noted to have active spermatogenesis. At the "peak" population phase, the suppression of spermatogenesis in most of the animals is due to the reduced functional activity of Leydig and Sertoli cells, i.e., the decreased content of testosterone, ABP, and inhibin. The observed degeneration of seminiferous cells is due to the reduced secretion of inhibin that leads to the increased synthesis of FSH. The high level of FSH serves as a marker of degenerative changes in sex cells. If the population is small, animals have a persistent trend towards the suppression of the germinal testicular function due to the endocrine situation in animals, which are at the "peak" population phase. The presence of androgens in blood is regulated by the activity of the hypothalamic–pituitary–gonadal system. The androgens, which are accumulated in the testicles themselves, have local effects on Sertoli cells. Thanks to testosterone being bound with ABP, which is pro-

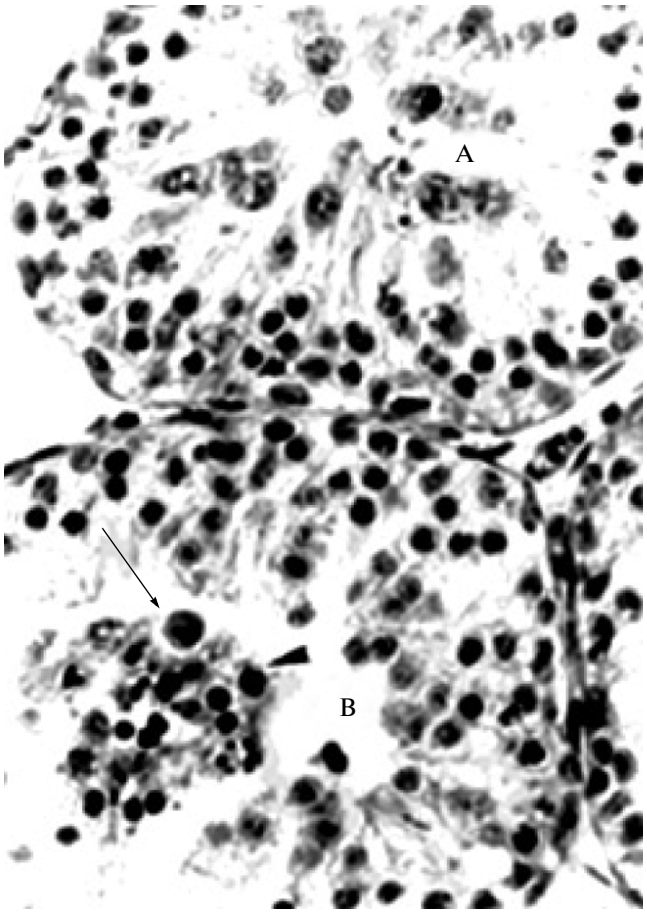

Fig. 4. Histological section of the testicle of a red-backed mice. A, seminiferous tubule without pathological changes; B, seminiferous tubule with degeneration of spermatids (designated with the arrow).

duced by Sertoli cells, a high level of testosterone is constantly supported in testicles. The intragonade level of androgens is an important factor in paracrine regulation of spermatogenesis.

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