Ontogenetic Changes in Bank Vole (Clethrionomys glareolus) Sperm Morphology

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SHORT COMMUNICATIONS

Ontogenetic Changes in Bank Vole (*Clethrionomys glareolus*) Sperm Morphology

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The stability of mammal populations in time and space largely depends on their reproduction, which is described using various reproductive characteristics of animals at population to suborganismal levels, e.g., age at maturity, fecundity, reproductive period length, and morphofunctional state of reproductive organs. Studies on the effect of environmental (including technogenic) factors on the reproductive sphere should be based on estimates of natural variation, primarily ontogenetic variation. This variation in muroid rodents from natural zones of temperate latitudes may be considered from two aspects: with regard to variants (pathways) of ontogeny or to stages of sexual development.

The first aspect is focused on the existence of two variants of individual development, with part of animals reaching maturity in the year or birth (variant I) and the other part, in the next year after overwintering (variant II) [1, 2]. One of the two variants (ontogenetic pathways) is implemented depending of the time of birth, the state of the population, and other factors. This type of ontogenetic variation accounts for differences not only in life span (3–6 months in variant I vs. 12–15 months in variant II) but also in many other traits: metabolic [3], bioenergetic [4], morphophysiological [1], odontological [2], etc. The rates of growth and maturation, reproductive period length, and fecundity also differ depending on the variant of ontogeny, which therefore should be taken into consideration in analyzing population dynamics [6–9]. The existence of two ontogenetic pathways is regarded as a particular instance of polyvariant ontogeny that provides for redistribution of reproductive effort during the life cycle of individuals [10]. The result of this redistribution—the functional heterogeneity of population—is reflected in its reproductive age structure, in which three groups are usually distinguished: mature and immature young of the year and overwintered individuals.

The second aspect of variation is related to stages of sexual development (immaturity, maturation, maturity, and senility) and is independent of ontogenetic pathway. Based on a set of exterior and interior traits and capture date, males can be divided into corresponding groups: immature, pubertal, sexually mature (potentially capable of reproduction), and senile (with decay of sexual function accompanied by testicular involution). The two aspects of variation are not mutually exclusive: the entire complex of ontogenetic (age-related and reproductive) changes may be analyzed in groups distinguished with regard to both the pathway of ontogeny and the stage of sexual development.

Characteristics of sperm are particularly important for the assessment of male fertility, but their variation in mammals from natural populations has not been studied sufficiently. The majority of relevant studies have been performed on men [11–13], less frequently on laboratory or agricultural animals [14–16], and only in rare cases on wild animals [17, 18]. As a rule, the authors conclude that the quality of sperm, its morphological characteristics and fertility deteriorate with age [11, 12], but other studies provide no evidence for the impairment of reproductive characteristics upon aging [19–21].

The purpose of this study was to evaluate ontogenetic variation in morphological parameters of spermatozoa in the bank vole (*Clethrionomys glareolus* Schreber, 1780), a widespread small mammal species. The question was addressed as to whether this variation should be taken into account in analyzing dimensions of normal spermatozoa and the frequency of defective cells.

The material for the study was obtained in the course of small mammal censuses taken in the Visim Nature Reserve (southern taiga, the Middle Urals; 57°22′ N, 59°46′ E) between 2007 and 2017. Three rounds of trapping with live traps were conducted: in

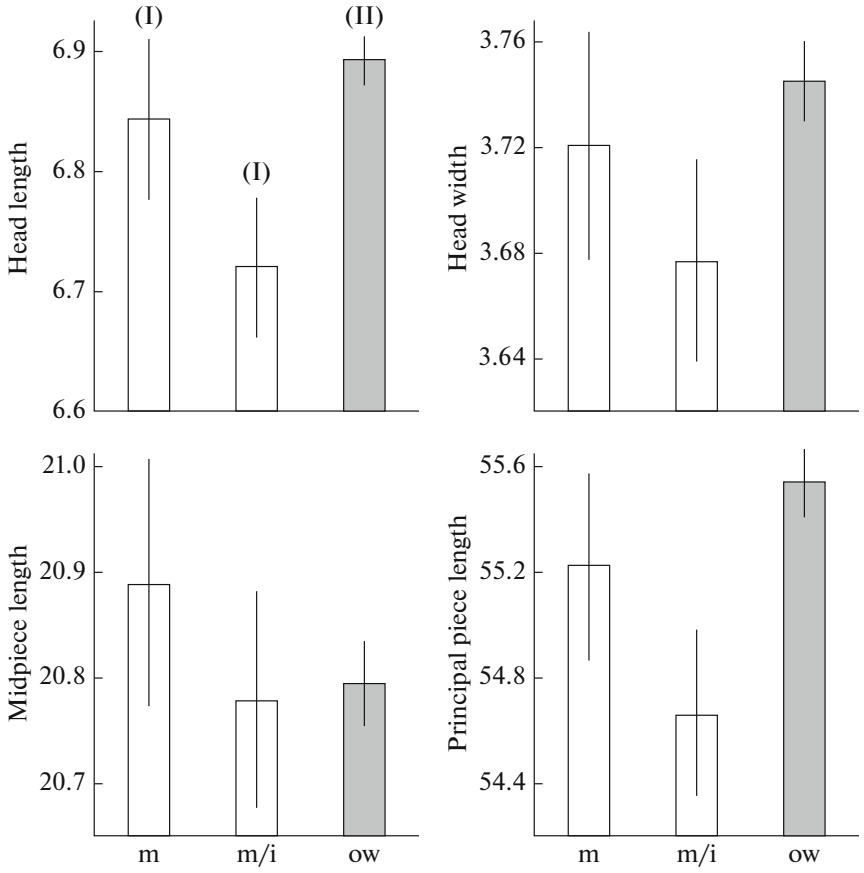

Fig. 1. Morphometric parameters of spermatozoa (μm, average values with confidence intervals) in bank voles of different reproductive age groups: (m) mature young of the year, (m/i) young of the year with testicular involution, (ow) overwintered males; (I, II) variants of ontogeny.

spring (late May–early June), in summer (the second half of July), and in autumn (late August–early September). The sample of males (n = 72) reflected the structure of bank vole population in the period of trapping. The prevalent population groups were as follows: in spring, overwintered animals; in summer, overwintered animals and sexually mature and immature young of the year; in autumn, immature young of the year and young of the year with testicular involution. Since pubertal young of the year and overwintered males with testicular involution were almost absent, variation of spermatozoa between reproductive stages was analyzed in young of the year (mature and with testicular involution), and between variants of ontogeny, in mature young of the year and overwintered males. On the whole, 7 mature young of the year (m), 9 young of the year with testicular involution (m/i), and 56 overwintered males (ow) were included in the study. Their calendar age was determined based on age-related dental changes [2].

Sperm morphology was analyzed using smear preparations from the cauda epididymis, which were imaged under a microscope with a digital camera (Leica Microsystems, Germany) at 400× magnification. The images were examined to distinguish normal and abnormal spermatozoa (with head and tail defects) [22]. The proportion of abnormal cells among 200 spermatozoa was estimated for each animal, and 30 spermatozoa were used to measure the maximum head length, head width, midpiece length, and principal piece length.

Variation in the size of normal spermatozoa and the frequency of defects was analyzed using generalized linear models (GLM) for continuous and dichotomous traits (linear and logit regressions). The odds ratios (OR) and their 95% confidence intervals (CI) are presented after exponentiation of logit regression coefficients (the logarithms of ORs): exp(lnOR) or 1/exp(lnOR). Mature young of the year were chosen as a reference group. The false discovery rate in multiple comparisons of statistical hypotheses was controlled using the Benjamini–Yekutieli procedure. Adjusted significance levels (p) are presented below. Statistical analysis was performed using Statistica 8.0 and R software.

No differences in morphometric parameters of spermatozoa were revealed between reproductive age groups (Fig. 1): for head length, p = 0.17; for head

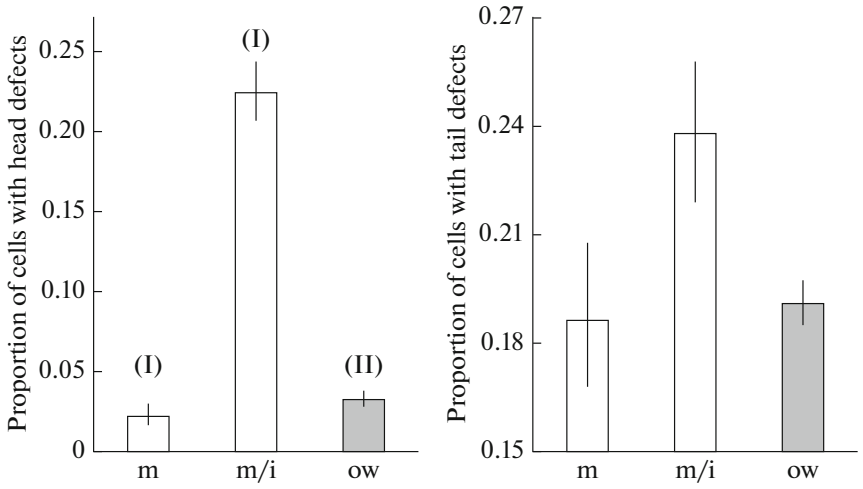

Fig. 2. Frequencies of abnormal spermatozoa (average values with confidence intervals) in bank voles of different reproductive age groups: (m) mature young of the year, (m/i) young of the year with testicular involution, (ow) overwintered males; (I, II) variants of ontogeny.

width, $p = 0.72$; for midpiece length, $p = 0.99$; for principal piece length, $p = 0.17$. At the same time, the proportion of abnormal spermatozoa proved to differ between these groups: for head defects, $\chi^2(2) = 807.5$; $p < 0.0001$; for tail defects, $\chi^2(2) = 22.8$, $p < 0.0001$. The lowest frequency of abnormal spermatozoa was observed in mature young of the year (Fig. 2). The proportion of cells with head defects in this group proved to be lower by a factor of 4.7 (CI 4.1–5.4) than in young of the year with testicular involution ($\chi^2(1) = 446.4$, $p < 0.0001$) and by a factor of 1.7 (1.5–2.0) than in overwintered males ($\chi^2(1) = 59.4$, $p < 0.0001$). The proportion of cells with tail defects in mature young of the year was lower by a factor of 1.2 (1.1–1.3) than in young of the year with testicular involution ($\chi^2(1) = 20.0$, $p < 0.0001$) and by a factor of 1.1 (1.0–1.2) than in overwintered males ($\chi^2(1) = 6.2$, $p < 0.01$).

The absence of differences in the dimensions of normal spermatozoa between reproductive age groups is evidence for the conservatism of sex cells. The high frequency of sperm head defects in young of the year with testicular involution is apparently related to structural and functional rearrangements accompanying seasonal or age-dependent blocking of the reproductive function.

The observed differences in the frequency of spermatozoa with head defects between mature young of the year and overwintered males are similar to those obtained in our previous study for a different sample of males [22], which may be evidence for a consistent pattern of such differences. A probable explanation is that such defects are accumulated with age and are more frequent in older animals. On the other hand, the higher frequency of head defects in young of the year with testicular involution than in overwintered males is evidence that the effects of reproductive function blocking are much stronger than the effects related to the calendar age of animals.

Thus, when the morphology of normal spermatozoa is analyzed, it is of no significance at what stage of maturity or senescence the animal is and when it has sexually matured, in the year of birth or after overwintering. The same applies to the frequency of spermatozoa with tail defects, since the observed effects are weak, despite their statistical significance. In contrast, the frequency of spermatozoa with head defects should be estimated taking into account the reproductive age status of the animal. It addition, no assessment has as yet been made of ontogenetic variation in other parameters of sperm, namely, the concentration and motility of spermatozoa.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The authors declare that they have no conflict of interest.

Statement on the welfare of animals. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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