Belg. J. Zool., 143(2) : 131-141 July 2013

# Reproductive capacity of male bank voles (*Myodes glareolus* SCHREBER, 1780) - age-dependent changes in functional activity of epididymal sperm

**Małgorzata Kruczek[1,*], Józefa Styrna[2] & Joanna Kapusta[1]**

[1] Institute of Environmental Sciences, Jagiellonian University, Gronostajowa 7, 30-387 Kraków, Poland, tel. +48-12-664-52-05.

[2] Department of Genetics and Evolution, Institute of Zoology, Jagiellonian University, Gronostajowa 9, 30-387 Kraków, Poland, tel. +48-12-664-50-79.

[*] Corresponding author: malgorzata.kruczek@uj.edu.pl

ABSTRACT. The influence of age on male bank voles' reproductive tract development, epididymal sperm quantity and functional activity was investigated. Experiments were carried out on male bank voles aged 1.5 to 15 months (n=10 each in 8 age groups). The developmental stage of the reproductive tract was assessed by the weight of testes, seminal vesicles and coagulation glands. In each age group the number of epididymal sperm and their functional activity were examined. Epididymal sperm functional activity was assessed by motility, viability, maturity, head morphology and integrity of the sperm tail membrane. Ageing males were heavier than pre-pubertal and mature ones. Male age also affected the testes, seminal vesicles and coagulation gland development. The heaviest accessory sex glands were noted in 3-month-old males and the lightest in pre-pubertal (1.5-month-old) and older (12- and 15-month-old) males. Sperm counts were significantly higher in 3-, 4- and 5-month-old males than in pre-pubertal and old males. Generally, adult males aged 3- and 4- months , produced sperm of better functional activity. In conclusion, the best male reproductive capacity is found in bank voles of 3 to 4 months of age.

KEY WORDS: age, bank vole, spermatozoa, reproductive tract, sperm activity

## INTRODUCTION

Interest in the effect of paternal age on physical conditions and reproductive efficiency of progeny has recently increased worldwide. Studies on humans and certain rodents show that among anomalous effects associated with advanced male age are pregnancy losses (SERRE & ROBAIRE, 1998; SLAMA et al., 2005), stillbirth (ASTOLFI et al., 2004) and increased postnatal mortality (ZHU et al., 2008). Mutation frequencies in male genomes also increase with age, which may evoke negative effects on individual health, infertility as well as genetic defects in offspring (for a review, see for example SLOTER et al., 2004). In the Brown Norway rat (*Rattus norvegicus*), ageing leads to considerable damage in the testes and to lower serum testosterone levels due to decreased pituitary hormonal activity, and to decreased sperm production (WANG et al., 1993). In old mice more atrophic testes with degenerated seminiferous tubules have been found (PARKENING et al., 1988).

The efficiency of spermatogenesis is a key to male reproductive success. Spermatozoa concentrations as well as their functional activity are parameters that can be used to assess the potential success of the sperm and, consequently, the reproductive success of males. Functional activity can be assessed by spermatozoa viability, motility, maturity, integrity of the sperm tail membrane and head morphology. The study of age-dependent changes in sperm quality of the black-footed ferret (*Mustela nigripes*) based on only two parameters showed that motile and structurally normal sperm was diminished in older males (WOLF et al., 2000). To our knowledge, there are few published reports assessing the

influence of male age on complex measurements of sperm functional activity. Studies on mice have shown that mating with males of 12 months of age and older significantly reduced fertilization and decreased implantation potential and fetal viability (KATZ-JAFFE et al. 2013). The influence of age has been also demonstrated in studies on the male mosquito, where the optimum male age for successful insemination of females was found to be 4-8 days (SAWADOGO et al. 2013). The aim of the present study was to investigate the effect of age on the reproductive development of bank vole (*Myodes glareolus*) males. The bank vole is presumably the most common small rodent in Europe. This species has become a research object in behavioural, population, and environmental protection studies and it is also used as a classical model for laboratory experiments. Reproductive biology of bank vole is rather well known, including such aspects as time of male sexual maturity (MARCHLEWSKA-KOJ, 2000), factors influencing mating behaviour (MARCHLEWSKA-KOJ et al., 2003) and multiple paternity (RATKIEWICZ & BORKOWSKA, 2000). In the wild, bank voles breed seasonally (CLARKE, 1981). The reproductive season lasts from the beginning of March to the end of October. Males living in a natural environment reach puberty at about two months of age (BUJALSKA, 1973). In a laboratory colony mature spermatozoa were observed for the first time in 1.5-month-old bank vole males (KRUCZEK, 1986). However the optimal male age for reproduction is still not well determined. In the present study we examined the effects of age on the quality and functional activity of epididymal sperm of bank vole males using different parameters.

## MATERIALS AND METHODS

### Animals

The bank voles used in the experiments came from the Institute of Environmental Sciences, Jagiellonian University in Kraków. The animals are maintained as outbred stock according to the system described by Green (GREEN, 1966). Briefly, each generation consists of at least 22 breeding pairs; the male and female in each mating pair do not have common parents or grandparents. This breeding system ensures the heterogeneity of the colony. It could be assumed that animals from such a colony are comparable to rodents living in a natural population. Bank voles were kept in polyethylene cages (36 x 21 x 17 cm) under a 14 h photoperiod (lights on at 06:00 a.m.) at 20±2° C. Standard pelleted chow and water were available *ad libitum*. Wood shavings were provided as bedding material and changed once a week. The males were housed, at least in 70% of cases, as three per cage from weaning at 19-20 days; only occasionally were there two or four males per cage.

The experiments were carried out on males aged 1.5 (1.5M), 3 (3M), 4 (4M), 5 (5M), 6 (6M), 9 (9M), 12 (12M), and 15 months (15M). There were 10 males in each tested group (n=10), all together 80 males. The males in each age group came from different mating pairs.

### Procedures of body and organ weights and of epididymal sperm evaluation

At the appropriate age, the males were sacrificed by cervical dislocation and weighed. The paired testes and seminal vesicles and coagulating glands were dissected out and weighed (the latter two jointly) too. The procedures and methods to count and observe spermatozoa were the same as used for mice (STYRNA & KRZANOWSKA, 1995; STYRNA et al., 2003) as well as for bank voles in our previous paper (KRUCZEK & STYRNA, 2009). The individual counting and analyzing of epididymal sperm were conducted without information on the males' ages.

*Preparation of epididymal sperm suspension*

After gentle pressing of each cauda epididymis with forceps, allowing sperm to pass to the vasa deferentia, the latter was dissected out and its content gently squeezed directly into 100 µl of M2 medium (Sigma-Aldrich, Germany) containing 2% albumin bovine fraction V,

placed in Petri dishes, and allowed to disperse at room temperature for 2 minutes (STYRNA AND KRZANOWSKA, 1995; KRUCZEK AND STYRNA, 2009).

*Epididymal sperm concentration*

A 1:20 dilution of epididymal sperm suspension with M2 medium (Sigma) was prepared, and the number of spermatozoa in 100 squares of a hemocytometer was counted under a light microscope at 400x magnification. The average of two epididymal sperm counts was used to estimate sperm concentration. Epididymal sperm functional activity was assessed by spermatozoa motility, sperm tail membrane integrity (hypo-osmotic swelling test), viability, maturity (spermatozoa without cytoplasmic droplet) and sperm head morphology.

*Epididymal sperm motility*

Spermatozoa motility was assessed in a hemocytometer. A small drop of sperm suspension was transferred to a hemocytometer, covered with coverslip and spermatozoa showing progressive movements were inspected. The number of spermatozoa without a cytoplasmic droplet among 200 counted spermatozoa with progressive movements from each male was reported (SEED et al., 1996).

*Epididymal sperm tail membrane integrity – water test*

The integrity of the epididymal sperm tail membrane was determined by the hypo-osmotic swelling test (WALCZAK et al. 1994; STYRNA & KRZANOWSKA 1995; KRUCZEK & STYRNA 2009). 20 µl of epididymal sperm suspension (as described in Epididymal sperm concentration) was mixed with 120 µl distilled water on a clean glass slide, then the mixture was gently covered with a coverslip and incubated for 5 min at 37° C before it was examined. The percentage of spermatozoa showing swelling among 200 counted spermatozoa from each male was estimated.

*Epididymal sperm viability – eosin-Y test*

The test reflects the structural and morphological integrity of the epididymal sperm membrane in human and mouse sperm (WALCZAK et al., 1994; STYRNA & KRZANOWSKA, 1995). To assess epididymal sperm viability, 20 µl of epididymal sperm suspension (as described in *Epididymal sperm concentration*) was mixed with 20 µl of 0.2% eosin Y, incubated for 10 min at 37° C and smeared on a slide. The percentage of spermatozoa with unstained sperm heads (viable spermatozoa) among 200 counted spermatozoa from each male was calculated.

*Epididymal spermatozoa without a cytoplasmic droplet*

20 µl of sperm suspension (as described in *Epididymal sperm concentration*) was transferred to a slide and gently covered with a coverslip. According to the classification described for mice (STYRNA et al., 2002), three sperm categories can be distinguished: (1) without a droplet, (2) with a droplet on the end of the middle piece (distal droplet), and (3) with a droplet situated more proximally (proximal droplet) (Fig.1). For the present experiments, the percentage of spermatozoa without a cytoplasmic droplet among 200 counted spermatozoa with progressive movements was given for each male.

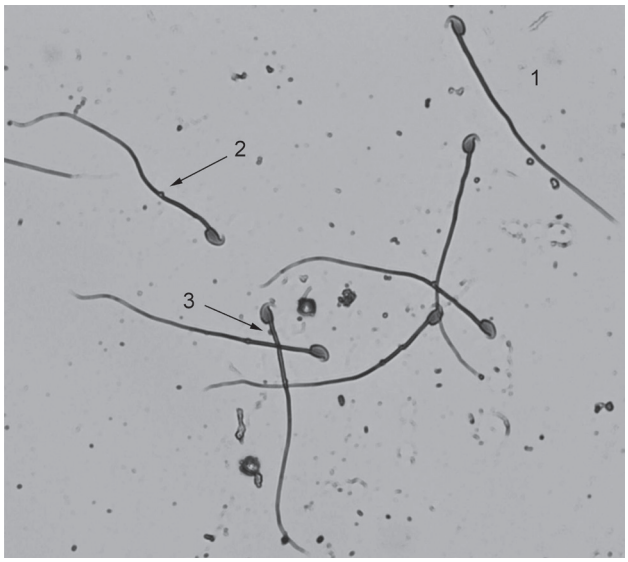


Fig. 1. – Three sperm categories of bank vole males: 1. without a cytoplasmic droplet, 2. with distal cytoplasmic droplet (with a droplet on the end of the middle piece), 3. with proximal cytoplasmic droplet (with a droplet situated more proximally)

*Epididymal sperm morphology*

For morphological examination a small drop of the epididymal sperm suspension was smeared on a slide, air-dried, fixed in acetic alcohol (absolute alcohol, glacial acetic acid, 3:1), and stained with Papanicolau to assess the proportions of different epididymal sperm head abnormalities. Based on our earlier study (KRUCZEK & STYRNA, 2009) for assessing bank vole epididymal sperm morphology, the following misshapen forms are distinguished : type 1- acrosomal abnormalities; type 2 - abnormalities in the distal part of the head; type 3 - serious abnormalities in the proximal part of sperm heads and type 4 - elliptic head. For statistical treatment, only classes of abnormal spermatozoa with a frequency of more than 0.5% of the total sperm in all tested animals were considered. They formed two classes: class 1 – slightly deformed with a small acrosomal part (type 1); and class 2 – other abnormalities, a group being composed of the remaining sperm defects described above, namely misshapen forms of types 2, 3 and 4.

The experimental procedures for this study were approved by the Regional Committee on Animal Experimentation in Kraków (Protocol No. 26/2007 and 82/2008) acting in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and conformed to the "Guidelines for the use of animals in research" (Animal Behaviour 1991, 41:183-186)

### Statistical analysis

The body weight, testes and accessory sex gland weights and epididymal sperm evaluation variables were compared by a one-way analysis of variance (ANOVA), followed by post hoc pair-wise comparisons using Tuckey's tests where the ANOVA gave significant results, as data had normal distributions. For statistical treatment (1) percentages were arcsine transformed (arcsine of the square root of the proportion) (2) the following classes of epididymal sperm morphology were taken as variables: class 1; the sum of classes 2, 3, and 4; and total percentage of abnormal shapes. All results were expressed as means ± S.E.M., and $p < 0.05$ was considered as statistically significant. All procedures were carried out using the Statistica PL ver. 8.0 statistical package.

## RESULTS

### Body and organ weights

Body weights of males increased with age up to 6 months and more. The 6M, 9M, 12M and 15M males were significantly heavier than 1.5M, 3M and 4M bank voles. Additionally, 3M, 4M and 5M males had significantly bigger body masses than did 1.5M males (Table 1). The relative weights of the testes of 1.5M males were significantly lower compared to other age groups. The biggest value of relative weight of accessory sex organs was noted for 3M males although significant differences were only observed between 3M males and the youngest 1.5M males as well as between 3M and the oldest 12M and 15M males (Table 1).

### Epididymal sperm evaluation

Epididymal sperm activity was influenced by male age. Mean sperm concentrations were significantly higher in 3M, 4M and 5M males than in 1.5M, 6M, 9M, 12M and 15M males (Fig. 2). The sperm of 3M and 4M males were characterized by a large proportion of progressive motile spermatozoa and this proportion was significantly larger than in 1.5M, 5M, 6M, 9M, 12M and 15M males (Fig. 3a). Swollen epididymal spermatozoa were significantly less frequent in groups 3M and 4M than in other groups. Moreover, the 5M and 6M bank voles had significantly lower proportions of swollen spermatozoa than did 9M, 12M and 15M males (Fig. 3b).

On the other hand higher proportions of viable epididymal spermatozoa were observed in the

TABLE 1

The age effects on the organometric parameters and proportion of abnormal epididymal sperm in bank vole males (mean ± S.E.M.).

| Age groups (months) | Body (g) | Relative weights of (mg/10 g body weight) testes | sem. ves. + coag. gl. |
|---|---|---|---|
| 1.5 | 15.98 ± 1.02$^{A,B,C,D,E}$ | 211.65 ± 6.98$^{A,B,C,D,E,F,G}$ | 107.17 ± 9.29$^{A}$ |
| 3 | 24.13 ± 0,94$^{A,B,C,D,E}$ | 303.78 ± 11 01$^{A}$ | 177.38 ± 13.65$^{A,B,C}$ |
| 4 | 25.21 ± 0.90$^{A,B,C,D,E}$ | 289.46 ± 9.24$^{B}$ | 150.09 ± 8.43 |
| 5 | 26.28 ± 1.66$^{E,}$ | 307.97 ± 11.57$^{C}$ | 145.54 ± 8.70 |
| 6 | 32.08 ± 1.25$^{A,}$ | 278.22 ± 7.84$^{D}$ | 134.42 ± 4.33 |
| 9 | 31.77 ± 0.92$^{B}$ | 321.90 ± 13.57$^{E}$ | 134.14 ± 15.12 |
| 12 | 31.75 ± 1.86$^{C}$ | 274.30 ± 11.81$^{F}$ | 127.33 ± 6.47$^{B}$ |
| 15 | 31.84 ± 1.33$^{D}$ | 296.21 ± 11.49$^{G}$ | 113.04 ± 6.64$^{C}$ |
| $F_{/7.72/}$ | 19.75 | 9.99 | 5.22 |
| $p$ | < 0.01 | < 0.01 | < 0.01 |

Means marked by the same letters differ significantly at A–O - $p < 0.01$, a, b - $p < 0.05$.

| Abnormal spermatozoa | | |
|---|---|---|
| total | class 1 | class 2 |
| 0.16 ± 0.02$^{A,D,G,J,M,O}$ | 0.12 ± 0.01$^{A,D,G,I,M,O}$ | 0.04 ± 0.01$^{A,B,C,a,b}$ |
| 0.05 ± 0.00$^{A,B,C}$ | 0.03 ± 0.00$^{A,B,C}$ | 0.02 ± 0.00$^{a}$ |
| 0.03 ± 0.00$^{D,E,F}$ | 0.02 ± 0.00$^{D,E,F,L}$ | 0.01 ± 0.00$^{A}$ |
| 0.06 ± 0.00$^{G,H,I}$ | 0.05 ± 0.00$^{G,H,a}$ | 0.01 ± 0.00$^{B}$ |
| 0.06 ± 0.01$^{J,K,L}$ | 0.05 ± 0.01$^{I,J,K}$ | 0.01 ± 0.00$^{C}$ |
| 0.07 ± 0.01$^{M,N}$ | 0.05 ± 0.01$^{L,M,N}$ | 0.02 ± 0.00$^{b}$ |
| 0.10 ± 0.01$^{B,E,H,K,O}$ | 0.08 ± 0.01$^{B,E,J,O,a,b}$ | 0.02 ± 0.00 |
| 0.14 ± 0.01$^{C,F,I,L,N}$ | 0.12 ± 0.01$^{C,F,H,K,N,b}$ | 0.02 ± 0.01 |
| 27.46 | 29.96 | 4.74 |
| < 0.01 | < 0.01 | < 0.01 |

3M and 4M males in comparison with other age groups (Fig.4a). Additionally, the proportion of viable sperm in 3M males was significantly higher than in 5M animals; and 5M males had significantly more viable sperm than did 6M, 9M, 12M and 15M bank voles (Fig. 4a).

Sperm without a cytoplasmic droplet was significantly more frequent in the vasa deferentia of 3M and 4M males than in all other ages, and 15M and 3M males had significantly more mature spermatozoa than did 4M bank voles (Fig. 4b). Additionally, 5M and 6M males had a significantly higher proportion of mature spermatozoa than did 9M, 12M and 15M males; and 1.5M males had significantly more mature spermatozoa than did 12M and 15M males. Finally 9M males had significantly more spermatozoa without a cytoplasmic droplet than did 15M bank voles (Fig. 4b).

There were also significant differences in sperm morphology between age groups of males (Table 1). The mean total proportion of abnormal sperm was significantly lower in 3M, 4M, 5M, and 6M males than in 1.5M, 12M and 15M males. The sperm of 9M animals was characterized by a significantly lower proportion of total abnormal

sperm in comparison with 1.5M and 15M males, and 12M animals had significantly less abnormal sperm than did 1.5M males. Similarly, 3M, 4M, 5M, and 6M males had significantly lower proportions of class 1 abnormalities than did 1.5M, 12M and 15M animals; additionally, the proportion of class 1 abnormalities in 4M bank voles was significantly lower than noted for 9M males. At 9M and 12M there were lower proportions of class 1 abnormalities than at 1.5M and 15M .The sperm of 3M, 4M, 5M, 6M, and 9M males was characterized by significantly lower values of class 2 spermatozoa abnormalities than that of 1.5M males (Table 1).

## DISCUSSION

A positive relationship between ageing and increase in body weight has also been documented for Spraque-Dawley rats (see for review WANG et al., 1993) while a negative correlation between body mass and age has been shown for Fisher 344 rats (BASKIN et al., 1979). The high positive dependence between bank vole male body mass and testes weight observed in our experiments are in agreement with earlier observations on bank voles (YLÖNEN et al., 2004). Since the development and secretory activity of the male accessory sex organs are under the direct control of androgens produced by the testes (FRANCA et al., 2006), the growth of these organs in 3-, 4- and 5- months-old bank vole males could be evoked by an increase of testosterone. It has been documented for Brown-Norway rats that ageing in the reproductive system is manifested by lower serum testosterone levels (WANG et al., 1993; HARDY & SCHLEGEL, 2004). A positive role of accessory sex organs on sperm functional activity has been observed in hamsters where secretions protect sperm against oxidative damage influencing sperm viability or motility (CHEN et al., 2002). So for adult bank vole males, it seems to be beneficial to have larger seminal vesicles and coagulating glands.

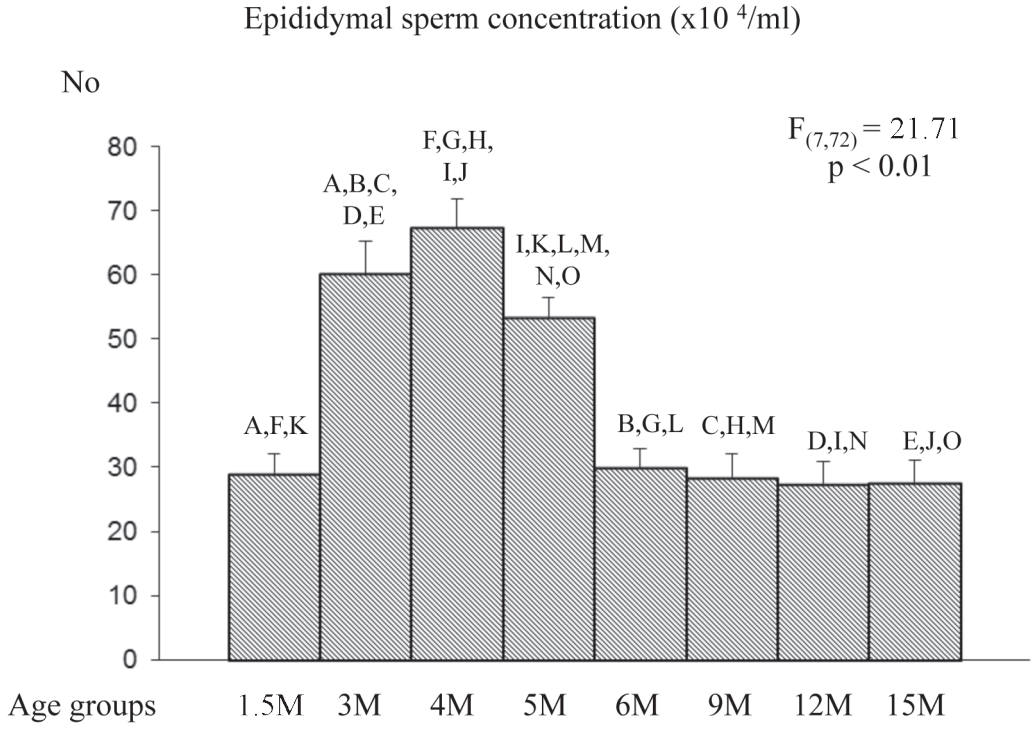


Fig. 2. – Epididymal sperm concentration (x$10^4$/ml) in 1.5- (1.5M), 3- (3M), 4- (4M), 5- (5M), 6- (6M), 9- (9M), 12- (12M) and 15-month-old (15M) bank vole males (means ± S.E.M.). Number of males in each age group = 10. Means marked by the same letters differ significantly at A-O - $p < 0.01$.

In the present study we also showed that adult bank vole males (3-, 4- and 5-month-old) produced more spermatozoa in comparison to prepubertal and aged males. Considering all parameters assessing epididymal sperm functional activity, i. e. its motility (Fig.3a) , viability (Fig. 4a), and maturity (Fig. 4b), mature males produced sperm of better functional activity. Their sperm

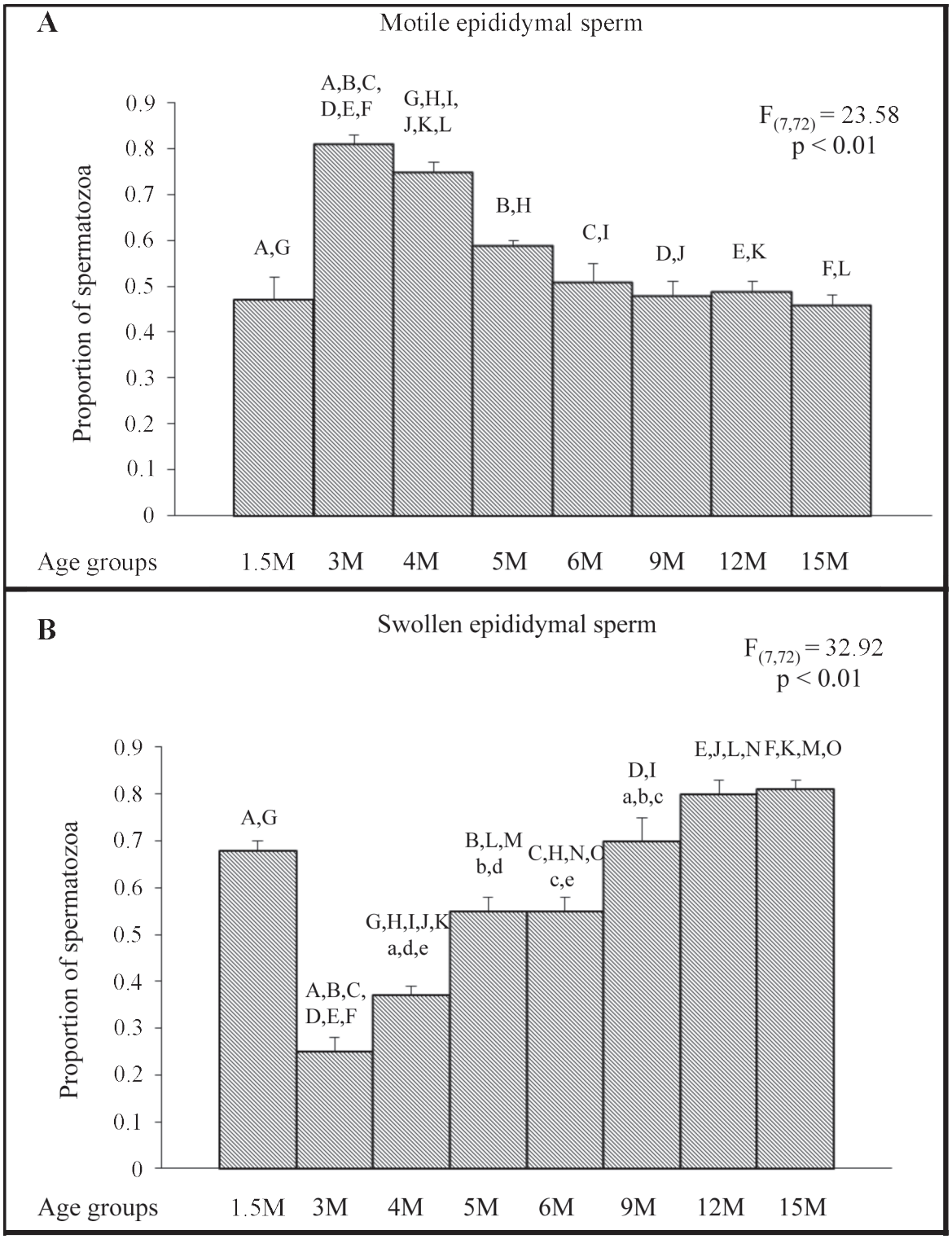


Fig. 3. – Proportions of motile (a) and swollen (b) epididymal sperm in 1.5- (1.5M), 3- (3M), 4- (4M), 5- (5M), 6- (6M), 9- (9M), 12- (12M) and 15-month-old (15M) bank vole males (means ± S.E.M.). Number of males in each age group = 10. Means marked by the same letters differ significantly at A-O - $p < 0.01$, a-e - $p < 0.05$.

also showed fewer morphological abnormalities and higher tail membrane integrity (Fig. 3b). Decline in daily sperm production with ageing has been also noted in Brown-Norway rats with complete cessation of spermatogenesis in very old males (WANG et al., 1993). The opposite

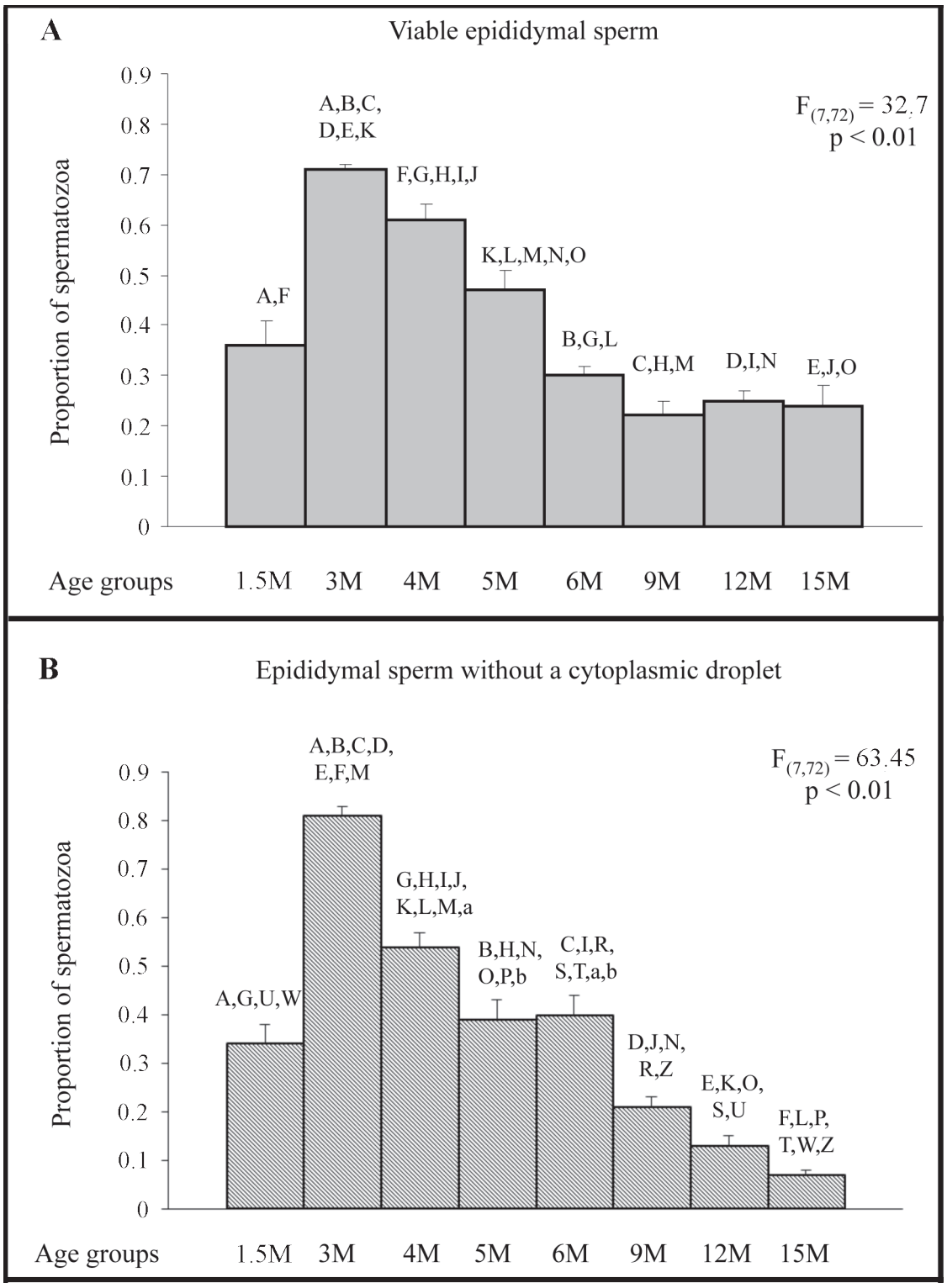


Fig. 4. – Proportions of viable epididymal sperm (a) and epididymal sperm without cytoplasmic droplet (b) in 1.5- (1.5M), 3- (3M), 4- (4M), 5- (5M), 6- (6M), 9- (9M), 12- (12M) and 15-month-old (15M) bank vole males (means ± S.E.M.). Number of males in each age group = 10. Means marked by the same letters differ significantly at A-Z - $p < 0.01$, a-b - $p < 0.05$.

pattern was observed in Spraque-Dowley rats, which did not show progressive decrease in sperm concentration with age (JOHNSON & NEAVES, 1983). Bank voles in captivity live till about 18 months and reproduce till 15 months although their reproductive activity significantly decreased with age (from 6 month) (breeding observation M. Kruczek). In our experiment older bank vole males (to 15 month) do not cease spermatogenesis totally but produce sperm of lower quality.

Progressive movement generated by flagella is necessary for the sperm to reach the ovum in the oviduct and to initiate fertilization (MORTIMER, 1997). In our experiments, the swelling of bank vole spermatozoa, in response to hypo-osmotic solution, was lowest in sperm of adult, 3- and 4-month-old. A strong negative relationship between a high score in the hypo-osmotic test and pregnancy rates has been observed in men (TARTAGNI et al., 2004). On the other hand there was no such correlation in mice (STYRNA & KRZANOWSKA, 1995). As bank voles are more closely related to mice it can be expected that spermatozoa swelling will not have strong influence on pregnancy rate. This aspect of bank vole reproduction has not been investigated before and deserves further study using eg. Sperm Select Penetration Test (STYRNA & KRZANOWSKA 1995).

For several mammalian species correlation between a high proportion of spermatozoa with attached droplets and infertility has been documented (GATTI et al. 2004; COOPER, 2005). Fertilizing potential of spermatozoa depends on their ability to undergo capacitation and acrosome reaction and only viable spermatozoa with intact cell membrane are able to achieve this (YANAGIMACHI, 1981).

As described in Materials and Methods, four categories of misshapen form can be distinguished in bank vole sperm. The most common abnormality is class 1. In this class, acrosomal parts of the head are shorter with very weak staining, which suggests the absence of enzymatic content, which in turn may lead to disorders in acrosomal function and consequent lower male fertility as has been shown in mice (STYRNA et al., 1991, 2002)

Spermatozoa with abnormalities in the distal part of the head, those with serious damage in the proximal part of the head, and spermatozoa with elliptical heads were rarely observed in the bank vole males from each tested age group. Since they did not exceed 0.5% of the total spermatozoa they were pooled together in one class of "other abnormalities". Our results showed that ageing in bank voles produces an increase in the frequency of altered sperm with acrosomal damage as well as in the number of other abnormal forms of spermatozoa. In hamsters, the number of abnormal spermatozoa dramatically increased in advanced-age males from 14-19% in adult to 39-50% in old males, but this increase was due more to an increase in the frequency of altered sperm than through an increase of the other misshapen forms (CALVO et al., 1997). How the misshapen head morphology influences the fertility rate is still under discussion but it is generally accepted that specific head abnormalities correlate with male infertility (for reviews see WEISSENBERG et al., 1987; PESCH & BERGMANN, 2006).

## ACKNOWLEDGEMENT

This work was supported by grants from the Polish State Committee for Scientific Research (DS/V/WBiNoZ/UJ/757/09). The authors are grateful to Dorota Buda-Lewandowska, Paulina Haltman and Magdalena Guzik for their technical assistance.

## REFERENCES

ASTOLFI P, DE PASQUALE A & ZONTA LA (2004). Late paternity and stillbirth risk. Human Reproductive, 19:2497-2501.

BASKIN SI, ROBERTS J & KENDRICK ZV (1979). Effect of age on body weight, heart rate and blood pressure in pair-caged male Fisher 344 rats. Age, 2:47-50.

Bujalska G (1973). The role of spacing behaviour among females in the regulation of reproduction in the bank vole. Journal of Reproduction and fertility. Supplement, 19:465-474.

Calvo A, Martinez E, Pastor L, Vazquez J & Roca J (1997). Classification and quantification of abnormal sperm along the epididymal tract. Comparison between adult and aged hamsters. Reproduction Nutrition Development, 37:661-673.

Chen H, Hardy MP & Zirkin BR (2002). Age-related decreases in Leydig cell testosterone production are not restored by exposure to LH in vitro. Endocrinology, 143:1637-1642.

Clarke JR (1981). Physiological problems of seasonal breeding in eutherian mammals. In: Finn CA (ed), Oxford Reviews of Reproductive Biology 3, Clarendon Press, Oxford:244-312.

Cooper TG (2005). Cytoplasmic droplets: the good, the bad or just confusing. Human Reproductive, 20:9-11.

Franca LR, Suescun MO, Miranda JR, Giovambattista A, Perello M, Spinedi E & Calandra RS (2006). Testis structure and function in a nongenetic hyperadipose rat model at prepubertal and adult ages. Endocrinology, 147:1556-1563.

Gatti JL, Castella S, Dacheux F, Ecroyd H, Metayer S, Thimon V & Dacheux JL (2004). Post-testicular sperm environment and fertility. Animal Reproduction Sciences, 82-83:321-339.

Green EL (1966). Breeding systems. In: Green EL (ed), Biology of the Laboratory Mouse. McGraw-Hill Book Company, New York:13-14.

Hardy MP & Schlegel PN (2004). Testosterone production in the aging male: where does the slowdown occur? Endocrinology, 145:4439-4440.

Johnson L & Neaves WL (1983). Enhanced daily sperm production in the remaining testis of aged rats following hemicastration. Journal of Andrology, 4:162-166.

Katz-Jaffe MG, Parks J, McCallie B & Schoolcraft WB (2013). Aging sperm negatively impacts in vivo and in vitro reproduction: a longitudinal murine study. Fertility and Sterility, 100:262-268.e2

Kruczek M (1986). Seasonal effects on sexual maturation of male bank voles (*Clethrionomys glareolus)*. Journal of Reproduction & Fertility, 76:83-89.

Kruczek M & Styrna J (2009). Semen quantity and quality correlate with bank vole males' social status. Behavioural Processes, 82:279-285.

Marchlewska-Koj A (2000). Olfactory and ultrasonic communication in bank voles. Polish Journal of Ecology, 48:11-20.

Marchlewska-Koj A, Kruczek M & Olejniczak P (2003). Mating behaviour of bank voles (Clethrionomys glareolus) modified by hormonal and social factors. Mammalian Biology , 68:144-152.

Mortimer ST (1997). A critical review of the physiological importance and analysis of sperm movement in mammals. Human Reproduction Update, 3:403-439.

Parkening TA, Collins TJ & Au WW (1988). Paternal age and its effects on reproduction in C57BL/6NNia mice. Journals of Gerontology, 43:79-84.

Pesch S & Bergmann M (2006). Structure of mammalian spermatozoa in respect to viability, fertility and cryopreservation. Micron, 37:597-612.

Ratkiewicz M & Borkowska A (2000). Multiple paternity in the bank vole (*Clethrionomys glareolus*): field and experimental data. Mammalian Biology, 65:6-14.

Sawadogo SP, Diabaté A, Toé HK, Sanon A, Lefevre T, Baldet T, Gilles J, Simard F, Gibson G, Sinkins S, Dabiré RK (2013). Effects of age and size on Anopheles gambiae s.s. male mosquito mating success. Journal of Medical Entomology, 50:285-293.

Seed J, Chapin RE, Clegg ED, Dostal LA, Foote RH, Hurtt ME, Klinefelter GR, Makris SL, Perreault SD, Schrader S, Seyler D, Sprando R, Treinen KA, Veeramachaneni DN & Wise LD (1996). Methods for assessing sperm motility,morphology, and counts in the rat, rabbit, and dog: a consensus report. Reproductive Toxicology, 10:237-244.

Serre V & Robaire B (1998). Paternal age affects fertility and progeny outcome in the Brown Norway rat. Fertility & Sterility, 70:625-631.

Slama R, Bouyer J, Windham G, Fenster L, Werwatz A & Swan SH (2005). Influence of paternal age on the risk of spontaneous abortion. American Journal of Epidemiology, 161:816-823.

Sloter E, Nath J, Eskenazi B & Wyrobek AJ (2004). Effects of male age on the frequencies

of germinal and heritable chromosomal abnormalities in humans and rodents. Fertility & Sterility, 81:925-942.

STYRNA J, BILIŃSKA B & KRZANOWSKA H (2002). The effect of a partial Y chromosome deletion in B10.BR-Ydel mice on testis morphology, sperm quality and efficiency of fertilization. Reproduction, Fertility & Development, 14:101-108.

STYRNA J, IMAI, HT & MORIWAKI K (1991). An increased level of sperm abnormalities in mice with a partial deletion of the Y chromosome. Genetics Research, 57:195-199.

STYRNA J, KILARSKI W & KRZANOWSKA H (2003). Influence of the CBA genetic background on sperm morphology and fertilization efficiency in mice with a partial Y chromosome deletion. Reproduction, 126:579-588.

STYRNA J & KRZANOWSKA H (1995). Sperm select penetration test reveals differences in sperm quality in strains with different Y chromosome genotype in mice. Archives of Andrology, 35:111-118.

TARTAGNI M, CICINELLI E, SCHONAUER MM, CAUSIO F, PETRUZZELLI F & LOVERRO G (2004). Males with subnormal hypo-osmotic swelling test scores have lower pregnancy rates than those with normal scores when ovulation and timed intercourse is used as a treatment for mild problems with sperm count, motility, or morphology. Journal of Andrology, 25:781-783.

WALCZAK R STRUMILLO E & KULA K (1994). Eosin and water tests and results of conventional semen analysis. Ginekologia Polska, 65:99-102.

WANG C, LEUNG A & SINHA-HIKIM AP (1993). Reproductive aging in the male brown-Norway rat: a model for the human. Endocrinology, 133:2773-2781.

WEISSENBERG R, BELLA R & LUNENFELD B (1987). The fertilization capacity of golden hamster epididymal spermatozoa in relation to age, number, motility and morphology. Andrologia, 19:47-53.

WOLF KN, WILDT DE, VARGAS A, MARINARI PE, KREEGER JS, OTTINGER MA & HOWARD JG (2000). Age-dependent changes in sperm production, semen quality, and testicular volume in the black-footed ferret (*Mustela nigripes*). Biology of Reproduction, 63:179-187.

YANAGIMACHI R (1981). Mechanisms of fertilization in mammals. In: Mastroianni L&, Biggers JD (eds). Fertilization and embryonic development in vitro. Plenum Press. New York:81-182.

YLÖNEN H, HORNE T.J & LUUKKONEN M (2004). Effect of birth and weaning mass on growth, survival and reproduction in the bank vole. Evolutionary Ecology Research, 6:1-10.

ZHU JL, VESTERGAARD M, MADSEN KM & OLSEN J (2008). Paternal age and mortality in children. European Journal of Epidemiology, 23:43-447.

*Received: February 28th, 2013*

*Accepted: September 17th, 2013*

*Branch editor: Huybrechts Roger*