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Semen quality parameters in outbred male mice from four different selected lines

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Abstract: *Semen quality parameters in outbred male mice from four different selected lines.* Breeding of (outbred) selective lines of laboratory mouse was initiated in Warsaw University of Life Sciences about 40 years ago. It bred Heavy (C) and Light (L) mice selected opposite for body weight at weaning (21st day of life), S mice line selected for higher testes weight, and control (K) mice without selection. All lines have identical genetic background, but different directions of selections caused diversification of specific phenotypic traits between them. The purpose of this study was to compare semen quantity and quality parameters in outbred C, K, L and S male mice in the context of measurements of average body and testes weight for each line.
Research materials were seminal fluids squeezed out of the vas deferens from 20 outbred C, K, L and S male mice (5 males per group). Animals had been euthanized, and necropsy was performed. Body and testes weight was measured. Also sperm concentration, viability (by Eosin test), cytoplasmic membrane integrity degree (HOS test), sperm head morphology and maturity were estimated.
It was shown that S male mice, which have much higher testes weight, also have a significant increase of viable spermatozoa according to control line. Moreover, sperm concentration from S males is at least two times higher than in other selective lines.

Key words: semen quality parameters, HOS, selective lines, Eosin test, mouse

INTRODUCTION

Breeding of selected lines of laboratory mouse was initiated in The Department of Genetics and Animal Breeding, Warsaw University of Life Sciences 43 years ago (Sławiński 1974). It bred Heavy (C) and Light (L) mice selected opposite for body weight, S mice line selected for higher testes weight and control (K) mice without selection. Next, lines were bred also in The Department of Genetics, The Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology in Warsaw, where it started getting inbred stocks from them, but until now none of selective lines is homozygotic inbred strain (G8-G13, depending on line).

All lines have identical genetic background, but different directions of selections caused diversification of specific phenotypic traits between them (Wirth-Dzięciołowska et al. 2005). It was showed differences in reproduction traits such as the ovulation rate, prenatal mortality and embryo number (Wirth-Dzięciołowska 1973). It was also correlation between body weight and lifetime reproduction

rate as well as time of maturation (Wirth-Dzięciołowska et al. 1996). Moreover L mice lived longer than C mice (Wirth-Dzięciołowska and Czumińska 2000).

MATERIAL AND METHODS

The purpose of this study was to compare sperm quantity and quality parameters in outbred C, K, L and S male mice in the context of measurements of average body and weight for each line according to previous studies (Wirth-Dzięciołowska 1973).

Tests were carried out in The Department of Genetics, The Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology in Warsaw.

Animals

Five adult (3–6 months old) males from each of the C, K, L and S lines were used in the study. Animals were maintained in a barrier facility (constant light cycle – 12L/12D, room temperature about 23°C, water and food (Labofeed H) were available *ad libitum*). It were mice post-selected, obtained from the herd.

Necropsy and morphometrical parameters

Male mice been euthanized by cervical dislocation, and necropsy was performed. Body and testes weight was measured (±0,001 g). Research materials were seminal fluids squeezed out of the vas deferens into 100 µl of M2 Medium – common medium used for *in vitro* culture of preimplantation stage embryos. It can be used for collecting viable gametes outside a CO_2 incubator and it also extend sperm fluids.

Sperm quality parameters

Tests were performed per 200 spermatozoa (Krzanowska 1962), and analyzed under light microscope – Olympus, type B091 and B201. Percentage of sperm without cytoplasmic droplet (mature spermatozoa), sperm with proximal (immature spermatozoa) and distal droplet (during maturation, but normal) were counted from fresh semen suspension (400× magnification).

First portion of suspension (10 µl) was diluted (1 : 5, v/v) with sterile water (50 µl) and integrity of cytoplasmic membrane of sperm tails was carried out by Hypoosmotic Swelling Test (HOS). Percentage of spermatozoa with integral membrane (swollen tails) and without integral cytoplasmic membrane (broken and straight tails) was counted (400× magnification).

Second portion of semen suspension (10 µl) and 0,2% eosin Y (10 µl) was mixed (1 : 1, v/v) and incubated for 10 min. Sperm viability was elucidated (Eosin Test), by counting of percentage of dead (red) and alive (green) sperm head (400× magnification).

Next, 10 µl of the sperm suspension was extended (1 : 5, v/v) with PBS (50 µl) and sperm number was evaluated per at least 5 large squares and at least 200 spermatozoa were counted in a haemocytometer.

Sperm head morphology was analyzed under 1,000× magnification. Smears of sperm suspension was fixed in a mixture of 99.8% ethanol and acetic acid (3 : 1, v/v) and stained with 1% eosin Y for 15 min. Percentage of normal sperm head, and abnormal spermatozoa was counted with classification of sperm head abnormalities by Krzanowska (1976).

The statistical significance of differences between the analyzed lines were calculated by ANOVA (IBM SPSS Statistics 21).

RESULTS AND DISCUSSION

Figure 1 shows the mean C, K, L, S male mice body weight. It was noted significant differences between average body weight of all lines. C (43.87 g) and S (35.96 g) lines were characterized by the highest body weight as well as L line (25.45 g) had the lowest body weight.

The highest testes weight (Fig. 2) was recorded in S males (0.56 g) in the main direction of this line selection, which was more than 2 times higher than in C line and more than 3 times higher than in the K and L male mice.

S male mice was characterized by 4 times higher sperm concentration $162.19 \cdot 10^6$/ml (Fig. 3) in comparison with C ($37.86 \cdot 10^6$/ml) and K line ($39.55 \cdot 10^6$/ml). Level of this parameter in L mice was about 1.5 times higher than in control and C males ($68.31 \cdot 10^6$/ml), but it was more than 2 times lower in relation to S males.

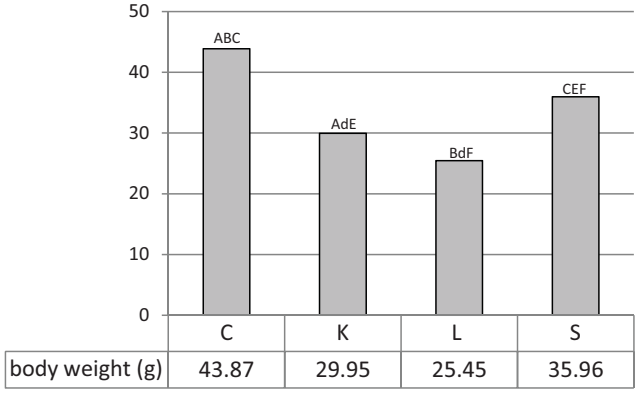


FIGURE 1. Average body weight (g) of selected mice lines: Heavy (C), Control (K), Light (L) and Testes Weight (S). ABCEF at $P \leq 0.01$, d at $P \leq 0.05$

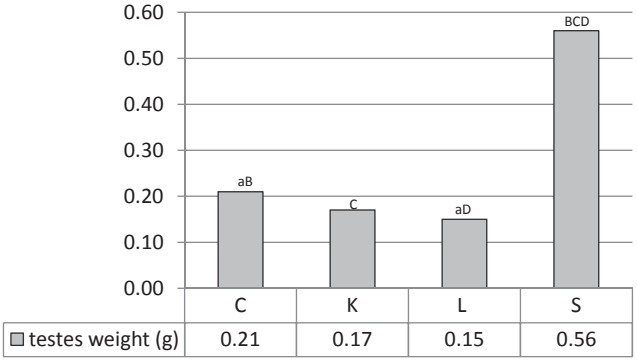


FIGURE 2. Average testes weight (g) of selected mice lines: Heavy (C), Control (K), Light (L) and Testes Weight (S). BCD at $P \leq 0.01$, a at $P \leq 0.05$

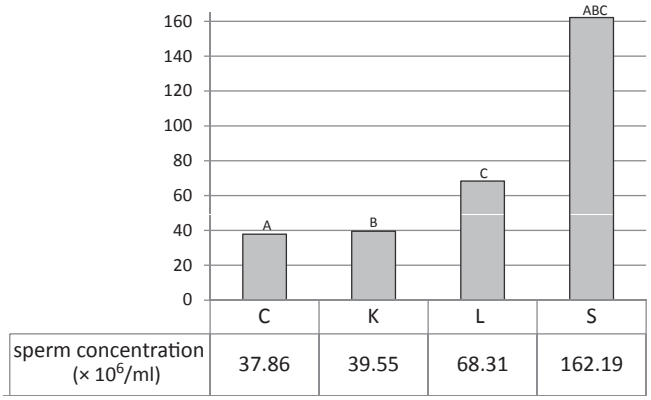


FIGURE 3. Sperm concentration [$\times 10^6$/ml] of selected mice lines: Heavy (C), Control (K), Light (L) and Testes Weight (S). ABC at $P \leq 0.01$

The study of sperm head morphology (Fig. 4) shows the highest percentage of morphologically normal S male mice sperm heads (89%). This parameter in C, K and L lines oscillated between 79.9––83.3%. Higher completely mature sperm (Fig. 4) percentage was detected mainly in the semen fluids of K and S male mice (60.9–62.35%), than in L and C male (respectively 47.07 and 47.9%), but observed differences were not statistically significant.

The results of HOS test and Eosin test (Fig. 5) showed that the largest sperm viability (alive, swollen tails) were observed in S male mice (alive – 59.70%, swollen tails – 71.70%). The differences among other lines, C (55.15%; 66.65%), K (50.85%; 61.20%) and L (55.90%; 71.70%), were smaller and not statistically significant.

Reproduction is one of the most important functions of the free-living and breeding organisms. Thus regular assessment of fertility parameters in

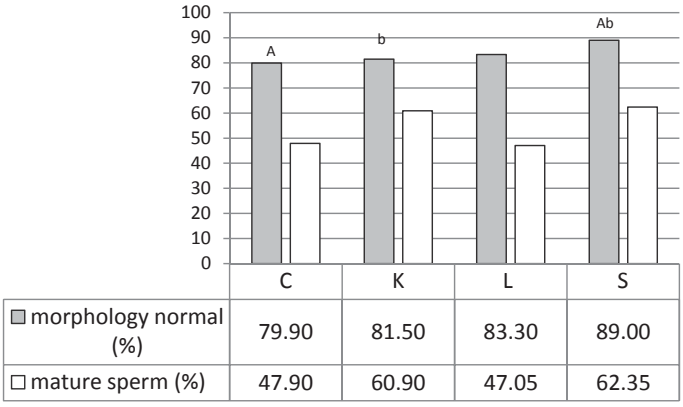
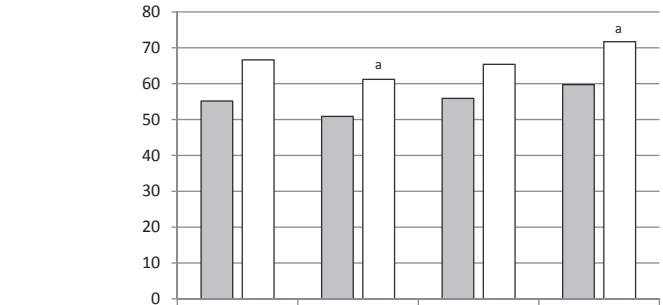


FIGURE 4. Sperm head morphology and tail membrane integrity of selected mice lines: Heavy (C), Control (K), Light (L) and Testes Weight (S). A at $P \leq 0.01$, b at $P \leq 0.05$



	C	K	L	S
alive sperm (%)	55.15	50.85	55.90	59.70
swollen tails (%)	66.65	61.20	65.40	71.70

FIGURE 5. Sperm viability (Eosin test and HOS test) of selected mice lines: Heavy (C), Control (K), Light (L) and Testes Weight (S). a at $P \leq 0.05$

animals seems to be necessary to maintain high-quality reproductive herd (Bakker et al. 1978). Assessing reproductive parameters is also of utmost importance in genetic control of outbred herds.

Sperm quality parameters were analyzed to determine viability, motility and maturity of spermatozoa, cytoplasmic membrane integrity of tails (Krzanowska 1962, Gołas et al. 2011), sperm head morphology (Krzanowska 1976) as well as sperm concentration was counted.

Our study reveals that K and C male mice have rather "basal" level of sperm parameters, nearing inbred strains parameters (Gołas et al. 2011). L males fertility parameters are gently higher in relation with C and K results, which can make them effective males in reproduction. S male mice have the highest sperm quality parameters and sperm concentration.

Testes size may affects sperm production, so in bigger testes much higher number of spermatozoa is produced (Le Roy et al. 2001, Gołas et al. 2011). Our tests shows that in S males, which are selected for high testes weight, at least 2.5 times more spermatozoa is produced, which is also alive and motile with normal morphology. It should also be noted that testis weight could be correlated ($r = 0.3$) with a body weight of lines analyzed. However, in other publications there found no correlation between these morphologically parameters (Hill et al. 1990, Le Roy et al. 2001). C and L line, which is selected for high and low body weight, respectively have also higher and lower testes weight, but probably these results are only the line effect and false correlation is caused by small number of animals in analyzed groups.

Probably long-term selection (more than 100 generations) can not affect sperm quality directly (Wirth-Dzięciołowska 1992). L males have smaller testes than C and K males, but L male mice semen quality parameters are higher than C and K average level.

Nevertheless, S males selected for high testes weight have also higher body weight and fertility levels which can be close to the maximum. Significantly better sperm parameters of S males are the effect of an increase of selection for

testes weight, what was confirmed by Hill et al. (1990) and Le Roy et al. (2001).

CONCLUSIONS

Increase of testes weight causes much higher sperm concentration and increase semen quality parameters in S males in comparison with C, K and L mice. According to our investigations, long-term selection for body weight seems not affect directly qualitative and quantitative parameters of semen in male mice.

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Streszczenie: *Parametry jakości plemników samców myszy z czterech outbredowych linii selekcyjnych.* Celem przeprowadzonych badań było dokonanie oceny jakości parametrów plemników nasieniowodowych, pobranych od 20 samców (po 5 samców z linii) myszy z linii selekcjonowanych przez wiele pokoleń: przeciwstawnie na masę ciała (C i L), masę jąder (S) oraz samców stanowiących linię kontrolną (K), w kontekście pomiarów średnich mas ciała i jąder dla poszczególnych linii. Materiał badawczy stanowiły

plemniki pobrane z nasieniowodów od zwierząt poselekcyjnych. Oszacowano liczbę plemników w 1 ml pożywki (M2). Dokonano analizy parametrów jakości plemników, wykonując test oceny żywotności plemników, test położenia kropli cytoplazmatycznej, który jest miarą dojrzałości plemników oraz test hipoosmotyczny (HOS) do oceny integralności błony cytoplazmatycznej witek plemników. Ponadto dokonano oceny morfologii główek plemników. Wykazano, że samce linii S w porównaniu z osobnikami z linii kontrolnej K oraz linii ciężkiej (C) i lekkiej (L) mają istotnie większą masę jąder, większy odsetek dojrzałych i żywotnych plemników, a także 2–4--krotnie większą koncentrację plemników liczoną w 1 ml medium.

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