Number of spermatozoa in the spermatheca of honey bee queens inseminated with small doses of semen and kept in an incubator in cages with different numbers of workers

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Summary

In mass production, instrumentally inseminated honey bee queens are usually kept in mailing cages with a low number of workers and inseminated with small doses of semen. Inseminated queens should have more than 4 mln sperms in their spermatheca. The aim of this study was to investigate the number of spermatozoa entering the spermatheca of queens inseminated with different small doses of semen. Ninety queens were divided into six groups and inseminated as follows: 1 μ l, 2 μ l or 3 μ l of semen at the age of 7 days and 2 $\times$ 1 μ l, 2 $\times$ 2 μ l or 2 $\times$ 3 μ l at the age of 7 and 9 days. Queens were kept in the mailing cages with 15 or 25 attendant bees before and after instrumental insemination. No significant differences were found in mortality and the condition of oviducts of queens inseminated with different small doses of semen and kept in cages with 15 or 25 workers. Queens inseminated with 2 $\times$ 2 μ l of semen had significantly less sperms in their spermatheca than those inseminated with 2 $\times$ 3 μ l but significantly more than 2 $\times$ 1 μ l and 1 μ l, 2 μ l or 3 μ l. The smallest dose of semen used for instrumental insemination of honey bee queens should be 2 $\times$ 3 μ l. Significant differences in the number of spermatozoa in the spermatheca of queens kept with 15 or 25 attendant bees were not found.

Keywords: honey bee queens, instrumental insemination, number of spermatozoa in spermatheca

Collection of honey bee drone semen is time consuming. For this reason, in mass production honey bee queens are usually inseminated with small doses of semen. Naturally mated queens store in their spermatheca 4 to 7 million sperm cells (5). Therefore, well inseminated instrumentally queens should have also at least 4 million spermatozoa. However Wilde (11) did not observe any differences in the strength of colonies during the entire season and honey production on the brood area and productivity in the colonies with instrumentally inseminated queens with a dose of 8, 4 and only 2 μ l of semen. The higher the dose of semen, the higher the number of spermatozoa in the spermatheca but the dose of 8 μ l is sufficient (14, 15). However, Bieńkowska et al. (1) received significantly more spermatozoa in queens inseminated with 3, 4 and 6 μ l of semen than with 8 μ l. Double insemination is more effective. Queens inseminated once have less sperms than those inseminated twice with the same divided dose of semen (1, 9, 14, 15). Gąbka (6) stated that queens inseminated once with

10 μ l of semen have similar number of sperms in their spermatheca as those inseminated with 2 $\times$ 2 μ l but significantly less than those inseminated with 2 $\times$ 4 μ l of semen. Two inseminations with each dose of 6 μ l give even better results than one insemination with 20 μ l (15). The access of attendant bees significantly affects the number of spermatozoa in the spermatheca (12, 13, 16, 18). It is best if queens are kept with at least 350 workers after insemination (16, 17). The access of workers also affects the clearing of excess semen from oviducts (16). According to Gontarz et al. (8) queens should be kept with attendant bees even before insemination because the period prior to insemination affects condition of their oviducts after insemination. However Wilde (10) did not observe any differences on the brood area and productivity in the colonies with instrumentally inseminated queens kept before and after insemination in different conditions.

The aim of this study was to investigate the number of spermatozoa entering the spermatheca of queens

inseminated with different small doses of semen and kept in cages with different number of workers.

Material and methods

The experiment was performed in the Apiculture Division of the Warsaw University of Life Sciences in 2013. Queens rearing was conducted in three strong *A. m. carnica* colonies with the method described by Büchler (4). The larvae grafted to rear queens were less than one day old. Queens emerged in incubator and after one day were put into the mailing cages with worker bees. Ninety *A. m. carnica* queens were analysed (Tab. 1). All queens originated from one reproductive queen. They were divided into six groups and inseminated as follows: 1 µl, 2 µl or 3 µl of semen at the age of 7 days and 2 × 1 µl, 2 × 2 µl or 2 × 3 µl of semen at the age of 7 and 9 days. Before and after instrumental insemination queens were kept in cages with 15 or 25 attendant bees and held in an incubator at 28°C. Directly after insemination, the queens were introduced into cages with new workers. The queens were killed three days after insemination, the condition of oviducts was checked and the number of spermatozoa was counted by a method described by Woyke (13).

Two-way ANOVA was carried out. The significance of differences between the means was determined by the Duncan's multiple range test. The difference in the number of dead queens in each group as well as the condition of oviducts was analyzed using χ^2 test.

Tab. 1. Mortality of queens

Semen dose	Number of						% of dead queens		
	inseminated queens			dead queens					
	A	B	Overall	A	B	Overall	A	B	Overall
1 µl	8	7	15	0	0	0	0	0	0
2 × 1 µl	7	8	15	0	1	1	0	12.5	6.7
2 µl	8	7	15	0	0	0	0	0	0
2 × 2 µl	7	8	15	1	0	1	12.3	0	6.7
3 µl	8	7	15	0	0	0	0	0	0
2 × 3 µl	7	8	15	0	1	1	0	12.5	6.7
Overall	45	45	90	1	2	3	2.2	4.4	3.3

Explanations: A – cages with 15 workers, B – cages with 25 workers

Tab. 2. Condition of the oviducts of queens

Semen dose	Number of						% of queens with semen residue in oviducts		
	inseminated queens			queens with semen residue in oviducts					
	A	B	Overall	A	B	Overall	A	B	Overall
1 µl	8	7	15	0	0	0	0	0	0
2 × 1 µl	7	7	14	0	0	0	0	0	0
2 µl	8	7	15	0	0	0	0	0	0
2 × 2 µl	6	8	14	2	0	2	33.3	0	14.3
3 µl	8	7	15	0	1	1	0	14.3	6.7
2 × 3 µl	7	7	14	1	1	2	14.3	14.3	14.3
Overall	45	43	87	3	2	5	6.8	4.6	5.7

Explanations: as in Tab. 1

Results and discussion

Queen mortality. Among 90 inseminated queens, 3 (3.3%) died. In each group of queens inseminated twice, one queen died and of those inseminated once no queen died. The differences were not statistically significant ($\chi^2 = 3.103$, $df = 5$, $p = 0.684$) (Tab. 1). A similar percentage (0-5.7) of dead queens after instrumental insemination with small doses of semen was obtained by Bieñkowska et al. (1).

Statistically significant differences between the mortality of queens kept with 15 or 25 workers were also not found ($\chi^2 = 0.345$, $df = 1$, $p = 0.557$) (Tab. 1). This confirms the investigations of Gąbka and Cobey (7). The attendance of workers after instrumental insemination of queens is necessary. Otherwise some queens will die (16). This also occurs when queens are kept in cages with a small number of bees (1-3).

Condition of oviducts. Among 87 queens, 5 (5.7%) had residues of semen in their oviducts after 3 days following insemination (Tab. 2). In groups 2 × 2 µl and 2 × 3 µl, 14.3% queens had residues of semen and in groups 1 µl, all queens in groups 2 × 1 µl and 2 µl had clear oviducts. Statistically significant differences between groups were not found ($\chi^2 = 6.475$, $df = 5$, $p = 0.263$). In the investigations of Bieñkowska et al. (1), all queens inseminated with 1 µl, 2 × 1 µl, 2 µl and 2 × 3 µl had clear oviducts but some of those inseminated with 2 × 2 µl and 3 µl did not clear the excess of semen from the oviducts (4 and 18%, respectively).

The clearance of oviducts was not affected by the number of attendant worker bees ($\chi^2 = 0.189$, $df = 1$, $p = 0.664$) (Tab. 2). Gąbka and Cobey (7) also stated that the number of queens with residue of semen in their oviducts did not significantly differ between the queens kept in cages with 15 or 25 workers. Queens kept after insemination without workers did not clear the excess of semen from oviducts (16). However, some queens kept in cages with a small number of bees also have residues of semen in their oviducts (1-3, 8).

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Number of spermatozoa in the spermatheca. Two-way ANOVA showed that the number of spermatozoa in the spermatheca was affected by the dose of semen ($F_{5,86} = 26.105$, $P < 0.001$). However, it was not affected by the number of attendant workers ($F_{1,86} = 0.026$, $P = 0.871$). Interaction between the two factors was not found to be significant ($F_{5,86} = 0.128$, $P = 0.986$).

Queens inseminated with 2 × 2 µl of semen had significantly less

Tab. 3. Number of spermatozoa in the spermatheca of queens inseminated with different doses of semen

Dose of semen	Number of queens	Mean $\pm$ SE	Min-Max
1 μ l	15	1.4 $\pm$ 0.13 ^a	0.5-2.3
2 $\times$ 1 μ l	14	2.2 $\pm$ 0.15 ^b	1.3-3.2
2 μ l	15	1.9 $\pm$ 0.16 ^{ab}	0.8-2.9
2 $\times$ 2 μ l	14	3.6 $\pm$ 0.23 ^c	1.3-4.6
3 μ l	15	2.4 $\pm$ 0.22 ^b	0.9-3.7
2 $\times$ 3 μ l	14	4.2 $\pm$ 0.29 ^d	2.0-5.7
Overall	87	2.6 $\pm$ 0.13	0.5-5.7

Explanations: a, b, c, d – means with superscript different letters differ significantly at $p < 0.05$

sperms (3.6 mln) in their spermatheca than those inseminated with $2 \times 3 \mu$ l (4.2 mln) but significantly more than those inseminated with $2 \times 1 \mu$ l (2.2 mln) and 1 μ l (1.4 mln), 2 μ l (1.9 mln) or 3 μ l (2.4 mln) (Tab. 3). In the investigations of Bieńkowska et al. (1), the number of spermatozoa in the spermatheca of queens inseminated with $2 \times 2 \mu$ l and $2 \times 3 \mu$ l did not differ significantly and was much higher (6.27 and 6.07 mln, respectively) than that obtained in the present study. We stated that the smallest dose of semen used for instrumental insemination of honey bee queens should be $2 \times 3 \mu$ l. The same dose was recommended by Mackensen (9). The results obtained here confirm other investigations (3, 14, 15) that an increase of the volume of semen resulted in a higher number of spermatozoa in the spermatheca.

Queens kept in cages with 15 or 25 workers had similar number of spermatozoa in their spermatheca (2.5 and 2.7 mln, respectively) (Tab. 4). It confirms the results obtained by Gąbka and Cobey (7). Woyke (13) did not state any differences in the number of sperms in spermatheca of queens kept in cages without workers or with only 10 workers. There was also no difference between 20 and 40 attendant bees, but increasing the number of workers to 80 resulted in a significant increase of the number of spermatozoa in the spermatheca (17, 18).

There are no significant differences in the mortality and condition of oviducts of queens inseminated with different small doses of semen and kept in cages with 15 or 25 workers. Honey bee queens inseminated with $2 \times 3 \mu$ l of semen had significantly more spermatozoa in their spermatheca than those inseminated with $2 \times 2 \mu$ l. The smallest dose of semen used for instrumental insemination of honey bee queens should be $2 \times 3 \mu$ l.

Tab. 4. Number of spermatozoa in the spermatheca of queens kept in cages with different number of workers

Number of workers in cage	Number of queens	Mean $\pm$ se	Min-Max
15	44	2.5 $\pm$ 0.19	0.5-5.5
25	43	2.7 $\pm$ 0.19	0.8-5.7
Overall	87	2.6 $\pm$ 0.13	0.5-5.7

The number of spermatozoa in the spermatheca of instrumentally inseminated queens kept in cages with 15 or 25 workers did not significantly differ.

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