THE INFLUENCE OF HONEY BEE (*APIS MELLIFERA*) DRONE AGE ON VOLUME OF SEMEN AND VIABILITY OF SPERMATOZOA

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S u m m a r y

A comparison was done of the volume of semen and viability of spermatozoa collected from drones at ages 15, 20, 25 and 30 days. The drones originated from different queens and were reared in different environments. Semen volume was determined by measuring the filled length of a capillary. Percentages of live and dead spermatozoa were determined by SYBR-14/propidium iodide fluorescence staining and flow cytometry. The volume of semen collected from drones ranged from 0.5 to 1.3 µL. The mean volume of semen significantly decreased with drone age. Sperm viability increased significantly with drone age.

Keywords: *Apis mellifera*, honey bee, drone, age, sperm viability.

INTRODUCTION

Honey bee (*Apis mellifera*) drones develop from unfertilized eggs. Their development takes 24 days: the egg 3 days, larva 6 days, prepupa and pupa 15 days (Winston, 1987). Spermatogenesis starts at the larval stage and spermiogenesis ends at the pupal stage (Bishop, 1920; Hoage and Kessel, 1968). During the first week of adult life, spermatozoa are transferred from the testes to seminal vesicles, where they are stored until copulation (Snodgrass, 1956). The testes are largest during the pupal stage, and later degenerate (Snodgrass, 1956; Winston, 1987; Page and Peng, 2001).

Drones are not able to copulate immediately after emergence. Copulation and transfer of the spermatozoa from the seminal vesicles to the everted endophallus becomes possible when drones are 10-12 days old (Woyke, 1955; Woyke and Ruttner, 1958, 1976). As drones age, the color and viscosity of their semen changes (Woyke and Jasiński, 1978; Cobey, 2007). The semen of 2-week-old drones is yellowish and fluid; the semen of 4-week-old drones is brown and more viscous. Spermatozoa of old drones are less viable but their motility does not change (Locke and Peng, 1993).

Drones aged 10-21 days are considered most suitable for natural and artificial insemination (Woyke and Ruttner, 1958; Woyke and Jasiński, 1978; Harbo, 1986). Drones younger than 10 days are immature, and the semen of those older than 21 days is too viscous. Queens inseminated with viscous semen transfer fewer spermatozoa in their spermatheca and have difficulty expelling excess semen from their oviducts, which become plugged (Woyke and Jasiński, 1978).

Fertility of the queen depends on the quantity and quality of semen stored in her spermatheca. During natural mating the queen is inseminated by 3-17 drones (Woyke, 1960; Adams et al., 1977; Kraus et al., 2003). To obtain 8 µl of semen for artificial insemination requires the use of 8–80 drones (Bobrzecki, 1968; Prabucki et al., 1992). When there are problems with semen collection, a large number of drones have to be used for artificial insemination (Woyke and Ruttner, 1958; Prabucki et al., 1992; Chuda-Mickiewicz and Prabucki, 1993; Woyke, 2008).

One drone can produce 1.5-1.7 µl of semen (Woyke, 1960). Usually 1.0 µl is collected to a syringe during artificial insemination. This is equivalent to 7.5 million spermatozoa (Woyke, 1960). Amount and quality of the semen is important for the success of both natural and artificial insemination. Queens inseminated with semen of poor quality can be superseded at an earlier age (Woyke and Ruttner, 1976; Cobey 2007). According to Locke and Peng (1993) spermatozoa viability decreases with drone age. Moreover, viability of semen produced by drones stored after emergence in higher temperatures is lower (Bieńkowska et al., 2011). It is not clear, however, if the environment in which drones develop, affect their sperm viability. In this study we compared the volume of semen and the viability of spermatozoa from drones of different ages. The drones originated from different queens and were reared in different environments.

MATERIAL AND METHODS

The research used 120 drones (*A. m. carnica*). The drones were sons of three unrelated queens and were reared in three different localities: Krakow, Wrocław, and Szczecin. The queens were caged on an empty drone comb for 24 hours. This procedure was repeated 4 times at 5-day intervals to obtain drones at the ages of 15, 20, 25, and 30 days. Drones were reared by Ruttner's (1976) method. Before the experiment the drones were transferred to wooden cages (130x115x70 mm) together with 150 workers. The cages were supplied with candy and water.

Semen was collected using a microcapillary calibrated to 1 µL. Semen volume was determined by measuring the filled length of the capillary with calipers. The semen was diluted in 1000 µL of Kiev buffer (0.3 g D+glucose, 0.41 g potassium chloride, 0.21 g sodium bicarbonate, 2.43 g sodium citrate per 100 mL deionized sterile water) (Moritz, 1984).

Each of the three queens (A, B, C) were represented by 10 drones in each of the four age groups (15, 20, 25, 30 days). In total, 120 samples of semen were analyzed. In each sample the percentages of live and dead spermatozoa were determined by SYBR-14/propidium iodide (IP) fluorescence staining with the LIVE/ DEAD Sperm Viability Kit (Molecular Probes L-7011). Using this sperm viability kit, live and dead sperm were stained green and red, respectively. From each sample, two 300 µL portions of diluted semen were collected; 5 µL SYBR 14 (stock solution, deionized sterile water 1:50) was added and then incubated for 5 minutes at 36°C. Then, 4 µL propidium iodide (PI) was added and stirred. The counts were obtained by flow cytometry (Becton Dickinson FACSCalibur, USA) with a 488 nm argon laser. Green florescence was measured in the LFL1 channel and red in channels LFL2 and LFL3, using the manufacturer's fluorescence compensation filters (Tofilski et al., 2012). The sperm viability was measured within 20 min from semen collection.

The data were analyzed using WinMDI 2.8 software. Nested ANOVA was used for statistical analysis. Percentage data were arcsin-transformed (Sokal and Rohlf, 1981).

Table 1.

Volume of semen and viability of sperm from drones originating from three different honey bee queens

Queen	N	Volume of semen (µl)		Sperm viability (%) (mean ± SD)
		mean ± SD	range	
A	40	0.95 ± 0.19	0.55 – 1.34	88.49 ± 4.53
B	40	0.91 ± 0.20	0.50 – 1.32	89.64 ± 3.71
C	40	0.95 ± 0.20	0.55 – 1.29	89.33 ± 4.60

Table 2.

Volume of semen and viability of sperm from drones of different ages

Age (days)	N	Volume of semen (µl)		Sperm viability (%) (mean ± SD)
		mean ± SD	range	
15	30	1.02 ± 0.22 a*	0.56 - 1.34	87.8 ± 4.93 a
20	30	0.93 ± 0.17 ab	0.63 - 1.27	88.0 ± 4.38 a
25	30	0.91 ± 0.21 b	0.55 - 1.32	89.4 ± 3.73 ab
30	30	0.88 ± 0.19 b	0.50 - 1.25	91.4 ± 3.12 b

Means with different letters within columns differ significantly ($p<0.05$).

RESULTS

With age, the viscosity of semen increased and its color changed from light cream to dark cream. The mean volume of semen collected from one drone was 0.93 µL (Tab. 1). The volume of semen collected from drones originating from different queens did not differ significantly ($F = 0.611$, $df = 2, 108$, $p = 0.545$). The mean volume of collected semen decreased with drone age ($F = 2.942$, $df = 3, 108$, $p = 0.036$) (Tab. 2).

The mean viability of sperm was 89.15% (Tab. 1). The viability did not differ between drones originating from different queens ($F = 1.100$, $df = 2, 108$, $p = 0.335$). Sperm viability increased significantly with drone age ($F = 6.270$, $df = 3, 108$, $p < 0.001$) (Tab. 2).

DISCUSSION

The observed reduction of semen volume with drone age, confirms earlier findings (Woyke and Jasiński, 1978; Locke and Peng, 1993). The viability of spermatozoa did not differ between drones originating from different queens. Locke and Peng (1993) reported reduction of sperm viability from 86% in 14-day-old drones to 81% in 28-day-old drones. In this study we found an increase of sperm viability from 88% in 15-day-old drones to 91% in 30-day-old drones.

One explanation for the increase of sperm viability with age may be related to the gradual maturation of drones. Possibly the drones are able to evert the endophallus and ejaculate before full sexual maturity. In younger drones, the amount of semen remaining in seminal vesicles after ejaculation is higher, and the number of spermatozoa in ejaculate increases with age (Rhodes, 2008). Full maturity of drones may be related not only to more efficient emptying of their seminal vesicles but also to higher sperm viability.

Another possible explanation of increased sperm viability with age might be that drones with higher sperm viability have a higher survival. Drones developing in a better environment might have both longer lives and more viable sperm. In these circumstances, the observed increase in sperm viability could be an effect of poor survival of drones with low sperm viability. In other studies, only 4% of drones survived to the age of 35 days (Rhodes et al., 2011).

CONCLUSIONS

The volume of semen collected from one drone decreased with its age.

The sperm viability increased with drone age.

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OBJĘTOŚĆ NASIENIA I ŻYWOTNOŚĆ PLEMNIKÓW TRUTNI PSZCZOŁY MIODNEJ (*APIS MELLIFERA*) W ZALEŻNOŚCI OD ICH WIEKU

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S t r e s z c z e n i e

Ilość i jakość nasienia trutni decyduje o skuteczności naturalnego i sztucznego unasieniania matek pszczelich. Unasienienie matek nasieniem o obniżonej żywotności plemników może mieć wpływ na czas ich użytkowania. Według wcześniejszych badań, żywotność plemników spada z wiekiem trutni. Nie wiemy czy obniżanie się żywotności plemników z wiekiem trutni zależy tylko od wieku czy też może mieć na to wpływ ich pochodzenie. Celem badań było porównanie objętości nasienia i żywotności plemników trutni w tym samym wieku, pochodzących od różnych matek, utrzymywanych w różnych warunkach.

Trutnie *Apis mellifera* pochodziły od niespokrewnionych matek z rodzin znajdujących się w trzech pasiekach, w Krakowie, Wrocławiu i w Szczecinie. W każdej rodzinie, w tym samym czasie, co 5 dni, rozpoczynano wychów trutni. Badania objętości nasienia i żywotności plemników wykonywano u trutni w wieku 15, 20, 25 i 30 dni. W sumie zbadano nasienie 120 trutni, pobrane od 30 osobników z każdej klasy wiekowej, po 10 od tej samej matki. Objętość nasienia mierzono mikrokapilarą o wyskalowanej objętości, z dokładnością do 0,1 µL. Udział plemników żywych i martwych oceniano metodą fluorescencyjną SYBR-14/jodek propidyny z zastosowaniem cytometru przepływowego FACSCalibur.

Objętość nasienia pobranego od jednego trutnia wynosiła od 0,50 do 1,34 µL, żywotność plemników od 87,8 do 91,4%. Objętość nasienia trutni pochodzących od różnych matek nie różniła się istotnie ($p=0,545$), natomiast malała wraz z wiekiem trutni ($p=0,036$). Żywotność plemników trutni w zależności, od której matki pochodziły nie różniła się ($p=0,335$), ale wraz z wiekiem trutni znacząco wzrosła ($p<0,001$). Wzrost udziału żywych plemników z wiekiem trutni

był prawdopodobnie efektem naturalnej selekcji polegającej na szybszym wymieraniu słabszych osobników o prawdopodobnie gorszej jakości nasienia.

Słowa kluczowe: *Apis mellifera*, pszczoła miodna, truteń, wiek, żywotność plemników.