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# THE INFLUENCE OF AGE ON THE RESULTS OF INSTRUMENTAL INSEMINATION OF HONEYBEE QUEENS*

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## SUMMARY

Altogether 237 queens, 1—47 days old, were instrumentally inseminated with 8 $mm^3$ of semen. The queens which survived were killed 48 hrs after insemination and the number of spermatozoa in their spermatheca was counted. A significant influence of the age of queens on the results of insemination was found. Heavy losses occurred (survival 14-23 %) and a low number of spermatozoa entered the spermatheca (2.658 million), after queens 1-3 days old were inseminated. The best results in survival (75-100 %) and the highest number of spermatozoa entering the spermatheca (avg. 3.975 million) were obtained, when queens 5-10 days old were inseminated. Instrumental insemination of queens 5-14 days old is recommended. Older queens can be inseminated, but significantly fewer spermatozoa (2.356 million) enter their spermatheca.

## INTRODUCTION

In order to increase the efficiency of the number of spermatozoa entering the spermatheca of instrumentally inseminated queens, several factors have already been investigated. Mostly small amounts of semen (1-2,5 $mm^3$) were used in the pioneer experiments conducted by MACKENSEN (1955). After the phenomenon of multiple mating in one mating flight was discovered,

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the volume necessary for sufficient instrumental insemination was studied. Woyke (1960) recommended two inseminations with 4 $mm^3$ or one insemination with 8 $mm^3$, and Mackensen (1964) two insemination with 3 $mm^3$. WOYKE (1971) showed that the conditions as early as in the grafting time of larva for queen rearing, influenced the results of inseminations. The number of spermatozoa entering the spermatheca increased when the queens were reared from the younger brood. There is also a significant influence of the temperature on the result of insemination (WOYKE and JASINSKI 1973). Queens inseminated with 8 $mm^3$ of semen and kept at 34° after insemination, had 808 thousand more spermatozoa in the spermatheca than those kept at 24° or in cages in colonies.

Before the influence of the age on the results of artificial insemination could be studied, the relation between age and natural mating had to be known. OERTEL (1940) found that queens mate naturally between their 6th and 13th day of life, and most of them between the 8th and 9th days. SOCZEK (1958) observed matings from the 6th to the 36th day of life, but most of the queens mated between the 10th and 12th day of life. WOYKE (1956) found that all queens but one mated between the 7th and 32nd day of life had semen in the oviducts from several drones, but the largest amounts were found in queens younger than 14 days. This means that the younger queens mated more often with a greater number of drones than did the older ones. There also exists a correlation between the development of eggs in the ovaries and the number of matings in one flight. WOYKE (1960) showed that, the larger the eggs in the ovaries, the smaller the volume of semen brought home by the queen from the mating flight. ZMARLICKI and MORSE (1962) showed that most queens prevented from flying for one month or longer did not mate, and those two which mated, had many fewer spermatozoa in their spermatheca than those mated at the normal mating time.

MACKENSEN (1955) instrumentally inseminated queens 1-10 days old with semen from one drone only. Except queens 1 day old, no difference was found in the number of spermatozoa entering the spermatheca of queens inseminated daily at different ages. FRESNAYE (1966) found by a different test, that on the average 87,17 % of the queens were normally fertilized after being instrumentally inseminated at the age of 10-12 days, while only 57,60 % of those inseminated at the age of 5-6 days were normally fertilized. Big differences occurred between queens inseminated at different periods of the year. But MACKENSEN (1975) obtained similar results, after queens 4-11 days old inseminated with 5-8 $mm^3$ were compared. CHWALKOWSKI (1969) showed that queens inseminated at an average age of 2, 7, and 18 days started to lay eggs 10-12 days after insemination, irrespectively of the age at insemination. But he presented neither the percentage of lost queens nor the number of spermatozoa in the spermatheca.

## MATERIALS AND METHODS

Altogether 237 sister caucasian queens were instrumentally inseminated in July with 8 mm³ of semen. The semen was collected from free flying drones. The queens were kept before, as well as after insemination in ZANDER rearing cages placed between brood combs in queenless colonies. Two experiments were conducted. In the first, young queens of all ages from 1-8 days were inseminated. In the second experiment queens were inseminated at the age of 4 and 7 days, then in one week intervals to the age of 35 days, and the oldest ones were 47 days old. All the queens were killed 48 hrs after insemination, the spermatheca was dissected and the number of spermatozoa in it was counted by the method already described (WOYKE 1971). FISHER's analysis of variance was applied, and Student t-test was used to find statistically significant differences between the means.

## RESULTS

Table 1 shows, that very heavy losses occured after queens 1-3 days old were instrumentally inseminated. The queens still had some semen in the oviducts, so probably more of them would have died if they were kept longer in the colony. Significantly more queens survived after being inseminated at the age of 4 days. Nevertheless, a 40 % loss of queens is too high for commercial beekeeping. Five days old and older queens survived in percentages similar to those characteristic for natural mating/from personal observations — 75 %/. The best results, 100 % survival were obtained when 7 and 8 days old queens were inseminated.

TABL. 1. — *Results of instrumental insemination of young queens with 8 mm³ of semen.*

| Age of queens in days | No. queens inseminated | % survivals | No spermatozoa in spermatheca, millions | |
|---|---|---|---|---|
| | | | Range | Mean |
| 1 | 21 | 19.0 | 1.798-3.168 | 2.555 a* |
| 2 | 21 | 14.1 | 1.507-3.399 | 2.393 a |
| 3 | 21 | 23.1 | 2.101-3.793 | 2.863 a |
| 4 | 25 | 60.0 | 3.454-4.422 | 4.011 b |
| 5 | 20 | 75.0 | 3.586-4.400 | 4.100 b |
| 6 | 17 | 82.0 | 2.139-4.325 | 3.914 b |
| 7 | 15 | 100.0 | 3.525-4.356 | 3.925 b |
| 8 | 15 | 100.0 | 3.256-4.290 | 3.859 b |

* Different letters indicate significant differences between means.

The number of spermatozoa in the spermatheca of queens inseminated at the age of 1-3 days was low. The average for all those queens was 2.658 million. Significant increase in the number of spermatozoa entering the sperma-

theca occurred after four days old and older queens were inseminated. No statistically significant differences were found in the mean numbers of spermatozoa in the spermatheca of queens inseminated at the age of 4-8 days. The average for all those queens was 3.975 millions. Thus, queens inseminated at the ages of 4-8 days had 1.317 millions more spermatozoa than the younger ones. Nevertheless, due to high losses of queens inseminated at the age of 4 days, it is not recommended to inseminate queens of this age for commercial apiaries.

Table 2 shows, that the survival of queens inseminated from a very broad range of ages was very similar to results presented earlier for queens inseminated at the ages of 4 and 7 days. The other queens survived at a rate similar to that occurring during natural mating. The high survival of queens inseminated when 35 days old is probably accidental due to the low number of investigated queens.

TABL. 2. — *Results of instrumental insemination of queens from a broad range of ages with 8 mm³ of semen*

| Age of queens in days | No. queens inseminated | % survivals | No. spermatozoa in spermatheca, millions | |
|---|---|---|---|---|
| | | | Range | Mean |
| 4 | 16 | 68.7 | 2.475-4.213 | 3.734 a* |
| 7 | 16 | 100.0 | 2.986-4.125 | 3.582 a |
| 14 | 15 | 86.7 | 2.915-3.685 | 3.209 b |
| 21 | 15 | 73.3 | 2.387-3.096 | 2.728 c |
| 20 | 11 | 90.0 | 1.386-2.480 | 2.097 d |
| 35 | 5 | 100.0 | 1.870-2.579 | 2.246 cd |
| 47 | 4 | 75.0 | 1.859-3.275 | 2.354 cd |

* Different letters indicate significant differences between means.

The number of spermatozoa entering the spermatheca was similar when 4 and 7 days old queens were inseminated. Significantly fewer spermatozoa entered the spermatheca when 14 days old queens were inseminated and still fewer, after older queens were inseminated. Queens inseminated at the age of 21 days had 0.854 million fewer spermatozoa in the spermatheca than those inseminated when 7 days old, and queens 28 days old and older had 1.228-1.485 million spermatozoa fewer than the 7 days old ones.

## CONCLUSIONS

A significant influence of age on the results of instrumental insemination occurs, when queens from a broad range of ages are investigated. The worst

results are obtained when queens younger than 4 days old are inseminated. To get the best results insemination of queens 5-10 days old, up to 14 days old is recommended. Older queens can be inseminated, but the results are worse, than those of queens inseminated at the age of 5-14 days.

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## ZUSAMMENFASSUNG

### DER EINFLUSS DES ALTERS AUF DIE ERGEBNISSE DER INSTRUMENTELLEN BESAMUNG VON BIENENKÖNIGINNEN (*Apis mellifica mellifica* L.)

MACKENSEN (1955) besamte 1-10 Tage alte Königinnen instrumentell mit dem Samen eines einzigen Drohn. Mit Ausnahme bei eintägigen Königinnen wurden keine Unterschiede in der Zahl der Spermatozoen festgestellt, die in die Spermatheka täglich besamter, verschiedenaltriger Königinnen gelangt waren. Nach Entdeckung der Mehrfachbegattung auf einem Begattungsflug empfahl WOYKE (1960) zwei instrumentelle Besamungen mit je 4 mm³ Samen oder eine Besamung mit 8 mm³ Samen. Aber der Einfluss des Königinnenalters auf die Zahl der Spermatozoen, die bei Besamung mit grossen Samenmengen in die Spermatheka der Königinnen gelangen, ist nicht bekannt.

*Methode*

Insgesamt wurden 237 1-47 Tage alte Königinnen einmal mit 8 mm³ Samen instrumentell besamt. Die Königinnen wurden in Weiselkäfigen zwischen Brutwaben weiselloser Völker gehalten. 48 Stunden nach der Besamung wurden die Königinnen abgetötet und die Zahl der Spermatozoen in der Spermatheka gezählt.

*Ergebnisse*

Bei instrumenteller Besamung von 1-3 Tage alten Königinnen traten hohe Verluste auf (Tab. 1). Die Anzahl der Spermatozoen, die in die Spermatheka gelangt waren, war bei diesen Königinnen gering, durchschnittlich betrug sie 2,678 Millionen.

Bei Königinnen, die im Alter von 4-8 Tagen besamt wurden, betrug die Zahl der Spermatozoen in der Spermatheka durchschnittlich 3,975 Millionen, d.h. 1,317 Millionen mehr als bei den zuvor erwähnten. Auch drei Wochen alte oder noch ältere Königinnen können künstlich besamt werden, doch ist die Zahl der in die Spermatheka gelangenden Spermatozoen gering (Tab. 2). Um die besten Ergebnisse bei der instrumentellen Besamung zu erlangen, wird empfohlen, 5-10 Tage alte oder auch bis zu 14 Tage alte Königinnen zu verwenden.

## RÉSUMÉ

### L'INFLUENCE DE L'AGE SUR LES RÉSULTATS DE L'INSÉMINATION ARTIFICIELLE DES REINES D'ABEILLE

MACKENSEN (1955) a inséminé des reines âgées de 1-10 jours avec le sperme d'un seul mâle. Les reines d'un jour mises à part, on n'a trouvé aucune différence dans le nombre de spermatozoïdes qui pénètrent dans la spermathèque des reines inséminées chaque jour à des âges divers. Après qu'on a découvert le phénomène de la fécondation multiple au cours d'un

seul vol nuptial, WOYKE (1960) a recommandé 2 inséminations de 4 mm³ de sperme ou une seule insémination de 8 mm³. Mais on ne connaît pas l'influence de l'âge sur le nombre de spermatozoides qui pénètrent dans la spermathèque des reines inséminées avec de fortes doses de sperme.

*Méthodes*

237 reines âgées de 1 à 47 jours furent inséminées artificiellement avec une dose de 8 mm³ de sperme. On a maintenu les reines dans des cages placées entre les rayons à couvain dans des colonies orphelines. On les a tuées 48 h plus tard et on a dénombré les spermatozoïdes présents dans la spermathèque.

*Résultats*

Des pertes sévères sont survenues après l'insémination artificielle des reines âgées de 1 à 3 jours (Tabl. 1). Le nombre de spermatozoïdes pénétrant dans la spermathèque de ces reines était faible, environ 2,678 millions. Le nombre moyen des spermatozoïdes dans la spermathèque des reines inséminées âgées de 4-8 jours était de 3,975 millions, soit 1,317 million de plus que précédemment. Il est possible d'inséminer artificiellement des reines âgées de 3 semaines ou plus, mais le nombre de spermatozoïdes qui pénètrent dans leur spermathèque est faible. (Tabl. 2). Il est recommandé d'utiliser des reines qui ont entre 5 et 10 jours, ou même jusqu'à 14 jours, si l'on souhaite obtenir les meilleurs résultats.

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