

DYNAMICS OF ENTRY OF SPERMATOOA INTO THE SPERMATHECA OF INSTRUMENTALLY INSEMINATED QUEEN HONEYBEES

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Summary

Queens were inseminated with 1, 2, 4 or 8 mm³ of semen, and either held, each with 250 workers, in an incubator at 34°C, or caged without workers in queenless colonies. In the first 4 h the amount of semen injected and the presence or absence of workers had little, if any, effect on the rate of entry of spermatozoa into the spermatheca, which was 200–300 thousand/h. In queens injected with 1 and 2 mm³ of semen, the process of entry had largely ended after 4–8 h, but in queens injected with 8 mm³ it continued, slowly, up to at least 40 h. The presence or absence of workers had little or no influence on the final number of spermatozoa in the spermatheca when 1 or 2 mm³ of semen were injected, but it clearly increased the final number when 4 or 8 mm³ were injected.

Introduction

Woyke (1979) showed that the presence of workers with instrumentally inseminated queens increased the number of spermatozoa entering the spermatheca, and Woyke and Jasinski (1979) found that 250 workers were enough to ensure the maximum entry of spermatozoa when queens were kept in an incubator at 34°C.

However, the internal process of entrance of spermatozoa in favourable and unfavourable conditions has not been investigated hitherto, and this has now been studied.

Material and Methods

Altogether 400 queens (*Apis mellifera*) were instrumentally inseminated with 1, 2, 4 or 8 mm³ of semen collected from free-flying drones. Half of the queens were introduced into small boxes, 12×12×4 cm, supplied with a piece of comb, candy and dilute syrup, and 250 workers of mixed ages. The boxes were placed in an incubator at 34°. The other queens were released after insemination into small screened cages without workers, which were placed in queenless colonies, in groups containing at least one replication of all insemination combinations. Two investigations were made. In the first, all four quantities of semen were used and queens were examined up to 24 h after insemination. In the second, queens were inseminated only with 1 or 8 mm³ of semen, but were examined up to 40 h after insemination. At each time of examination 5 queens of each treatment were killed (Fig. 1, Fig. 2, Fig. 3) and the numbers of spermatozoa in the spermatheca were counted by the usual method.

Analysis of variance was applied to the results and the new multiple range test was used to detect significant ($P < 0.05$) differences between the means.

Results

Dynamics of entry of spermatozoa into the spermatheca of queens with workers examined up to 24 h after insemination

Queens caged with workers were examined at times up to 24 h after insemination. At the end of the first 2 h it was impossible to detect differences between the results for queens inseminated with different amounts of semen (Fig. 1), but after 4 h an average of 0.814 million spermatozoa had entered the spermatheca in queens inseminated with 1 mm³ of semen, and 1.217 million in queens inseminated with 8 mm³ of semen; the difference was not found to be statistically significant. During the first 4 h, 200–300 thousand spermatozoa per hour entered, this being the highest rate for the whole period of 24 h. Of the spermatozoa that entered in the 24 h, 86% had done so by the end of the first 4 h in the queens inseminated with 1 mm³ of semen.

When the numbers of spermatozoa in the spermatheca of queens inseminated with 1, 2, 4 and 8 mm³ of semen were compared 8 h after insemination, statistically significant differences were found between every second average. In queens given 2 mm³ of semen, 73% of the total at 24 h had already entered after 8 h.

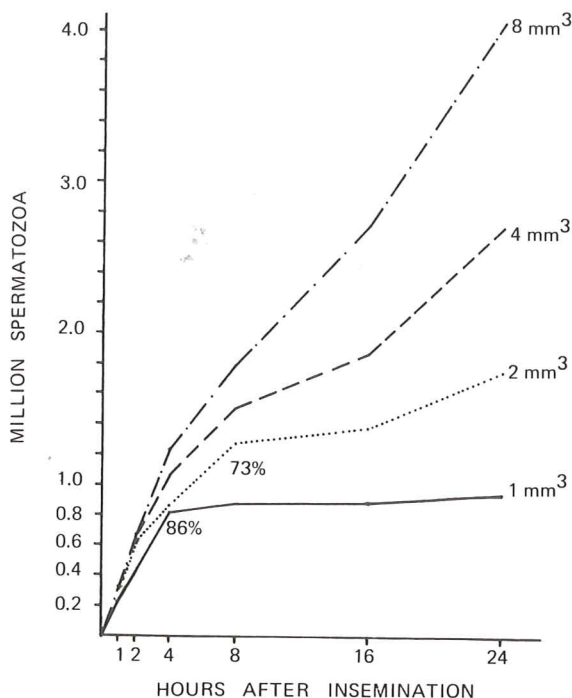


FIG. 1. Dynamics of entry of spermatozoa into the spermatheca of queens inseminated with different amounts of semen and attended by 250 workers, during the first 24 h.

In the queens inseminated with 4 and 8 mm³ of semen the numbers of spermatozoa in the spermatheca rose substantially and significantly between 16 and 24 h and it seems clear that the process of transfer had not finished by the end of that period. By 24 h after insemination, 0.951, 1.773, 2.692 and 4.058 million spermatozoa were found in the spermatheca of queens inseminated with 1, 2, 4 and 8 mm³, respectively, and the differences between these numbers were statistically significant. During the period 16–24 h the rates of entry were 7, 48, 103 and 167 thousand per hour, respectively.

The speed of entry of spermatozoa was thus highest during the first 2–4 h, and very similar for different amounts of semen injected. Later on, the speed decreased considerably for smaller doses, but remained high for larger doses until 24 h after insemination, although it was by then lower than in the beginning.

Dynamics of entry of spermatozoa into the spermatheca of queens without workers examined up to 24 h after insemination

Again there was little detectable difference during the first 2 h in results for queens inseminated with different amounts of semen (Fig. 2).

Four hours after insemination the average number of spermatozoa was lowest (0.331 million) in the spermatheca of queens inseminated with 1 mm³ of semen and highest (0.767 million) in queens inseminated with 8 mm³, but none of the differences between the 4 averages were found to be statistically significant. The speed of entry during first 4 h was 80 to 190 thousand per h. Spermatozoa continued to enter the spermatheca subsequently, and statistically significant differences were found between some averages. However, the speed of entry was lower than in the first 4 h. Entry finished after 16 h for doses of 1 mm³, but it continued for the other doses.

By 24 h after insemination 1.119, 1.831, 2.271 and 3.499 million spermatozoa were found in the spermatheca of queens inseminated with 1, 2, 4 and 8 mm³ of semen, respectively, and the differences between most of these averages were statistically significant.

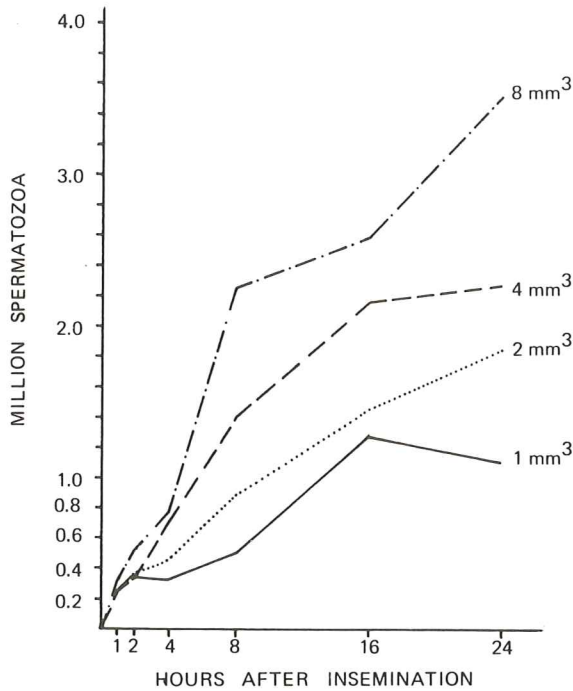


FIG. 2. Dynamics of entry of spermatozoa into the spermatheca of queens caged without workers in a queenless colony, during the first 24 h.

Comparison of the dynamics of entry of spermatozoa into the spermatheca of queens with and without workers

One hour after insemination 241–338 thousand spermatozoa were found on average in the spermatheca of queens inseminated with different amounts of semen and attended by worker bees (Fig. 1), and 266–309 thousand in those without workers (Fig. 2). The numbers are similar, independently of the amount of semen injected and of presence or absence of workers.

By 8 h after insemination, significantly fewer spermatozoa had entered the spermatheca of queens without workers than those of queens with workers and inseminated with the same amounts of semen. In queens inseminated with 1 or 2 mm³ of semen, entry had largely finished after 4–8 h if the queens were attended by workers (Fig. 1), but it continued in queens without them (Fig. 2). As a result, after 24 h similar numbers of spermatozoa had entered whether the queens were with or without workers.

For queens inseminated with larger amounts of semen, the results were different: 24 h after insemination significantly more spermatozoa had entered the spermatheca of queens with workers than of those without them.

Entry of spermatozoa into the spermatheca during the first 40 h after insemination

Since the above results suggested that the entry of spermatozoa into the spermatheca was not always complete 24 h after insemination, further queens were inseminated with 1 and 8 mm³ of semen and investigated for 40 h after insemination.

After 1 h, 327–430 thousand spermatozoa had entered the spermatheca and after 4 h, 0.914–1.221 million (Fig. 3). No statistically significant differences were found between any of the means. The speed of entry during the first 4 h was 228–305 thousand per h. Entry had practically ceased 8 h after insemination in queens inseminated with 1 mm³ of semen, and almost identical numbers had entered the spermatheca of queens with and without workers. At 40 h after insemination, 1.214 and 1.062 million spermatozoa were found in queens inseminated with 1 mm³ of semen and with and without workers, respectively, but the

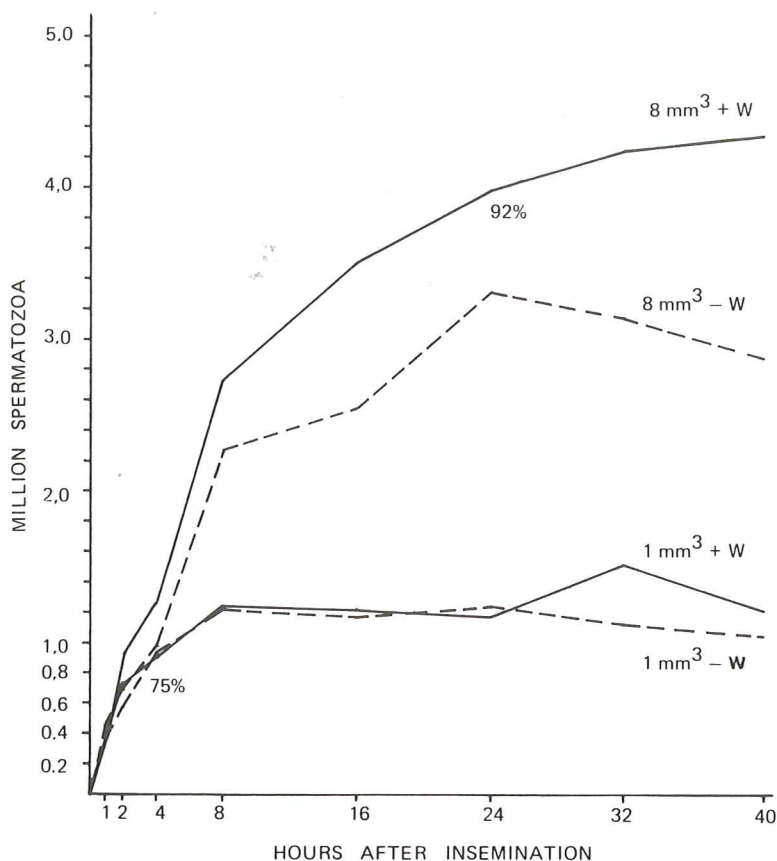


FIG. 3. Dynamics of entry of spermatozoa into the spermatheca of queens with workers (+W) and without them (-W), during the first 40 h.

difference between these means was found not to be significant.

A rapid increase in the number of spermatozoa in the spermatheca occurred during the first 8 h in both groups of queens inseminated with 8 mm³ of semen. The speed of entry decreased later, especially in queens without workers; 16 h after insemination there were significantly more spermatozoa in queens with than in those without workers. Entry of spermatozoa had nearly ended 24 h after insemination in queens with workers, but a slow increase continued up to 40 h, at which time 4.329 and 2.881 million spermatozoa were found in the spermatheca of queens inseminated with 8 mm³ of semen, with and without workers, respectively. The difference between these two means was highly significant.

With small amounts of semen, queens without workers performed slightly differently in the two experiments. In the first, but not in the second, the number of spermatozoa was less in queens without workers than in queens with workers, 4 and 8 h after insemination.

Conclusion

The speed of entry of spermatozoa into the spermatheca of instrumentally inseminated queens was highest during the first 4 h after insemination, and similar numbers of spermatozoa entered the spermatheca of queens inseminated with different amounts of semen, whether the queens were caged with or without workers.

Process of entry finished earlier with small doses of semen than with larger amounts, where it continued slowly up to 40 h from insemination.

At the end of the investigation in queens inseminated with a small amount of semen, similar

numbers of spermatozoa were found in spermatheca whether the queens were with or without workers. But in queens inseminated with large amounts of semen, significantly more spermatozoa had entered the spermatheca of queens caged with workers than those without them.

Thus the presence of workers in the cage with the queen is important when large doses of semen are used.

References

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