

## Effect of collection month on the freezability of drone semen of *Apis mellifera iberiensis* reared under field conditions

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### Introduction

Honey bees are vital pollinators, playing a crucial role in enhancing fruit and seed production while maintaining ecological balance. The health and reproductive quality of the queen are essential for ensuring colony stability.

As the sole fertile female in the colony, the queen's reproductive capacity directly affects the productivity and resilience of the hive. To produce strong and viable offspring, the queen must mate with a sufficient number of drones, which promotes greater genetic diversity and reduces the risk of inbreeding within the colony. However, the queen's reproductive success largely depends on the quality of the sperm she receives. Poor sperm quality can impair egg fertilization, reduce brood viability, and ultimately disrupt colony population dynamics.

During Spain's spring reproductive season, colonies produce numerous drones whose primary role is to mate with virgin queens. As the season progresses, drone numbers decline due to factors such as resource availability and colony size. Despite the critical importance of drone quality for colony viability, this aspect has been understudied, underscoring the need for further research.

### Our goal

To analyze the effect of the month of semen collection from drones reared under field conditions on sperm quality and sperm survival to the cryopreservation procedure.

### Results



#### Drone capture

Drones of *A. m. iberiensis* were collected from 10 hives (56/month) on warm, sunny days during their return from unsuccessful mating flights.



#### Semen extraction

Drones were immobilized with chloroform, and semen was extracted via manual eversion of the endophallus using a capillary tube.



#### Sample preparation

Semen from 224 drones was pooled in groups of 8, diluted in Kiev medium with 10 % egg yolk plasma and 10 % DMSO for cryoprotection.



#### Cryopreservation

##### Cooling stages:

- From 20 °C to 5 °C at 0.5 °C/min.
- From 5 °C to -40 °C at 3 °C/min.
- Immersion in liquid nitrogen (-196 °C).

##### Storage:

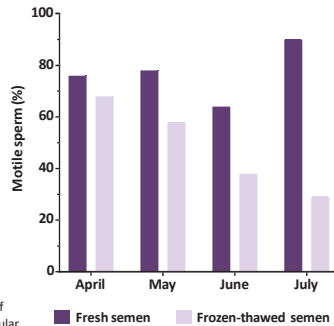
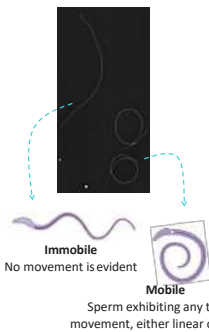
- Minimum 24 h before post-thaw analysis.



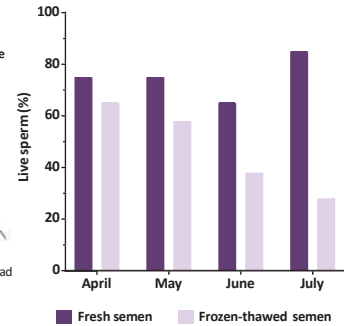
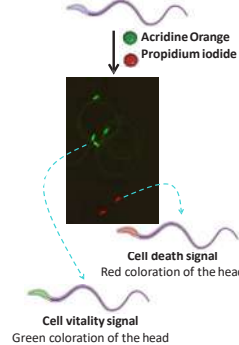
#### Sperm quality analysis

Motility and viability were assessed before and after thawing to evaluate cryopreservation success and suitability for queen insemination.

#### Effect of the month of semen collection on sperm motility in drone fresh and frozen-thawed semen



#### Effect of the month of semen collection on sperm viability in drone fresh and frozen-thawed semen



No significant differences in sperm quality of fresh samples were observed between months. Cryopreservation reduced sperm quality in all groups ( $p < 0.01$ ), with a sharper decline in samples from June and July: post-thaw motility and viability ranged from 58–66% in April–May, and dropped to 31–38% in the following months.

### Conclusion

Sperm freezability may be reduced in the drones reared under field conditions collected after the reproductive season, although these results should be confirmed in different years and conditions.

### Acknowledgement

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PLAY & LEARN



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Honeybee colonies raise large numbers of drones during the reproductive season, which occurs in spring in Spain. From that point onward, the number of drones declines to varying degrees, depending on numerous factors such as colony size and food availability. But not only the quantity but the quality of drones may be affected by food scarcity or lower food quality, although this has been hardly studied. This study aims to analyze the effect of the month of semen collection from drones reared under field conditions on their sperm quality and sperm survival to the cryopreservation procedure. Mature flying drones of the *Apis mellifera iberiensis* subspecies were recovered from 10 hives, in the afternoon of days with good weather, on their return to the hive after blocking the entrance with a queen excluder. Semen was collected from the ejaculates of 224 drones (56/moth) and were pooled in groups of eight, diluted in kiev medium to a final concentration of 10% egg yolk plasma and 10% of DMSO, and packed in 0.25 ml straws. Cooling rate was 0,5°C/min from 20 to 5 °C and 3°C/min from 5 to -40 °C, when the straws were submerged in liquid nitrogen, and kept frozen for a minimum of 24 hours prior to evaluating post-thaw quality. Sperm quality analysis of fresh and cryopreserved samples included individual sperm motility and sperm viability assessments. No significant differences were observed between moths for sperm quality of fresh samples. Freezing decreased the sperm quality parameters in all groups ( $p < 0.01$ ), but this decrease was more pronounced for the samples collected in June and July with values of individual sperm motility and viability after freezing ranging between 58% to 66 % in May and April, and between 31% and 38% afterwards. It is concluded that sperm freezability may be reduced in the drones reared under field conditions collected after the reproductive season. This work was supported by the Spanish AEI-MICIN (grant PID2023-148475OB-I00), the EU Horizon Europe (grant 101082073) and the DGA-FSE (grant A07\_23R).