Survival of Honey Bee Spermatozoa in Liquid Nitrogen[1]

JOHN R. HARBO

Bee Breeding and Stock Center Laboratory, Agric. Res. Serv., USDA, Baton Rouge, LA 70808

ABSTRACT

Worker progeny were produced by queen honey bees (*Apis mellifera* L.) inseminated with semen that had been stored in liquid nitrogen. The cryoprotectant was DMSO (dimethyl sulfoxide). Queen bees inseminated with a semen mixture (60% semen, 30% saline, and 10% DMSO) that was stored in liquid nitrogen retained 41% as many spermatozoa in their spermathecae as control queens.

Dimethyl sulfoxide (DMSO) was successfully used by Brown et al. (1971) as a cryprotectant for *Drosophila* cells stored in liquid nitrogen (−196°C). With hemolymph as a cryoprotectant, Melnichenko and Vavilov (1976) successfully stored honey bee (*Apis mellifera* L.) semen in liquid nitrogen. My plan was to test DMSO as a possible cryoprotectant for honey bee spermatozoa.

I began by testing the ability of queen bees to tolerate inseminations of semen containing DMSO. These nonfrozen mixtures of semen, saline, and DMSO did not reduce sperm transport to spermathecae, and the resulting progeny seemed normal. The highest DMSO concentration tested (12½%) apparently caused some premature queen supersedure.

I then established that spermatozoa containing 10 and 12½% DMSO were motile after storage in liquid nitrogen, and all 40 queens inseminated with this frozen sperm retained sperm in their spermathecae. However, the number of sperm retained in the spermathecae was much less than that of nonfrozen controls.

Finally, frozen sperm produced progeny. The eye markers snow and tan (Laidlaw et al. 1964) provided genetic proof of biparental origin, for red-eyed worker and queen progeny were produced from heterozygous snow queens inseminated with tan sperm that had been stored in liquid nitrogen.

METHODS AND MATERIALS

Using my most successful semen dilution, I designed an experiment to establish whether or not DMSO was responsible for sperm survival and to measure the effect of nitrogen storage on the subsequent transport of spermatozoa to the spermathecae of queens.

I established 4 test groups (Table 1) and stored semen mixtures for each group in 2 capillary tubes sealed at both ends with petrolatum (Harbo 1973). Tubes for groups A and B contained 60% semen, 30% saline, and 10% DMSO. (Because concentrated DMSO generates heat when added to water, I mixed DMSO with saline, 0.85 percent NaCl, before mixing with the semen.) Tubes for groups C and D contained only semen (60%) and saline (40%).

A tiny thermocouple (No. 36 copper-constantan) inserted into one of the tubes of group C monitored the cooling and thawing rates (Fig. 1). The leveling off in the cooling curve just below 0° very likely reflected the freezing point of tube C.

RESULTS AND DISCUSSION

Liquid nitrogen storage significantly reduced the number of spermatozoa that reached the spermatheca,

[1] In cooperation with Louisiana Agric. Exp. Stn. Received for publication Oct. 15, 1976.

and without DMSO as a cryoprotectant, sperm did not survive liquid nitrogen storage (Table 1).

Spermatozoa in the spermatheca may not be a direct measure of success, but their absence is a measure of failure because sperm transport to the spermatheca is an intermediate step in the fertilization of honey bee eggs. Therefore, I used this measure to screen out unsuccessful inseminations and as an indicator of success. Absolute survival can be proven only by producing genetically marked progeny from frozen spermatozoa; this was done with 10 queens, but not with the queens in Table 1.

As yet I have not thoroughly tested a storage period longer than 48 hours. Well-established storage techniques at nonfreezing temperatures (Taber and Blum 1960) are more practical when storing semen for only 48 h, but the 48-h storage in liquid nitrogen showed that bee spermatozoa with 10% DMSO can survive the harsh transition into and out of extreme cold.

ACKNOWLEDGMENT

I thank the personnel at the Dairy Improvement Center of Louisiana State University for their advice and cooperation.

Table 1.—The effect of storage temperature and 10% DMSO on the movement of sperm to the spermathecae of sister, hybrid queens. Each queen received 2.5 μl of semen.

Group	Storage temperature	No. of queens	Millions of sperm in the spermatheca ($\bar{x} \pm s_{\bar{x}}$)[a]
A (DMSO)	−196°C	8	1.18±0.17
B (DMSO)	14°C	8	2.87±0.22
C (No DMSO)	−196°C	9	0 ±0
D (No DMSO)	14°C	7	2.97±0.39

[a] Spermatozoa were counted spectrophotometrically (Harbo 1975).

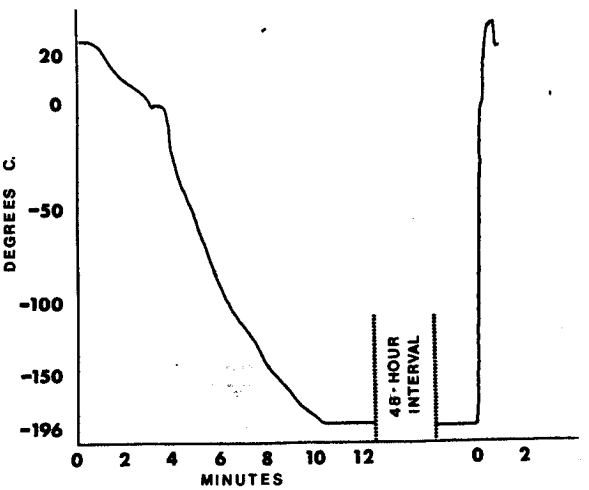

FIG. 1.—Cooling and thawing rates of spermatozoa in groups A and C as measured by a thermocouple in a tube containing 60% semen and 40% saline.

REFERENCES CITED

Brown, B. L., S. C. Nagle, Jr., J. D. Lehman, and C. D. Rapp. 1971. Storage of *Aedes aegypti* and *Aedes albopictus* cells under liquid nitrogen. Cryobiology 7: 249–51.

Harbo, J. R. 1973. Handling stored semen of honey bees. Proc. XXIVth Int. Apic. Congr. 338–44.

1975. Measuring the concentration of spermatozoa from honey bees with spectrophotometry. Ann. Entomol. Soc. Am. 68: 1050–2.

Laidlaw, H. H., M. A. el Bamby, and K. W. Tucker. 1964. Five new eye-color mutants in the honey bee. J. Hered. 55: 207–10.

Melnichenko, A. N., and Yu. L. Vavilov. 1976. Many years keeping of drone semen when freezing in liquid nitrogen. Dokl. Vses. Akad. Skh. Nauk: 25–26.

Taber, S., and M. S. Blum. 1960. Preservation of honeybee semen. Science (Wash., D.C.) 131: 1734–5.

Reprinted from the
ANNALS OF THE ENTOMOLOGICAL SOCIETY OF AMERICA
Volume 70, Number 2, pp. 257–258, March 1977