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Sterility in honey bees caused by dimethyl sulfoxide

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Abstract: Honey bee (*Apis mellifera*) semen was treated as follows: 1) diluted in saline with 10 percent dimethyl sulfoxide (DMSO) and stored at −196°C; 2) same treatment but stored at 12°C; 3) diluted in saline and stored at 12°C; and 4) undiluted, unstored semen. Daughters (queens) produced from the treated spermatozoa were evaluated for total sterility. Only sterile eggs were produced from 3 percent of the queens in both groups that had DMSO (5/166 in group 1, and 6/234 in group 2). They were different ($P < 0.05$) from groups 3 and 4 in which no queens were produced that laid only sterile eggs (0/151 and 0/137, respectively). These results demonstrate that, under the conditions used, a low level of sterility is induced by DMSO, and this F_1 sterility raises questions about possible genetic damage by DMSO.

Dimethyl sulfoxide (DMSO) has been used as a cryoprotectant for honey bee spermatozoa since 1976[1]. Many different freezing rates and diluents have proven satisfactory, but a freezing rate of about 20°C/minute was most common, and the final dilution always contained 10 percent DMSO[2].

Eggs fertilized with frozen bee spermatozoa were tested for percent viability, and those developing into adults were evaluated for physical anomalies. Egg viability was lower when fertilized with frozen spermatozoa[3], and some of the surviving bees became mosaic adults[4]. The production of mosaics did not persist beyond the first generation, and possible genetic damage was not detected until the viability of F_2 eggs was evaluated[5].

This paper deals with the viability of eggs from females (queens) that were progeny of treated semen. I first observed total nonhatching of eggs from such a queen in 1977, and recognized it as extremely rare and perhaps important. I reasoned that the egg production of these females marks the first time that chromosome replicates from the treated spermatozoa enter meiosis, so sterility at this point may indicate genetic damage. The objective of this study was 1) to learn if this total nonhatching of eggs (total sterility) was a chance occurrence, or if it occurred more often in treated than in nontreated queens; and 2) to find out which aspect of semen treatment (freezing, DMSO, dilution, or storage) caused the nonhatching of eggs.

Materials and Methods

Spermatozoa were given four different treatments: *frozen*—a final dilution of 10 percent DMSO, 30 or 40 percent saline, and 50 or 60 percent semen (salines and dilution rates varied from trial to trial, see Table I) was stored in liquid nitrogen for 2 days; *nonfrozen*—diluted as above but stored at 12°C for 2 days; *no DMSO*—DMSO replaced by 10 percent more saline, but stored as above at 12°C for 2 days; and *control*—undiluted unstored semen. My technique for diluting, storing, freezing, and recovering bee semen has been described[2].

Nine trials were conducted between 1978 and 1984. Within each trial the semen was collected from a common pool of drones on the same day. Semen for the control was collected 2 days later on the day of insemination.

Table I. Female progeny of treated semen that produced only sterile eggs; numbers in the table are number of females producing only nonhatching eggs/number of females tested

Trial	Semen dilution*	Semen treatment†			
		frozen	nonfrozen	no DMSO	control
1	a	0/22	—	—	0/11
2	b	1/12	0/4	—	0/9
3	b	0/14	1/9	—	0/9
4	b	1/10	—	—	0/8
5	c	2/54	0/28	0/13	0/18
6	b	0/8	1/26	0/15	0/33
7	a	1/37	1/33	0/10	0/30
8	b	0/9	1/40	0/11	0/19
9	a	—	2/94	0/102	—
Totals		5/166	6/234	0/151	0/137
		(3.0%)	(2.6%)	(0%)	(0%)

*Dilution of semen varied among trials. Three different salines were used: *saline a* = 0.85% NaCl, 0.25% dihydrostreptomycin sulfate; *saline b* = 1.1% $NaH_2PO_4 \cdot H_2O$, 0.85% $NaHPO_4 \cdot 7H_2O$, 0.25% dihydrostreptomycin sulfate; *saline c* = 2.43g sodium citrate, 0.21 g $NaHCO_3$, 0.04g KCl, 0.3g glucose, 0.1g dihydrostreptomycin sulfate, 100 ml tris at pH 7.2, titrated with HCl to pH 7.9. The final dilutions of trials 1-3 contained 60% semen; 4-9 contained 50% semen. DMSO and egg yolk were added to the saline portion so that the final dilutions contained 10% DMSO and 10% egg yolk; all trials contained DMSO, whereas only trials 2, 4, 6, and 8 contained egg yolk

†The "frozen" and "nonfrozen" treatments contained 10% DMSO, saline replaced DMSO in the "no DMSO" treatment, and the "control" consisted of undiluted, unstored semen

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Female parent (P_o) breeders consisted of sister queens inseminated with the treated or control semen. Daughter (F_1) queens produced by these breeders were mated to wild-type males and tested for total nonhatching of eggs.

The test consisted of putting the daughter queens into small colonies with worker bees and allowing the queens to lay eggs. If eggs developed into larvae, then the queen in that colony was tallied as normal. If the eggs failed to develop into larvae, that queen was rechecked in another colony and a third sample of eggs was collected and checked for development in an incubator (without worker bees present). Thus, all queens that produced nonhatching eggs were triple checked with at least 200 eggs from each of the three samples. Eggs of a queen that failed to hatch in her first colony, always failed to hatch in another colony and in the incubator.

The Fisher exact probability test was used to decide if the totals between two treatments reflected significant differences. A 1-tailed probability <0.05 was accepted as different.

Results and Discussion

Data in Table I indicate that a 10 percent concentration of DMSO in diluted semen caused about 3 percent of the female progeny to be totally sterile. The freezing process had no apparent effect. Therefore, attention focused on the groups that were not frozen. Six of 234 queens in the "nonfrozen" group were significantly different from 0/151 in the "no DMSO" group ($P<0.05$). Those two groups differed only in the presence or absence of DMSO in the diluent. The groups with DMSO (the "frozen" and "nonfrozen" groups) were similar (3.0 and 2.6 percent of the queens were sterile), and those without DMSO ("no DMSO" and "control") were identical (neither had a sterile queen).

Sterility appeared only among queens produced from spermatozoa that had been treated with DMSO. Among those queens, the frequency of total sterility was 3 percent. This is not to say that 3 percent of the eggs were nonviable but that 3 percent of the queens produced only nonviable eggs. Such total sterility does occur naturally in honey bees, but it is so rare that it had been noted in the literature as an oddity[6].

Of course, there was considerable sterility in the eggs from the P_o generation, especially in the frozen group, but not in the control group[3]. The gametes from each normal P_o queen had the chance to combine with thousands of treated spermatozoa, and some combinations caused lethal eggs[3]. In other cases the spermatozoa were so damaged that they could not even enter the egg to cause sterility; this expressed itself as a normal male because male honey bees are haploid and normally develop from unfertilized eggs (females are diploid and develop from fertilized eggs). Some combinations successfully produced viable offspring and it was these apparently normal females that were tested in this experiment.

Instead of combining the normal gametes from a female with treated spermatozoa, this experiment reversed the condition. The gametes to be tested were produced by the female and the spermatozoa were normal. Thus, the evaluation of eggs from an F_1 queen was an evaluation of a single gamete rather than of thousands of gametes, and this single gamete had already fertilized an egg to produce an apparently normal queen. The question was, could this gamete (now a genome of a queen) successfully complete the cycle and produce viable gametes? Of those treated with DMSO, 3 percent could not.

Although the mechanism of DMSO-induced sterility is not yet understood, it may be caused by genetic damage. DMSO has been identified as a mutagen in yeast[10] and rats[7], but other studies have shown DMSO to be nonmutagenic[8,9]. Kapp and Eventoff[7] attempt to resolve this by suggesting that DMSO may appear nonmutagenic when measuring only genic changes, whereas the prime action of DMSO may be to change chromosomal structure such as the breakage of chromatids that they found in rats treated with DMSO.

A similar type of chromosome change may be the cause of sterility in honey bees. Such chromosomes may not be lethal in honey bees until they encounter meiosis. At that point, they may sometimes cause total sterility.

This study suggests caution in the use of DMSO. DMSO should not be used as a cryoprotectant for the spermatozoa of honey bees, and perhaps other usage should be reviewed. Those using DMSO in tissue storage or as a solvent should be aware of its potential as a mutagen and may want to consider alternate solvents or cryoprotectants.

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