PROCTODEAL GLAND FOAM ENHANCES COMPETITIVE FERTILIZATION IN DOMESTIC JAPANESE QUAIL

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ABSTRACT.—Foam produced by the proctodeal gland of male Japanese Quail (*Coturnix japonica*) may help sperm transportation along the oviduct by inducing higher motility of the sperm via aeration. It is possible that foam can also suspend sperm in the proctodeum of the female to avoid sperm elimination by the egg as it travels down the oviduct. We demonstrated that when quail semen was mixed with foam in vitro, sperm motility was prolonged significantly. We labeled foam with Tc-99m sulfur colloid to demonstrate that foam deposited by the male through natural copulations may be retained by the female for more than 2 h and is not eliminated during oviposition. We concluded that the proctodeal gland of the male Japanese Quail may have evolved to produce a large amount of foam under domestication. This may allow the males to fertilize more females in competition with other males. *Received 29 April 1988, accepted 15 December 1989.*

SEVERAL hypotheses propound the function of proctodeal gland foam of the male Japanese Quail (*Coturnix japonica*) (Perez and Juarez 1966, Renzoni 1968, Schleidt and Shalter 1972), but no conclusive evidence nor a satisfactory explanation has been presented (King 1981). While the presence of foam may not be important for good fertility in artificial insemination where semen has been deposited in the vagina (Lepore and Marks 1966, Kobayashi et al. 1972), it is crucial for achieving good fertility in natural copulations (Cheng et al. 1989), where semen may be deposited in the proctodeum of the female. It is likely that foam acts as a medium for sperm transportation, but the reasons why foam is limited to male *Coturnix* and why the foam-semen mixture is deposited in the proctodeum during copulation remain unclear (Cheng et al. 1989).

Chickens and ducks lay early in the morning (Wilson 1964, Tanabe and Nakamura 1980), turkeys lay mostly during late morning and early afternoon (Wilson 1964), but Japanese Quail lay during the 2–4 h before sunset (Wilson 1964, Konishi 1980). In all these species, ovulation normally occurs 15–75 min after oviposition of the previous egg (Sturkie 1985) and fertilization occurs in 15–30 min after ovulation (Gilbert 1971). Sperm normally take an hour to traverse the oviduct (Allen and Grigg 1957), but near the time of ovulation, sperm can traverse the oviduct in 10–15 min (Bobr et al. 1964b, Howarth 1971). On the other hand, an egg in the oviduct, especially a hard-shelled egg, effectively blocks the sperm (Bobr et al. 1964a). Before shell membranes are deposited around the egg, albumen can also trap sperm and apparently lower the number of sperm stored in the uterovaginal (UV) sperm storage tubules (Bobr et al. 1964a). It is unlikely that sperm would survive in the lumen of the oviduct during periods of albumen and shell secretion (Howarth 1974).

Artificial insemination at times when a hard-shelled egg is in the oviduct results in lowered fertility (Moore and Byerly 1942, Parker 1945, Wyne et al. 1959). Thus a male should copulate within an hour postoviposition to make use of this "insemination window" (Cheng et al. 1983) to optimize his chance of fertilizing an ovum. If he inseminates shortly before oviposition, most of the semen may be carried out by the egg. If he inseminates the hen shortly after oviposition, he has a good chance of fertilizing the next ovum ovulated. If he delays, the ovum is no longer fertilizable and, although the sperm inseminated may be stored in the UV tubules, they are at risk of being covered by semen from other males via subsequent copulations and have little chance of fertilizing subsequent ova. In a flock situation, it may be difficult for a male to determine the egg-laying time for each female or to have the opportunity to copulate with a female at the appropriate time (Cheng and Burns 1988). The male would do best to copulate when most or all of the females in the flock have laid to maximize his chance of fertilization. This must balance with the chance of getting most sperm

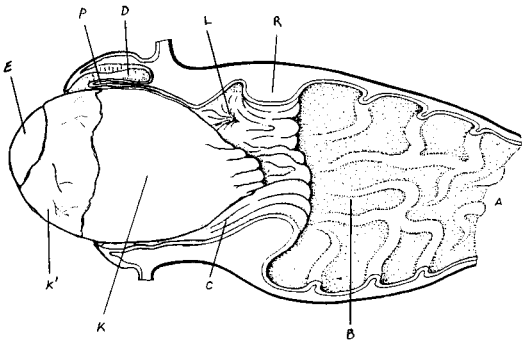

Fig. 1. Schematic illustration of foam-semen mixture position during oviposition (modified from Komarek [1971]; the egg is not drawn to scale): (A) rectum; (B) coprodeum; (C) urodeum; (D) proctodeum, filled with the foam-semen mixture; (E) the exposed blunt end of the egg; (K) left oviduct extended with an egg inside; (K') the everted edge of the oviduct exposing the inside wall; (L) the ostium of the left ureter; (P) uroproctodeal fold (*plica proctodeourodealis*); and (R) coprourodeal fold (*plica urodeocoprodealis*). (See fig. 1, Cheng et al. 1989, for comparison.)

stored by the females for subsequent fertilizations. This prediction is true for chickens (Wood-Gush 1971, Cheng et al. 1985), turkeys (Smyth and Leighton 1953), and ducks (Balthazart and Hendrick 1979, Cheng et al. 1982). In these species, copulation frequencies peak daily near the end of the normal period for egg laying.

In Japanese Quail, the predicted time for copulations would be after dark because egg laying peaks just 2–4 h before dark and some females lay consistently after dark (Wilson and Huang 1962, Opel 1966). Japanese Quail are not active after dark. There is no clear peak in copulation frequency in Japanese Quail and only a significant low that corresponds to the peak of egg-laying activities (Ottinger et al. 1982). It is possible that the foam gland developed in the Japanese Quail because its exudate compensates for the lack of an "insemination window" as occurs in other domestic birds. Foam may act as a medium for suspending sperm in the female's proctodeum (a pocket out of the way of the egg as it is being laid; Fig. 1) to avoid excessive loss of sperm during oviposition. As the foam dissipates, sperm may be released slowly (even after dark) for a better chance of fertilization or storage in the UV tubules, or both. In order to support this hypothesis, it must be shown that foam prolongs sperm motility and that the sperm-foam mixture stays in the female and is not eliminated through egg laying. We determined experimentally if foam would prolong sperm motility in vitro. Subsequently, we examined the length of time foam remained in the female after copulation, and if foam was eliminated by oviposition or defecation.

Materials and Methods

Five wildtype UBC-A males (see Cheng et al. 1989) were obtained from the Quail Genetic Stock Centre and trained for semen collection. We collected semen by the method of Marks and Lepore (1965) with modifications suggested by H. P. Van Krey (pers. comm., Hickman 1984). Foam was squeezed out of the proctodeal gland and eliminated or collected separately before semen was collected.

The experiment consisted of two treatments with two replications for each treatment. In Treatment 1, semen obtained from a male was divided into two portions. One portion was mixed on a microscope slide with about 20 μl of thin albumen from a fresh quail egg and covered with a cover-slip. The second portion was treated the same except a small amount of foam from the same male that provided the semen was added. Slides were observed simultaneously under two microscopes at room temperature. In Treatment 2, we followed the same procedure except that the foam added was a mixture from males other than the male providing the semen. This would determine if foam interacted immunologically to sperm from other males.

Six sexually mature UBC-A males and 12 UBC-A females were maintained for a second experiment. Two weeks before the start of the experiment, we placed the males in individual cages and habituated them to copulating with females (not experimental females) in the cage. The experimental females were also kept in individual cages and the approximate time of egg laying for each female was recorded daily.

We used a Technecium isotope, Tc-99m sulfur colloid (Frosstimage Sulfur Colloid Kit, Frosst Radiopharmaceuticals; Phan and Wasnich 1981) to label the foam. Tc-99m sulfur colloid has been used in human intravenous injection to monitor blood flow and restrictions, and for liver scanning. It has a physical half-life of 6 h. Colloid was used in this experiment because it is not viscous and will not alter the consistency of the foam. It will not irritate the birds as radiopaque substances would, it efficiently adsorbs other substances to its surface, and it is more likely to adhere to the foam. Only a minute quantity is required for labeling.

Radiographic (gamma ray) pictures were taken with a Picker Dyna Camera 4 connected to a Picker Image Programmer and an Adac Laboratories DPS-2800 computer. We monitored images from the camera on

a video screen and recorded them on hard disk for further analyses.

A first trial was conducted as a control to determine if the Tc-99m sulfur colloid adequately labeled the foam. We injected two males each with 0.025 ml (0.05 millicuries) of Tc-99m sulfur colloid directly in the proctodeal gland through the dorsal wall of the cloaca. Radiographic pictures were taken of the males. Then the foam from each male was squeezed out on a petri dish and a picture was taken of the foam alone.

In each additional trial, colloid (0.05 millicuries) was injected into the proctodeal gland of each male and a female was put into the cage with each male. Two completed copulations were allowed to increase the chance of sperm (and foam) transfer. The first 3 females that completed the copulations were used for the trial. We conducted the trials in early afternoons and used females with a hard-shelled egg in the oviduct. In the three trials, a total of 9 females were tested.

After the copulations, we restrained each female on her side on a wire platform with 1" × 2" mesh, with wings folded close to the body and legs stretched. We covered the bird's head with a piece of tissue paper to minimize excitability and stress to the bird. Feathers around the vent were clipped before the trials to minimize interference with the feces in case of defecation. The platform and the bird were placed on the camera stage for gamma ray pictures at regular intervals and after each defecation or oviposition, until about 3 h after the copulations. If the bird defecated, the feces fell through the wire mesh onto a piece of cellophane under the platform so that the feces could be separated to avoid overlapping images on the pictures. We removed the feces and replaced the cellophane before the next picture was taken.

RESULTS

The volume of ejaculate from quail was small (4–7 μl; Buxton and Orcutt 1975) and the semen was thick and viscous. The addition of thin albumen decreased viscosity and increased sperm motility. In all cases where no foam was mixed with the semen, sperm motility slowed 3–5 min after being placed on the slide; motility ceased within 10 min (Table 1). However, when foam was added to the mixture (whether it was foam from the same male that provided the semen or foam from other males), sperm remained vigorously motile even 45 min after they had been placed on the slide. The difference between the slides with and without foam was obvious, and observations ceased after about 45 min. Casual observation on one of the slides with foam added to semen revealed that the sperm were still motile after 95 min at room temperature.

TABLE 1. Duration of quail sperm motility at room temperature.

Treatment	Last observation (min)	Motility
Male 1		
Semen	4	Ceased
Semen + own foam	16	Vigorous
Male 2		
Semen	10	Ceased
Semen + own foam	55	Vigorous
Male 3		
Semen	45	Poor[a]
Semen + others' foam	45	Vigorous
Male 4		
Semen	11	Ceased
Semen + others' foam	45	Vigorous

[a] Small number of sperm with heads in air bubbles trapped under the cover-slip still had slow tail movements. Motility of all others ceased by 8 min.

Results from the trial run confirmed that the injected colloid adsorbed to the foam. When foam was squeezed out of the bird, most of the radioactivity was with the foam and not in the bird.

In 9 females tested, 2 showed very little or no radioactivity. They were probably not inseminated. Of the remaining 7 females, 1 laid an egg while she was restrained on the platform. No foam was observed on the egg. The egg was put beside the bird while a radiograph was taken. Another picture was also taken with the egg alone on the camera stage. No radioactivity was detected on the egg and there was no appreciable loss of radioactivity from the bird. A total of 10 defecations occurred. In 3 of these, no foam was observed on the feces and no radioactivity detected. Again, there was no observable decrease of radioactivity in the birds. In the other 7, radioactive foam was observed on the feces. When foam was observed on the feces, in most cases it retained its original consistency. In two cases where fecal material was liquid, the foam was diluted.

Three of the females lost >50% of radioactivity through defecation by 49, 60, and 51 min after copulation. The intervals for 2 other females were 88 and 145 min, after copulation. The remaining 2 retained the foam through the last observations at 150 and 206 min. The mean time that females retained foam was 107 min.

Discussion

Chicken sperm remains motile for about 25 min at room temperature (Sarvella and Marks 1970). Without mixing with foam from the proctodeal gland, quail sperm in vitro lost motility within minutes after collection. This observation is consistent with that of Ogasawara and Huang (1963). However, with the addition of foam, motility of the sperm was maintained for a much longer period even at room temperature. Schindler and Nevo (1962) reported that aeration of chicken and bull semen generally increased overall motility but decreased the duration of sperm motility. Mixing turkey frothy fluid with turkey semen did not affect sperm motility or fertility (Fujihara et al. 1987). The addition of quail foam to chicken semen did not affect sperm motility (Sarvella and Marks 1970) or may have decreased sperm motility (Hickman 1984). Adding foam to quail semen both increased and prolonged sperm motility, indicating that this is a special reaction in Japanese Quail. Presumably, stimulation by foam facilitates sperm movement into the UV sperm storage tubules once they are in the oviduct, and it lessens the chance of elimination (Lake pers. comm.).

Only one female laid during our trials. Oviposition did not cause the foam and semen mixture to be eliminated from the female body along with the egg. Other females probably delayed oviposition because of the stress of being restrained (Opel 1966). Nevertheless, the single incident of oviposition provided strong evidence that foam in the proctodeum of the female was unaffected by oviposition. Defecation could eliminate some of the foam but foam stayed in females for 2 h or more. The mean time of 107 min was a conservative estimate because birds defecate more often when they are frightened or stressed. Two of the seven females retained all the foam and all of the others had measurable radioactivity at the end of the observation period.

The secretion from the proctodeal gland in wild Common Quail (*C. coturnix*) and Japanese Quail may serve to aerate sperm to facilitate sperm transportation in the oviduct. In domestic Japanese Quail, the proctodeal gland may have further developed to produce a large amount of foam to suspend sperm in the proctodeum of the female away from the path of the egg. Such mechanism would enhance competitive fertilization (Clayton 1972, Haase and Donham 1980) by minimizing sperm loss due to oviposition. In this case, foam may be a neutralizing agent to protect the sperm from the hostile environment (e.g. uric acid and excrement) of the proctodeum, an idea to be explored. If the proctodeum of the female contains foam from a previous copulation, additional deposits from subsequent copulations may have a much higher chance of being eliminated. Under this situation, males which produce more foam per ejaculate would have an advantage. Additional indications that fertilization is highly competitive in males of domestic quail is that they have relatively large testes (2.3% of body mass) and a high daily output of sperm (308×10^6 per bird) (Clulow and Jones 1982).

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