A STUDY ON THE FUNCTION OF FOAM FROM THE PROCTODEAL GLAND OF THE MALE JAPANESE QUAIL (COTURNIX COTURNIX JAPONICA) WITH RESPECT TO ITS EFFECTS ON SPERM COMPETITION

BY

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ABSTRACT

The effect of foam from the male Japanese quail on sperm competition was investigated by comparing fertility and mating behaviour of foam-producing (sham-operated) and foamless (foam gland cauterized) males in two male / five female mating group situations. In vitro examinations of sperm behaviour in foam were also carried out.

Over an eight-week experimental period, foam-producing males sired more than 90% of the progeny produced when in competition with a foamless male. Pens in which two foamless males were competing had significantly lowered fertility and hatchability of eggs, while pens with two foam-producing males had the highest fertility. Foamless males attempted and completed copulations as often or more often than foam-producing males and examinations of females post-copula revealed that sperm transfer was occurring during copulations by both types of males. Results indicate that foam allows a male to copulate less frequently and yet maintain good fertility levels.

Foam significantly hindered the penetrating ability of quail sperm in thin albumin-filled capillary tubes (75 mm long x 1.2 inner diameter). On the other hand, foam significantly prolonged quail sperm motility. The behaviour of quail sperm when mixed with foam from the same or from a different male, was indistinguishable when examined on microscope slides at 400x magnification.

I found that during copulation by quail, sperm and foam are mixed and deposited into the female's coprodeum, rather than in the oviduct

as with most other birds examined. In conclusion, because the temporal pattern of egg laying in the Japanese quail (late in the day) precludes a peak in copulations at the optimum time for fertilization (within an hour post-oviposition), foam may aid a male's reproductive success by suspending and sustaining his sperm in the female's coprodeum, thus avoiding excessive loss of sperm via oviposition. These experiments also suggest a hypothesis about a role for foam in sperm competition: its physical presence fills space which may preclude normal foam and semen deposition by subsequent copulations and thus increase the chances of the foam/sperm mass from subsequent copulations being excreted by the female.

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INTRODUCTION

The male Japanese quail (Coturnix coturnix japonica) produces a thick foam from the proctodeal gland in the dorsal wall of the cloaca (Coil and Wetherbee, 1959; Ikeda and Taji, 1954; Nagra et al., 1959) (Fig 1). Although the production of foam is unique to the genus Coturnix, homologous vestigial glandular tissue has been found in chickens and turkeys (King, 1975, 1981; Komarek, 1970), in ducks and geese (Komarek, 1971), and possibly also in Northern Fulmars (Hatch, 1983). Such findings may indicate a much wider occurrance of these glands in the Class Aves. The production of foam has resulted in the common name "foam gland" for the proctodeal gland of the Japanese quail. The term "cloacal gland" had also been used in the literature. In this thesis I shall use the common name foam gland in referring to the proctodeal gland of the male Japanese quail.

The proctodeal gland occurs in both sexes of the afore-mentioned domestic birds including the Japanese quail (King, 1975; Komarek, 1971). In the female quail, a small amount of foam is sometimes produced, especially in the older birds (McFarland et al., 1968). Females, immature males, and castrates can be stimulated to produce foam by the administration of testosterone (Adkins and Adler, 1972; Hutchison, 1978; McFarland et al., 1968; Nagra et al., 1959; Schumacher and Balthazart, 1983).

The development (Schafersman and Klemm, 1977; Siopes and Wilson,

FIGURE 1: Sagittal section of cloaca of adult male Japanese quail.

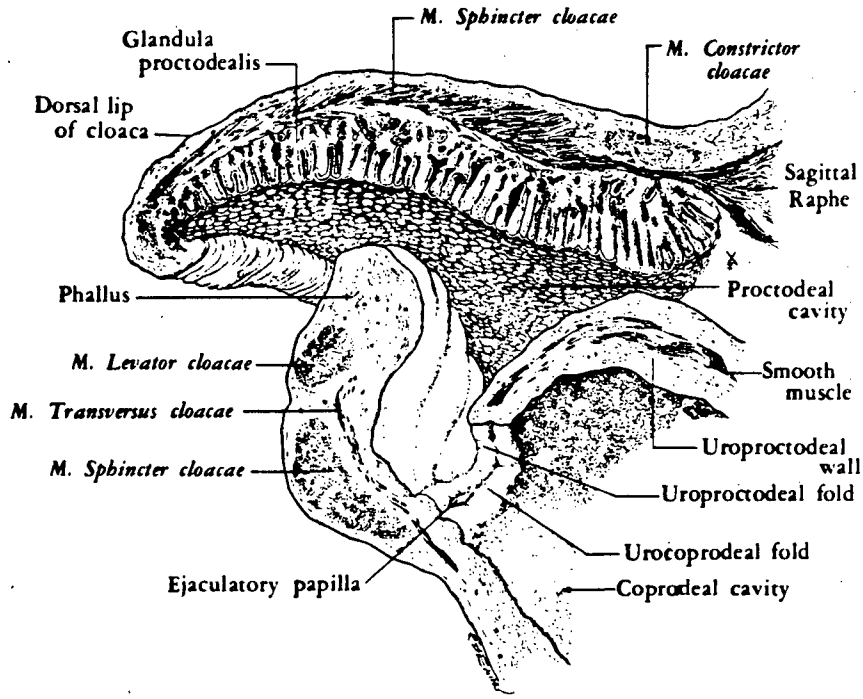


Figure taken from Klemm et al., 1973.

1975) and the morphology (Klemm et al., 1973; McFarland et al., 1968; Tamura and Fujii, 1967) of the male's foam gland have been studied. The foam gland is actually an "aggregate gland" of discrete glandular units united by connective tissue (Klemm et al., 1973). Hypertrophy of the glandular tissue is seasonally (photoperiodically) controlled; the glands are androgen-dependent with the size of the resulting protuberance highly correlated with sexual behavior (Sachs, 1967, 1969). Wilson et al. (1962) found that the foam glands started producing foam only after the testes weight had reached 0.75 gm. Thus, the cloacal protuberance of the male Japanese quail is a good indicator of the male's sexual condition and androgen levels (Sachs, 1967, 1969) and can be used to measure testicular activity over time without having to kill the male to obtain testicular weight (Siopes and Wilson, 1975). In this context, there has been much research done recently on testosterone metabolism in the foam gland, in comparison with metabolism in the brain of the male Japanese quail, and its effects on behaviour and sexual development (e.g. Adkins and Pniewski, 1978; Adkins et al., 1980; Balthazart and Schumacher, 1984; Balthazart et al., 1979, 1980, 1984; Cohen-Parsons et al., 1983; Davies et al., 1980; Massa et al., 1979, 1980; for review, see Adkins-Regan, 1981; Ottinger, 1983; Martini, 1982).

The secretion of the foam gland is a viscous mucoid--referred to as a glycomucoprotein by McFarland et al.(1968) and as a mucopolysaccharide by Fujii and Tamura (1967) and Klemm et al. (1973)--that apparently foams with CO_2 and H_2 produced from cloacal

bacteria (Escherichia coli and Proteus mirabilis) metabolizing glucose (McFarland et al., 1968). Pellerano-Domini and Renzoni (1969) found the secretion to be 44 % protein, 4.4 % polysaccharides, and 5.7 % uronic acid. Uronic acid is involved with the oxidative breakdown of glucose (Martin et al., 1983). Secretions from active foam glands are sulfated while secretions from the non-stimulated female and immature and castrated male foam glands are non-sulfated (Fujii and Tamura, 1967).

No conclusive evidence has been presented on the function of the foam but because it is deposited along with semen into the female during copulation and its production is androgen-dependent (Sachs, 1967, 1969), it is most commonly thought to be concerned with reproduction (Ikeda and Taji, 1954; Klemm et al., 1973; McFarland et al., 1968; Ogawa et al., 1974; Wetherbee, 1961). Other suggestions as to the foam's function have been: (i) it prevents post-copulatory sperm leakage from the female (Perez and Juarez, 1966), (ii) it acts as a lubricant for the male's phallus (Renzoni, 1968), and (iii) because it is also occasionally excreted with feces, it might function as a territorial marker (Schleidt and Shalter, 1972).

Although accummulated evidence suggests that foam from the male Japanese quail plays a role in reproduction, Marks and Lepore (1965) and Lepore and Marks (1966) obtained fair fertility using artificial insemination (AI) without mixing foam with the inseminated semen. The AI may have bypassed a factor in the process of normal mating, but foam is apparently not necessary for achieving fertilization in the Japanese quail.

A comparison of daily oviposition and peak copulation times between Japanese quail and other domestic birds provides some insights for the development of a hypothesis on the function of foam in natural mating. Chickens and ducks lay most of their eggs early in the light portion of the day (Tanabe and Nakamura, 1980; Wilson, 1964), turkeys lay mostly during late morning or early afternoon (Wilson, 1964), and Japanese quail lay during the last 2-4 hours of the day (Konishi, 1980; Wilson, 1964). In all of these birds, ovulation normally follows oviposition of the previous egg by 15-75 minutes (Sturkie, 1976). Fertilization of an ovulated ovum occurs in the infundibulum (Allen and Grigg, 1957; Howarth, 1974). A second vitelline membrane formation (Bellairs et al., 1963) and albumin deposition around the yolk sufficient to preclude fertilization occurs within 15-30 minutes after ovulation in these birds (Gilbert, 1971; Woodard and Mather, 1964). Sperm normally takes an hour to traverse the oviduct (Allen and Grigg, 1957) but near the time of ovulation, sperm can traverse the oviduct within 10-15 minutes (Bobr et al., 1964b; Howarth, 1971).

On the other hand, an egg in the oviduct, especially a hard-shelled egg, is an effective block to the upward travel of sperm and apparently lowers the concentrations of sperm subsequently stored in the uterovaginal (UV) sperm host glands (Bobr et al., 1964a). Artificial insemination at times when a hard-shelled egg is in the oviduct also results in lowered fertility (Moore and Byerly, 1942; Parker, 1945; Wyne et al., 1959). Thus, a male should copulate within an hour post-oviposition to optimize his chance of fertilizing an ovum. However, in a flock situation it will 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; the male would do best to copulate when most or all of the females in the flock have laid to maximize his chance of copulating with a female without a hard-shelled egg in her oviduct. Under these circumstances, the male's evolutionary strategy of timing copulation for the best chance of fertilization would be a compromise between two opposing time factors. The male should time copulation so that:

1) it's late enough in the day that if copulating with a female chosen at random from the flock, there would be no hard-shelled egg in the female's oviduct, but it's early enough so that
2) copulation would occur near the time of ovulation so his sperm would have the best chance of fertilizing the ovulated ovum.

Such prediction is true in chickens (Wood-Gush, 1971), turkeys (Smyth and Leighton, 1953), and ducks (Raitasuo, 1964); in these species, copulation frequencies peak daily near the end of the normal time curve for egg laying. However, in Japanese quail, the predicted peak for copulations would be after dark because egg laying peaks just 2-4 hours before dark with some females laying consistently after dark (Opel, 1966; Wilson and Huang, 1962). As it is, there's no clear peak in copulation frequency in Japanese quail, with only a significant low corresponding to the peak of egg laying activities (Ottinger et al., 1982). Thus, perhaps, the foam gland developed in the quail because its exudate played a role in compensating for the lack of a convenient

"insemination window" (Cheng et al., 1983) as occurs in other domestic birds. The role of the foam might be to aid sperm in reaching the oviduct and to also block sperm from subsequent copulations.

Further evidence to argue for a role by the male's foam in natural mating can be seen from a comparison of wild and domestic Japanese quail. In the wild, Japanese quail are mostly monogamous but polygyny often occurs with a surplus of females (Moreau and Wayre, 1968; Wetherbee, 1961); under about 600 years of domestication (Howes, 1964; Yamashina, 1961), Japanese quail have become promiscuous (Wetherbee, 1961). This condition has drastically increased the intensity of sperm competition within the female. At the same time, the size of the foam gland has increased significantly under domestication (Schleidt and Shalter, 1972). The physical characteristics of foam make it an ideal vehicle for spermicides in human vaginal contraceptives (Bernstein, 1971; Dingle and Tietze, 1963; Greenstein, 1965; Hafez, 1980; Sobrero, 1970); perhaps the quail's foamy exudate also evolved in response to its effects on sperm competition within the female.

Intrasexual selection will be especially intense for males to reduce competition from sperm of another male (Parker, 1970; Trivers, 1972). Sperm competition occurs in insects (Parker, 1970), reptiles (Devine, 1975; Gibson and Falls, 1975), mammals (Dewsbury, 1984), fish (Van Tienhoven, 1983), as well as in birds (Allen and Champion, 1955; Cheng et al., 1983; McKinney et al., 1984; Payne and Kahrs, 1961). There have evolved numerous strategies by males to reduce sperm competition in their favor, e.g. prolonged or repeated copulations

(insects: Parker, 1970; Smith, 1979; mammals: Ogelsby et al., 1981; birds: Welty, 1979), mate guarding (insects: Parker, 1970; salamanders: Arnold, 1976; birds: McKinney et al., 1984), sperm blocks or copulatory plugs (insects: Parker, 1970; reptiles: Devine, 1975, 1977; Ross and Crews, 1977; mammals: Hartung and Dewsbury, 1978; Voss, 1979). There has been no reported example of a sperm block occuring in birds (McKinney et al., 1984).

This thesis research was conducted to determine the function of the foam from the foam glands of the male Japanese quail and its relationship to sperm competition. Two hypotheses were tested:

1) The foam gland exudate of the male Japanese quail plays a role in the male's natural mating, and
2) The foam of the male Japanese quail increases that male's reproductive success by enhancing its own fertilizing capacity and/or by hindering sperm of competing males from achieving fertilization.

GENERAL MATERIALS AND METHODS

A) EXPERIMENTAL BIRDS

The birds used in all the experiments were either wild-type (UBC-A) or white (UBC-W) plumage Japanese quail. Both strains are maintained as randombred lines at the UBC Quail Genetic Stock Centre. The wild-type plumage UBC-A line was acquired from the University of Washington (Pullman) in 1964. The line has been closed since 1968 (Somes, 1981) and maintained with a population size of 100 females and 50 males each generation for at least 40 generations at the time of this experiment.

Birds from the UBC-W line carry an autosomal recessive mutation producing white feathers with occasional patches of darker feathers (Roberts et al., 1978). This line originated in 1976 from a pair of white birds acquired from a local breeder. The original birds were outcrossed to the UBC-A line and the F_1's were intermated to obtain the white progeny. UBC-W has since been maintained as a randombred line of 66 females and 33 males. This line had gone through at least 12 generations of closed random breeding at the start of these experiments.

These two lines were chosen for the experiments because the recessive white plumage gene of the UBC-W birds could be used as a genetic marker. Using only UBC-W females, unequivocal determination of paternity was possible--the homozygous dominant UBC-A males would

produce only heterozygous wild-type offspring and the recessive UBC-W males would produce only homozygous white offspring.

B) INCUBATION AND REARING

Birds to be used in the experiments were raised in groups of 70 with approximately equal proportions of UBC-A and UBC-W birds of both sexes. This was done to minimize bias due to preference for own phenotype in mating (Immelmann, 1972; Gallagher, 1976, 1977; Bateson, 1978; Truax and Siegel, 1982). Groups were reared in "Marsh Farm" game brooders (71 cm x 92 cm x 15 cm) (Marsh Mfg., Garden Grove, Ca.) until about 5 weeks of age. UBC-A females were then removed from the experiment and the remaining experimental birds were moved to and maintained in community holding cages until the start of experiments. Males of both lines were mixed but were kept separate from the UBC-W females. All experimental birds were wingbanded for individual identification and debeaked at about 4 weeks of age to reduce feather-pecking and cannibalism (Mahn and Blackwell, 1967). Feed (26% protein turkey starter ration) and water were available ad libitum during the rearing and experimental periods.

In experiments where fertility, hatchability, and phenotype of the offspring were to be determined, eggs were collected daily and identified by pen or cage number and date. When more than one female was kept per pen, eggs from a given female could be matched by the

specific patterns, colors, shapes, and sizes of the egg shells (Jones et al., 1964). Eggs were stored in a cooler room at about 11°C for a maximum of 6 days and then set for incubation in batches once a week. These eggs were artificially incubated and transferred on the 14th day of incubation for hatching in individual pedigree baskets in two Jamesway Model 252 incubators. All eggs not hatched after 18 days of incubation were stored at 11°C and were broken out later to determine if they were infertile (Kosin, 1944) or were embryonic deaths. Embryonic deaths were classified as early deads (ED; embryos that died before 8 days of incubation), late deads (LD; embryos that died between 8 days of incubation and pipping), and pips (embryos that pipped but were unable to emerge from the shell). Plumage phenotypes could be determined during the LD stage (Padgett and Ivey, 1960).

C) OPERATIONS

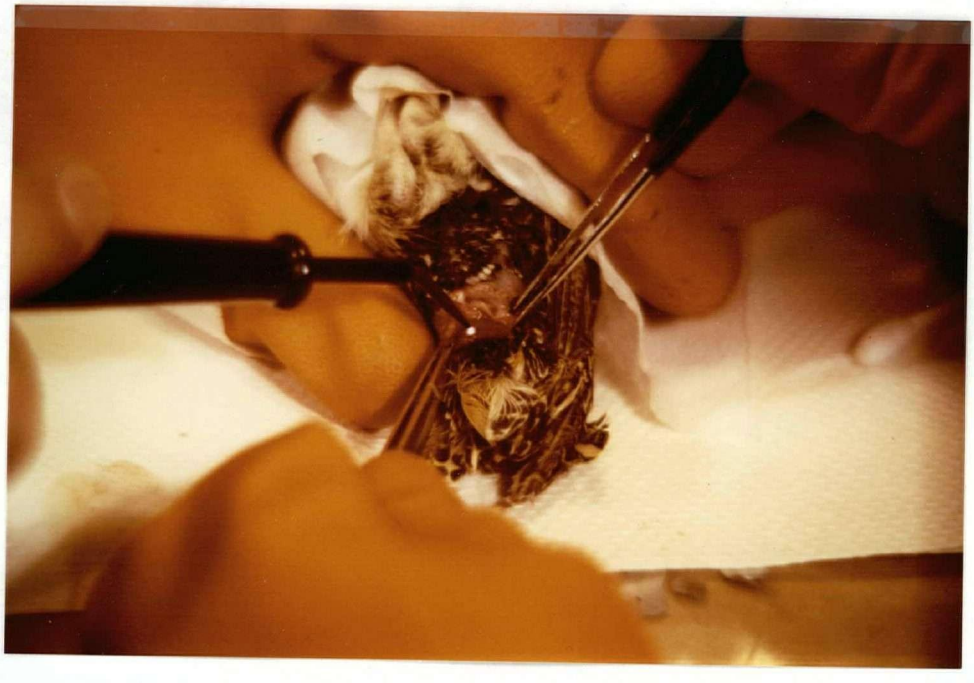
In two of the experiments, some treatments required males not producing foam and yet maintaining spermatogenesis and sexual behaviour. Therefore, it was necessary to operate on the foam glands of these males. As the foam gland is hypertrophied after sexual maturity, the best time for the operation would be before the gland is fully developed. Rather than trying to remove the gland surgically, it was destroyed by cautery. This was done to minimize distortion of the cloacal area. A similar procedure has been successful in

rendering other glands non-functional (e.g. avian salt gland, Hughes, 1977) A preliminary study was conducted using 3, 4, and 5-week old non-experimental chicks. Both destroying the gland by using dry ice applied locally and by electric cautery (Hyfrecator) were tried. I found that cautery was most effective between 4 and 5 weeks of age; the best results were obtained with approximately 90 volts.

All birds to be operated on were trimmed of feathers in the cloacal region. They were restrained by wrapping with a piece of folded "Kimwipes" tissue and then securing with masking tape (Fig. 2). Approximately 0.15 to 0.20 ml of local anesthetic (Xylocaine) was injected into the dorsal lip of the cloaca and 60 to 90 seconds were allowed for the anesthetic to take effect. A single 1 cm cut with a number 3 surgical scalpel was made longitudinally from the centre of the cloaca's dorsal lip to expose the inner cloacal surface. One person with insulated forceps kept the surface to be cauterized exposed while a second person operated the Hyfrecator (Fig. 3). Cotton swabs (Q-Tips) were used to clear the area when needed. After the glandular tissue was destroyed, talc powder was sprinkled over the area to help stop any bleeding. The bird was then released into a holding box with water available and was periodically observed to ensure that bleeding had stopped, and that the bird was moving around before being replaced in its original cage. The time required to operate on one bird was less than 10 minutes. Sham-operated birds used as controls were treated identically to the cauterized birds except that the cauterization itself was not performed.

FIGURE 2: Method of restraint for cauterization of foam gland.

FIGURE 3: Cauterization of foam gland.



EXPERIMENT 1

There is no empirical data in the literature about the role of the male Japanese quail's foam gland secretion in natural matings. This experiment was designed to determine whether the foam plays a role in natural mating by examining its effects in a 2-male/5-female mating group situation using sexually mature males either producing foam or with foam glands cauterized. One male can induce above 61% fertility when kept with 5 females (Woodard and Abplanalp, 1967). Two males were used in order to study the effects of foam on sperm competition within the female.

A) HISTORY AND REARING OF EXPERIMENTAL BIRDS

The birds used in this experiment (UBC-A and UBC-W strains) were hatched on 20 August, 1982, and were reared with the sexes and genotypes mixed. Forty-five males from each line and all 66 UBC-W females were used in the experiment. Sexes were separated at four

weeks of age and the UBC-W females were then maintained in community holding cages until the beginning of the experiment. After eight weeks of age, 60 UBC-W females were placed in their experimental floor pens for an acclimatization period of 10 days.

All experimental males were operated on before they were 6 weeks of age (30 males from each line were cauterized and 15 males from each were sham-operated). Males were separated by genotype and placed in community holding cages two days after all operations were completed.

Prior to the experiment, a "fertility test" was performed with 20 cauterized males (10 males from each line). Males were individually paired with females in cages for 6 days; eggs were collected, incubated for 5 days, and broken out to determine fertility. Males from pairs where the female was consistently laying infertile eggs were eliminated from the experiment. Because of time and cage availability, not all the cauterized males used in the experiment were tested for fertility.

B) EXPERIMENTAL PROCEDURE

Six chicken-wire floor pens with wood shavings litter (each pen = 122cm x 92cm x 46cm high) were placed in each of two rooms with the same dimentions (3.45 m x 3.65 m). Each pen contained five mature UBC-W females and two mature males (one UBC-A and one UBC-W) (Fig. 4).

FIGURE 4: Floor pen as used in Experiment 1.

The sides of the pens were covered with black plastic sheets to provide visual isolation between pens. The floor pens were used for three replications of these four treatments:

1) UBC-W male sham-operated; UBC-A male cauterized,

2) UBC-W male cauterized; UBC-A male sham-operated,

3) both males cauterized, and

4) both males sham-operated.

During the experiment, eggs were collected from the experimental pens, identified, and artificially incubated. Phenotypes of progeny were recorded and the percent progeny sired by foam-producing (sham-operated) and foamless (cauterized) males was determined. Fertility, hatchability, and % embryonic death were calculated for each female.

Behavioural observations were carried out on two of the replicates to monitor any changes in mating performance due to the operations and to detect the occurrance of any mate preference by females. Eighteen 20-minute observations were done on each of the eight pens --six during each of the three time periods spanning the light portion of the day (morning: 0700-1100 hrs; afternoon: 1100-1700 hrs; evening: 1700-2100 hrs). All observations were made by the same observer sitting on a stool on a raised platform next to the pens. A clipboard with data sheets, pen and a watch were used for recording observations. Only reproductive behaviours and aggressive behaviours involving at least one male were recorded. The observer sat quietly for 5 minutes before beginning to record observations. The UBC-W females in each pen were individually identifiable from specific

plumage patterns on their heads and backs; UBC-W males were marked with a spot of paint on their backs for quick identification.

The experiment lasted eight weeks. In order to minimize bias due to any particular male, two sets of males were used and rotated every two weeks so that the first set of males was used on weeks 1,2,5, and 6, while the second set was used on weeks 3,4,7, and 8. Within each set, the males were never placed with the same group of females during rotation, but each female treatment group always received males with the same operations as before. Eggs were collected and hatched for another two weeks after the removal of males at the end of the eighth week.

Eight females needed replacing during the 10 weeks of egg collection-- three due to death and a treatment group of five due to excessive aggression towards males. Eight males also needed replacing during the experiment due to excessive aggression from the females.

C) DATA ANALYSES

Data analyzed included developmental condition of eggs collected from the experimental pens after 18 days of incubation (infertile, embryonic death, fertile, hatch), phenotypes of progeny (wild-type or white feathers), and copulatory behaviour ("mount" indicating a copulation attempt and "tail-bend" indicating a completed copulation). Analysis of variance (ANOVA) was applied for the three fertility and

hatchability traits. Arcsine transformation (Steel and Torrie, 1980, p. 236) was applied to all percentages before analyses. The statistical model used was:

$$Y_{ijk} = \mu + R_i + T_j + E_{ijk},$$

and i=1,2,3; j=1,2,3,4; k=1,2,...,5; where Y_{ijk} = one of the dependent variables (% fertility, % hatch, or % embryonic death). Y_{ijk} is the condition of the incubated eggs of the k^{th} female in the j^{th} treatment of the i^{th} replication, u = the theoretical population mean, R_i = effect of the i^{th} replication, T_j = effect of the j^{th} treatment, and E_{ijk} = random error.

Percent white progeny phenotyped (number of white identified / number of chicks phenotyped) and percent white progeny hatched (number of white hatched / number fertile eggs) were also analysed by ANOVA. Percentages were again transformed using the arcsine transformation. A similar statistical model was used:

$$Y_{ijkl} = \mu + R_i + O_j + C_k + OC_{jk} + E_{ijkl},$$

and i=1,2,3; j=1,2; k=1,2; l=1,2,...,5; where Y_{ijkl} = one of the dependent variables (% white chicks phenotyped, % white chicks hatched). Y_{ijkl} is the percent white progeny produced by the l^{th} female from the i^{th} replication sired by the UBC-W male with the j^{th} type of operation in competition with the UBC-A male with the k^{th} type of operation, u = the theoretical population mean, R_i = effect of the i^{th} replication, O_j = effect of whether the UBC-W male was cauterized or sham-operated , C_k = effect of whether the competing UBC-A male was cauterized or sham-operated, OC_{jk} = effect of the 2-way interaction involving the main effects, and E_{ijkl} = random error.

Analyses were performed on the males of both genotypes to determine frequencies of attempted copulations ("mounting") and completed copulations ("tail-bending"). Because of the small number of copulations involved, all values were transformed using the square root transformation $((Y + 0.5)^{.5})$ (Steel and Torrie, 1980, p. 234) before analysis. The statistical model used was:

$$Y_{ijklm} = \mu + R_i + O_j + C_k + OC_{jk} + El_{ijk} + T_l + TO_{lj} + TC_{lk} + TOC_{ljk} + E2_{ijklm},$$

and $i=1,2$; $j=1,2$; $k=1,2$; $l=1,2,3$; $m=1,2,\ldots,6$; where Y_{ijklm} is the number of copulations by the UBC-W male with the j^{th} type of operation (1 = cauterized; 2 = sham-operated) in competition with the UBC-A male with the k^{th} type of operation (1 = sham-operated; 2 = cauterized) during the m^{th} observation of the l^{th} time period in the i^{th} replication, u = the theoretical population mean, R_i = effect of the i^{th} replication, O_j = effect of whether the UBC-W male was cauterized or sham-operated, C_k = effect of whether the competing UBC-A male was cauterized or sham-operated, OC_{jk} = effect of the interaction of the main effects, El_{ijk} = error term for testing the main effects, T_l = effect of the l^{th} time period, TO_{lj} and TC_{lk} = effects of the 2-way interactions involving time, TOC_{ljk} = effect of the 3-way interaction, and $E2_{ijklm}$ = error term for testing the sub-plot effects.

The mean separations between treatments were tested using Duncan's New Multiple Range Test (Steel and Torrie, 1980, p.187) in all of the above ANOVAs.

D) RESULTS

1. Effect of quail foam on fertility and hatchability under natural mating conditions:

Male Japanese quail that were producing foam fertilized more eggs than males that were foamless. Females in pens with two foam-producing males had the highest fertility (97.5%) while females in pens with two foamless males had the lowest fertility (26.4%) (Table 1). In between these extremes was the fertility of females in pens with one foam-producing and one foamless male (75.6% and 73.2%, Table 1). There was no significant difference in hatchability of fertile eggs from pens with two foam-producing males (70.2%) as compared with hatchability from pens with one foam-producing and one foamless male (73.6% and 75.9%); hatchability of fertile eggs from pens with two foamless males, however, was significantly lower (53.4%, Table 1). There were no significant differences in the three replications in these fertility and hatchability data.

2. Effect of quail foam on sperm competition:

In competition for fertilizing the females' eggs, UBC-W and UBC-A males were about equally as effective (51.5% and 48.5%, respectively; Table 1) when both males were foam-producing. In situations where one of the males was foam-producing and the other male was foamless, the

foam-producing male (whether UBC-W or UBC-A) had a distinct advantage and sired almost all of the progeny produced by the females (98.7% for UBC-W males and 99.4% for UBC-A males, Table 1). On the other hand, when two foamless males were competing the UBC-A males had an apparent advantage and sired 74.5% of the offspring while UBC-W males sired only 25.5% of the offspring ($X^2 = 25.77$, df = 1, $p < 0.001$).

Another interesting observation is shown in Table 2. In pens where both males were foam-producing, all of the females had progeny from both males. In pens where a foam-producing male was competing with a foamless male, females either produced progeny sired by both males or produced progeny sired exclusively by the foam-producing male. However, in pens where both males were foamless, some females produced progeny sired exclusively by UBC-W males, some produced progeny sired exclusively by UBC-A males, and others produced both types of progeny.

3. Effect of condition of the foam gland on the male's mating behaviour:

There was no significant difference in the frequency of attempted or completed copulations between foam-producing and foamless UBC-W males (Table 3) (However, see significant 3-way interaction described on page 25). There was also no significant difference in these frequencies whether the UBC-W male (foam-producing or foamless) was competing with a foam-producing or foamless male, i.e. the condition

TABLE 1: Fertility, hatchability, and phenotypic ratio of progeny in the four treatments (summed over three replications).[1]

TREATMENT	CONDITION OF UBC-W MALE	CONDITION OF UBC-A MALE[2]	NUMBER OF EGGS SET	PERCENT** FERTILITY	PERCENT[3]* HATCHABILITY	NUMBER OF CHICKS TYPED	% WHITE[4]** PROGENY TYPED	% WHITE[5]** CHICKS HATCHED
1	Foam	Foamless	663	75.6^{b}	73.6^{b}	418	98.7^{d}	70.7^{d}
2	Foamless	Foam	660	73.2^{b}	75.9^{b}	424	0.6^{a}	0.4^{a}
3	Foamless	Foamless	696	26.4^{a}	53.4^{a}	109	25.5^{b}	9.5^{b}
4	Foam	Foam	666	97.5^{c}	70.2^{b}	540	51.5^{c}	32.3^{c}

1 Comparisons only within a column by Duncan's Multiple Range Test; means followed by different letter subscripts are significantly different.

2 UBC-A males were used as markers for proportion of progeny sired by UBC-W males.

3 % hatchability of fertile eggs.

4 (number of white offspring typed / number of offspring typed) x 100

5 (number of white chicks hatched / total number of fertile eggs) x 100

* $p<0.01$ ** $p<0.005$

TABLE 2: Phenotypes of progeny from females in the four treatment groups*.

Rep.	Female ID	UBC-W:Foam UBC-A:Foamless	UBC-W:Foamless UBC-A:Foam	UBC-W:Foamless UBC-A:Foamless	UBC-W:Foam UBC-A:Foam
1	1	both	both	UBC-W	both
	2	both	both	both	both
	3	UBC-W	UBC-A	UBC-A	both
	4	UBC-W	both	both	both
	5	both	UBC-A	UBC-W	both
2	6	both	UBC-A	both	both
	7	UBC-W	UBC-A	UBC-A	both
	8	UBC-W	both	both	both
	9	both	both	UBC-A	both
	10	UBC-W	UBC-A	both	both
3	11	UBC-W	UBC-A	both	both
	12	UBC-W	UBC-A	both	both
	13	UBC-W	UBC-A	both	both
	14	UBC-W	UBC-A	UBC-A	both
	15	both	UBC-A	UBC-A	both

* Each treatment had 15 females (5 females / pen, with 3 replications).

of the competing male's foam gland had no significant effect on the UBC-W males' mating frequencies (Table 3). Furthermore, there were no significant differences in the frequencies of both attempted and completed copulations between the three time periods in a day for the UBC-W males (Table 3). None of the 2-way interactions involving the main effects were significant. There was, however, a significant 3-way interaction involving the main effects (condition of the UBC-W male's foam gland, condition of the competing male's foam gland, and time of day) in frequency of attempted copulations (Table 4). Although the separation was not clear cut by Duncan's New Multiple Range Test, the trend was that foamless UBC-W males attempted copulation more often when in competition with foam-producing males than when competing with foamless males (0.286 and 0.092 respectively, Table 3). The significant 3-way interaction may have masked this effect. Again, there were no significant replication effects involved.

Since in all the treatment pens a UBC-W male was competing with a UBC-A male for the five females, the mating frequencies of the competing males were not independent and could not be compared in the same analysis. The frequencies of attempted and completed copulations by UBC-A males were therefore examined in separate analyses. The frequencies of both attempted and completed copulations differed significantly between foam-producing and foamless UBC-A males (Table 5). Foamless UBC-A males attempted and completed significantly ($p<0.01$ and $p<0.05$, respectively) more copulations than foam-producing UBC-A males. Neither the condition of the competing male nor the time of day had a significant effect on the mating frequencies (Table 5).

TABLE 3: Mean number of mating attempts (MA) and completed copulations (CC) by UBC-W males per observation period (20 minutes)[1].

TREATMENT	MA	CC
	se	se
Condition of UBC-W males:		
FOAM	.184 ± .027	.148 ± .025
FOAMLESS	.188 ± .029	.118 ± .026
Condition of competing UBC-A males:		
FOAM	.286 ± .032	.212 ± .030
FOAMLESS	.092 ± .021	.059 ± .018
Time of Day:		
0700-1100 h	.231 ± .036	.124 ± .030
1100-1700 h	.158 ± .032	.124 ± .030
1700-2100 h	.171 ± .035	.153 ± .034

[1] None of the treatment effects was significantly different.

TABLE 4: Significant 3-way interaction between foam gland operation, operation of competing male, and time of day; mean number of mating attempts (MA) by UBC-W males per observation[1].

TIME OF DAY	TREATMENT 1 UBC-W:Foam UBC-A:Foamless se	TREATMENT 2 UBC-W:Foamless UBC-A:Foam se	TREATMENT 3 UBC-W:Foamless UBC-A:Foamless se	TREATMENT 4 UBC-W:Foam UBC-A:Foam se
0700-1100	.200 ±.068^{ab}	.407 ±.092^{a}	.129 ±.058^{ab}	.200 ±.068^{ab}
1100-1700	.063 ±.043^{ab}	.327 ±.090^{ab}	.000 ^{b}	.224 ±.074^{ab}
1700-2100	.177 ±.081^{ab}	.327 ±.090^{ab}	.000 ^{b}	.200 ±.068^{ab}

1 Means followed by different letter superscripts are significantly different ($p<0.05$) by Duncan's Multiple Range Test.

TABLE 5: Mean number of mating attempts (MA) and completed copulations (CC) by UBC-A males per observation period (20 minutes).

TREATMENTS	MA	se	CC	se
Condition of UBC-A males:				
Foam	.294	± .034*	.157	± .027*
Foamless	.540	± .051	.365	± .036
Condition of competing UBC-W males:				
Foam	.384	± .043	.265	± .034
Foamless	.442	± .044	.249	± .031
Time of Day:				
0700-1100 h	.413	± .056	.232	± .041
1100-1700 h	.423	± .054	.330	± .043
1700-2100 h	.403	± .050	.212	± .030

* $p<0.05$

On the other hand, there was a significant 2-way interaction involving the condition of the male and time of day in the frequency of attempted copulations (Table 6)--UBC-A foamless males attempted significantly more copulations than the UBC-A foam-producing males only during the 11:00-17:00 hrs time period of each day (0.678 by foamless males and 0.200 by foam-producing males). Mating is usually infrequent during this part of the day in Japanese quail (Ottinger, et al., 1982). There were no significant replication effects.

Although there was no direct comparison, UBC-A males attempted and completed copulation twice as often as UBC-W males (Table 7). While there was no difference in the mating frequencies between foam-producing and foamless UBC-W males (Table 3), foamless UBC-A males mated significantly more often than foam-producing UBC-A males (Table 5) . This may be the reason why in pens where two foamless males were competing, the UBC-A males were able to sire more progeny than the foamless UBC-W males (Table 1).

4. Differences between genotypes in homosexual copulations:

During the observation periods, homosexual copulations between the experimental males were recorded. UBC-W males mounted UBC-A males 33 times resulting in nine apparently completed copulations. UBC-A males, however, were observed mounting UBC-W males only once during the experiment. Foamless UBC-W males attempted homosexual copulation 21 times while foam-producing UBC-W males attempted only 12 times.

TABLE 6: Significant time of day x condition of foam gland interaction; mean number of mating attempts (MA) by UBC-A males per observation[1].

TIME OF DAY	FOAM	FOAMLESS
	se	se
0700-1100 h	.379 ± .059^{ab}	.448 ± .098^{ab}
1100-1700 h	.200 ± .047^{b}	.678 ± .093^{a}
1700-2100 h	.310 ± .068^{ab}	.502 ± .073^{ab}

[1] Means followed by different letter superscripts are significantly different ($p<0.05$) by Duncan's Multiple Range Test.

TABLE 7: A comparison of the total number of mating attempts (MA) and completed copulations (CC) by UBC-W and UBC-A males.

MALES	MA	CC
UBC-W	35	26
UBC-A	79	48

EXPERIMENT 2

Results from Experiment 1 showed that, in natural matings, foam from the cloacal foam glands aids the male in fertilizing more eggs when in competition with another male. However, it was not conclusive as to:

1) how the foam aids the male in fertilization, or
2) whether the foam can act as a block to sperm from subsequent matings.

Experiments 2 and 3 address these two questions. In this experiment, the second question was examined by attempting to determine the order of transfer of foam and semen to the female during an ejaculation. If foam was transferred following insemination or mixed with the semen, then I expect that foam would act as a blocking agent to hinder sperm travel from any subsequent inseminations. On the other hand, if foam transfer was found to preceed semen transfer, then the sperm block hypothesis would have to be reconsidered.

A) MATERIALS AND METHODS

Pair copulations were staged and fluid and foam samples were scraped from three different cloacal regions of the females

immediately post-copula and examined microscopically. These three regions were:

1) just inside the lip of the cloaca,
2) foam expelled from the female's cloaca with light squeezing, and
3) scrape of the everted oviduct opening.

The slides were examined under 400x magnification to determine where the greatest concentration of sperm occurred. Mature UBC-A wild-type Japanese quail were used and copulations were staged after oviposition in the late afternoon so there would be no hard-shelled egg in the uterus to hinder everting the females' cloacae. A male was placed in the female's cage and observed until copulation with cloacal contact had occurred. If no copulation occurred within 5 minutes (Schein et al., 1972's "rule of thumb" for whether a male will perform sexually), a different male was used with the same female. After copulation, the female was removed and held ventral-side up with the head downwards in the experimenter's left hand. Without squeezing, the cloaca was slightly opened and a sample taken with a small vinyl spatula (a straightened paper clip with electrical tape trimmed to a bulb shape at one end) and transferred directly to pre-labelled Slide 1. After squeezing out some foam, a second sample was taken with a new spatula and transferred to Slide 2. After fully everting the female, the third sample (obtained with a third spatula) was taken from the everted oviduct opening and placed on Slide 3.

Slides were examined microscopically and scored for the presence of foam and relative density of sperm. A score of 1 was given for the

presence of sperm, a 2 was given for higher sperm concentrations compared to a 1, and a 3 was given for the presence of the high concentration sperm mass. Absence of sperm was given the score 0. Only the presence or absence of foam was recorded.

B) RESULTS

As reflected in the fluid and foam samples from the cloacal opening and the partially squeezed cloaca, sperm were always found mixed with foam; sperm were also found in the oviduct, but with no foam preceding them (Table 8). Thus, sperm are apparently always mixed with foam at insemination, and enter the oviduct without foam occurring between sperm and the infundibulum (site of fertilization; Olsen and Neher, 1948; Burke et al., 1969). Sperm from subsequent matings may have the preceeding male's foam to traverse before entering the oviduct.

In chickens (Lake, 1966), turkeys (Hale and Schein, 1962), and most waterfowl (Guaryahu et al., 1984; Van Tienhoven, 1983), the semen is deposited directly into the female's oviduct during natural matings. However, during this experiment, I found that the foam / semen mass of the male Japanese quail is deposited into the coprodeum of the female rather than her oviduct as had been expected (Fig 5). The coprodeum is the most frontal of three cloacal sections (with the urodeum and proctodeum, respectively, more caudal) and stores fecal

TABLE 8: The presence of sperm and foam in different parts of the female's cloaca immediately after copulation.

SAMPLE	CLOACAL OPENING		PARTIALLY SQUEEZED		OVIDUCT OPENING	
NUMBER	Sperm	Foam	Sperm	Foam	Sperm	Foam
1	1	1	1	1	1	0
2	1	1	2	1	1	0
3	1	1	3	1	1	0
4	1	1	3	1	1	0
5	1	1	2	1	1	0
6	1	1	-	-	0	0
7	2	1	1	1	1	0
8	-	-	1	1	1	0
9	0	1	0	1	0	0
10	1	1	-	-	1	1

0 = absence of sperm / foam

1 = presence of sperm / foam

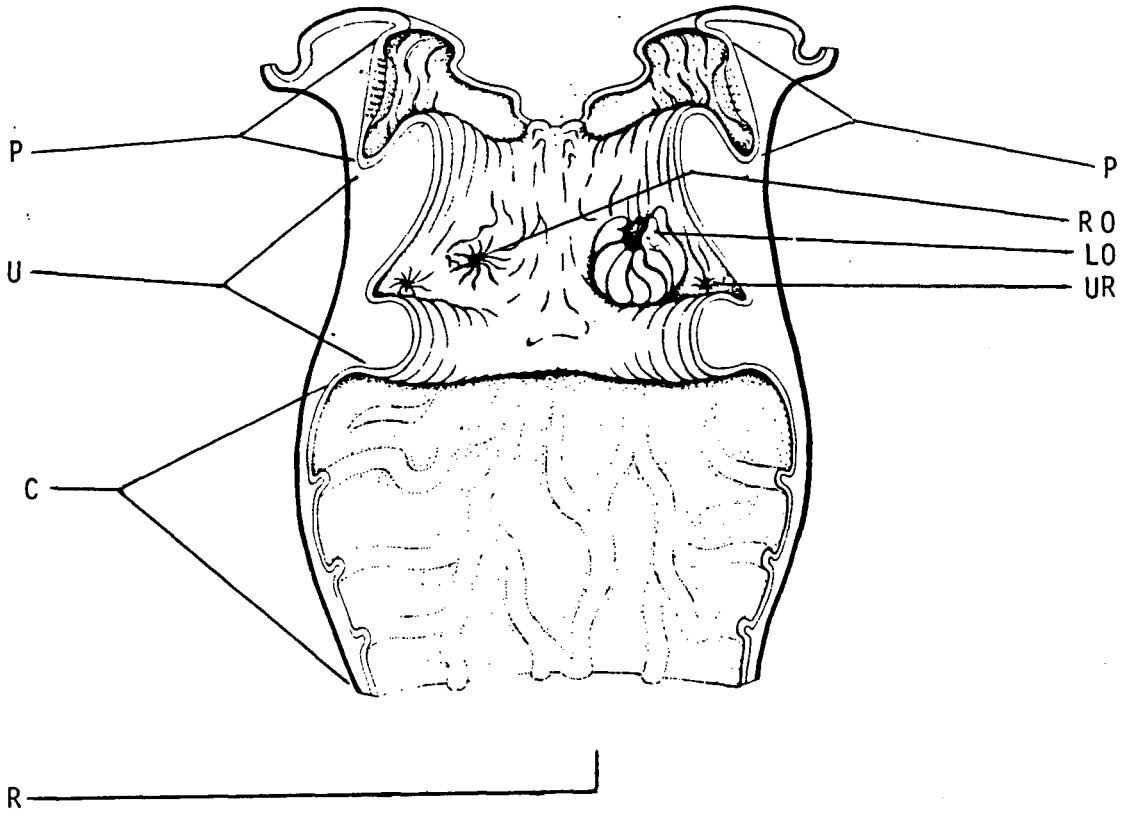
2 = higher sperm concentrations compared to 1

3 = presence of high concentration sperm mass

- = no observation

FIGURE 5: Foam being expelled from the female's coprodeum.

FIGURE 6: Cloaca of a female chicken, opened along the dorsal midline and laid flat.



P proctodeum
U urodeum
C coprodeum
R rectum
RO right oviduct
LO left oviduct
UR ureter

Figure taken from Komarek, 1971.

material from the rectum for defecation (King, 1981) (see Fig 6 for the cloacal anatomy of a female chicken). Chicken and quail are related species within Family Phasianidae (Welty, 1979) and have a similar cloacal anatomy. The implications of this discovery for sperm competition will be discussed.

EXPERIMENT 3

Results from Experiment 1 indicated that in a competitive situation, foam-producing males fertilized more eggs than foamless males. This provides evidence that his foam aids the male in fertilization in a competitive situation. However, I obtained no information on how fertility was enhanced or if foam may hinder sperm from subsequent matings in traversing the oviduct.

In Experiment 2, I found that:

i) foam and sperm are mixed at insemination and sperm can enter the oviduct ahead of the foam, and

ii) foam and sperm are deposited into the coprodeum (rather than the oviduct itself) and sperm and foam from subsequent matings are deposited posterior to the first male's foam.

These results indicate that the foam is placed in the female's cloaca

where it could play a role in sperm competition.

Experiment 3 was designed to study the ability of foam to block sperm penetration *in vitro* and at the same time examine the possibility that foam aids fertilization by enhancing or prolonging sperm motility. Because semen from domestic chickens can be obtained easily and in large quantity, this *in vitro* study was first conducted using chicken sperm (Experiment 3A) and then later repeated using quail sperm (Experiment 3B).

EXPERIMENT 3A

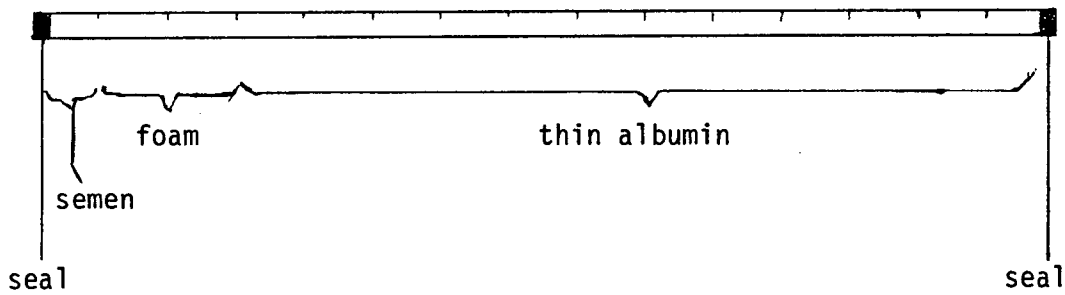
A) MATERIALS AND METHODS

The technique used to measure sperm penetration through foam was based on a method described by Birrenkott *et al.* (1977) for evaluating the quality of avian semen. Glass capillary tubes measuring 75 mm long and 1.2 mm inner diameter were marked with indelible ink every 5 mm along their length. Foam from mature male quail was collected by a gentle squeeze of the cloacal protuberence immediately before the experiment in 2 ml plastic auto-analyzer cups. Foam accidentally mixed with feces was not used. Eight marked capillary tubes were filled

with foam up to the 10 mm mark by suctioning and then further filled with thin albumin (at room temperature, 21°-22° C) from chicken eggs until only 5 mm was left empty at the end of the capillary tube. The foam was closest to the open end (Fig 7). The opposite end was then sealed with a silicon gel sealer. For the control, eight more marked capillary tubes were filled with only thin albumin (again leaving 5 mm empty at one end and sealing the opposite end). Each prepared capillary tube was embedded vertically with the open end uppermost in one of two styrofoam holders (styrofoam blocks, each with holes punched for eight capillary tubes) so that each holder contained four experimental-control pairs of capillary tubes. Semen was collected from a rooster by the massage method (Burrows and Quinn, 1935,1937; Lake, 1957) and transferred to a 1 cc tuberculin syringe. Equal volumes (about 5 µl) of the semen were gently deposited with a 23-guage needle into each of the 16 capillary tubes, making sure that the semen did not pass the 5 mm mark at the tubes' open ends and that no air bubbles occurred between the semen and the contents of the tube. The order of semen deposition was randomized between holders and between pairs. Each capillary tube was sealed with silicon gel immediately after semen deposition. When all the tubes were sealed, one of the styrofoam holders was placed in an incubating oven at 41° C (the bird's body temperature) and the time was recorded. The other styrofoam holder was left at room temperature (21°-22°C) and was loosely covered with a paper towel to reduce the amount of light reaching the incubating sperm.

After 15 minutes of incubation, eight capillary tubes were

FIGURE 7: Diagram of a prepared capillary tube.



examined in sequence by each of two observers under microscopes at 400x magnification for about 2 minutes. The order of examination was randomized both by pairs and by holders (temperature) with the two observers examining capillary tubes in an opposite order, i.e. if Observer A started with an "experimental" capillary tube at "room temperature", then Observer B started with a "control" capillary tube at "body temperature". Each capillary tube was replaced into its original position after examination. The capillary tubes were again examined 30 minutes after the first examination.

For each examination, both concentration and motility of sperm within each 5 mm section of the capillary tubes were examined. The scale used for scoring the sperm concentration per section of capillary tube was:

A: sperm present en masse,

B: more than 10 sperm present, but not en masse,

C: between 5 and 10 sperm present, and

D: fewer than 5 sperm present.

The scoring scale for motility was modified from the 5-class scale used by Wilcox (1959):

A: active movement of all sperm,

B: moderate movement of most sperm,

C: sluggish movement of sperm at best, and

D: no movement of sperm.

Semen from five roosters was used; each rooster was sampled twice. All semen samples from one rooster constituted a replication.

B) ANALYSES

The maximum distance travelled by sperm in the capillary tube was determined by the 5 mm section furthest away from the starting point that scored a "C" or above for concentration. This dependent variable was analyzed by ANOVA with repeated measures. The statistical model used was:

$$Y_{ijklmn} = \mu + R_i + C_j + T_k + O_l + CT_{jk} + CO_{jl} + TO_{kl} + CTO_{jkl} + El_{ijkl} + S_m + SC_{mj} + ST_{mk} + SO_{ml} + SCT_{mjk} + SCO_{mjl} + STO_{mkl} + SCTO_{mjkl} + E2_{ijklmn},$$

and i=1,2,3,4,5; j=1,2; k=1,2; l=1,2; m=1,2; n=1,2,3,4; where Y_{ijklmn} is the maximum distance travelled (in mm) by the sperm from the i^{th} male in the tube with the j^{th} contents (1=foam; 2=no foam) incubated at the k^{th} temperature examined by the 1^{th} observer during the m^{th} time of observation from the n^{th} sample, μ = the theoretical population mean, R_i = effect of the i^{th} replication, C_j = effect of whether there was foam in the tube, T_k = effect of whether incubation was at room or body temperature, O_l = effect of examination by the 1^{th} observer, S_m = effect of time of examination, CT_{jk}, CO_{jl}, TO_{kl}, SC_{mj},

ST_{mk}, and SO_{ml} = effects of the 2-way interactions, CTO_{jkl}, SCT_{mjk}, SCO_{mjl}, and STO_{mkl} = effects of the 3-way interactions, $SCTO_{mjkl}$ = effect of the 4-way interaction, El_{ijkl} = error term for testing the effects of the main plot, and $E2_{ijklmn}$ = the sub-plot error term. The mean separations between treatments were tested using Duncan's New Multiple Range Test.

Sperm motility was analyzed by the Kruskal-Wallis k-Sample Signed Rank Test (Steel and Torrie, 1980, p. 544). The average motility score for each capillary tube was calculated by first summing the scores for each section and then dividing by the number of sections scored. These averages were then ranked and the Kruskal-Wallis test was applied. Pairwise comparisons of sperm motility in capillary tubes were made between each of these treatments:

1) tubes with foam at the 15 minute examination,
2) tubes with foam at the 45 minute examination,
3) tubes without foam at the 15 minute examination, and
4) tubes without foam at the 45 minute examination.

C) RESULTS

1. Effect of foam on sperm penetration.

The presence of quail foam significantly hindered the travel of chicken sperm through capillary tubes (Table 9, $p<0.005$). Sperm travelling through capillary tubes without foam averaged 20.0 mm per observation averaged across temperature, time of observation, observer, and replication, while sperm with foam to penetrate travelled an average of only 11.6 mm per observation.

2. Effect of incubation temperature on sperm penetration.

Sperm were able to travel further when incubated at body temperature (41°C) than when incubated at a room temperature of 21°-22°C (Table 9, $p<0.005$). At room temperature, sperm travelled an average of 14.4 mm per observation averaged across tube contents, time of observation, observer, and replication, but travelled 17.2 mm per observation at body temperature.

3. Effect of time of examination on sperm penetration.

As expected, sperm had penetrated significantly further when examined at the second observation (45 minutes) than when examined at the first observation (15 minutes) of the capillary tubes (Table 9,

$p<0.005$). After 15 minutes of incubation, sperm had averaged 12.4 mm of travel while after 45 minutes of incubation, sperm had penetrated an average of 19.3 mm through the tube.

4. Effect of observers on measurements of sperm penetration.

Sperm was recorded as travelling further through tubes by Observer B than by Observer A (Table 9, $p<0.05$); however, the difference was consistent--both observers recorded further sperm penetration through tubes without foam, at body temperature, and at the second examination. Observer A recorded an average penetration of 15.0 mm per observation averaged across all factors while Observer B recorded an average penetration of 16.7 mm per observation.

5. Effect of replication on sperm penetration.

A replication was represented by semen collected from one of the five roosters. There was a significant replication effect; the average penetration of sperm for each replication varied from a low of 13.2 mm to a high of 18.6 mm (Table 9, $p<0.005$). These replication differences reflect individual variation in sperm motility between the different males.

TABLE 9: Mean distance (mm) travelled by rooster sperm through capillary tubes per examination.

TREATMENT	DISTANCE TRAVELLED se
Effect of contents of tubes**:	
Foam	11.66 ± .05
Foamless	20.05 ± .06
Effect of incubation temperature**:	
21°-22° C (Room)	14.45 ± .06
41° (Body)	17.25 ± .07
Effect of observer*:	
A	14.98 ± .06
B	16.72 ± .07
Effect of time of examination**:	
15 minutes	12.39 ± .04
45 minutes	19.31 ± .07
Effect of replication**:	
1	18.55 ± .09
2	15.70 ± .10
3	17.11 ± .10
4	14.65 ± .10
5	13.24 ± .08

* $p<0.05$ ** $p<0.005$

6. Effects of the significant interactions involving the main effects on sperm penetration.

Of the 2-way interactions examined, only those involving time were significant. The Time of Observation x Contents of Tube interaction shows that not only had the sperm in tubes without foam already penetrated significantly further by 15 minutes than sperm in tubes with foam, but also the sperm's increase of penetration between 15 and 45 minutes was significantly greater in the tubes without foam (Table 10, $p<0.005$). It was also observed that by 15 minutes the sperm in tubes without foam had already travelled significantly further than sperm in tubes with foam would travel by 45 minutes.

The Time x Temperature interaction was also significant (Table 11, $p<0.005$). Although sperm penetrated further at body temperature than at room temperature, the difference between the two temperatures in distance penetrated was larger at 45 minutes than at 15 minutes.

The significance in the Time x Observer interaction was due to the fact that although there was no significant difference in the distance of penetration recorded by the two observers at the 15-minute examination, Observer B recorded a greater increase in sperm penetration than Observer A at the 45 minute examination (Table 12, $p<0.005$).

None of the 3-way interactions examined were significant. The 4-way interaction was highly significant ($p<0.005$), but no clear trend could be interpreted.

TABLE 10: Significant 2-way interaction between time and contents of tube; mean distance (mm) travelled by rooster sperm through capillary tubes per examination[1].

CONTENTS OF TUBE	TIME OF EXAMINATION	
	15 MIN	45 MIN
	se	se
Foam	9.12 ± .04[a]	14.19 ± .08[b]
Foamless	15.66 ± .05[c]	24.44 ± .07[d]

1 All means are significantly different ($p<0.01$) by Duncan's Multiple Range Test.

TABLE 11: Significant 2-way interaction between time and temperature; mean distance (mm) travelled by rooster sperm through capillary tubes per examination[1].

TEMPERATURE	TIME OF EXAMINATION	
	15 MIN	45 MIN
	se	se
21°-22° C (Room T.)	11.59 ± .05[a]	17.31 ± .08[c]
41° C (Body T.)	13.19 ± .06[b]	21.31 ± .10[d]

[1] All means are significantly different ($p<0.01$) by Duncan's Multiple Range Test.

TABLE 12: Significant 2-way interaction between time and observer; mean distance (mm) travelled by rooster sperm through capillary tubes per examination[1].

OBSERVER	TIME OF EXAMINATION	
	15 MIN	45 MIN
	se	se
A	11.91 ± .05[a]	18.06 ± .09[b]
B	12.88 ± .06[a]	20.56 ± .10[c]

1 Means followed by different letter superscripts are significantly different ($p<0.01$) by Duncan's Multiple Range Test.

TABLE 13: Significant 2-way interaction between time and contents of tube; mean motility ranks for rooster sperm in capillary tubes[1].

CONTENT OF TUBES	TIME OF EXAMINATION	
	15 MIN	45 MIN
Foam	45.72[b]	24.00[a]
Foamless	51.82[b]	40.45[b]

1 Means followed by different letter superscript are significantly different ($p<0.05$) by Kruskal-Wallis k-Sample Signed Rank Test.

7. Effect of foam on chicken sperm motility.

Japanese quail foam caused a significant decrease in chicken sperm motility over time. There was no significant difference in motility of sperm in tubes with or without foam at 15 minutes. However, at 45 minutes, sperm motility in capillary tubes with foam was significantly lower than the motility of sperm in tubes without foam at the same time (Table 13). Moreover, in tubes without foam, sperm motility at 45 minutes was not significantly different from motility in tubes with foam at 15 minutes.

EXPERIMENT 3B

Results from Experiment 3A showed that foam from the male Japanese quail hinders the travel of chicken sperm in vitro. In order to determine if the foam would have a similar effect on Japanese quail sperm, the experiment was repeated using quail sperm and thin albumin from quail eggs. However, due to the inconsistency of obtaining semen from any given male quail, a balanced experimental design similar to the one used for examining chicken sperm was not possible.

A) MATERIALS AND METHODS

1. Semen collection from male Japanese quail.

Semen was collected using a modification of the method by Marks and Lepore (1965) after suggestions by H. P. VanKrey (personnal communication). The male, with feathers near the cloacal opening clipped, was held in the left hand with the bird's breast in the palm and the collector's thumb was placed just below the ventral lip of the cloaca for support. The foam was first removed by two or three squeezes to the cloacal protuberance with the thumb and forefinger of the right hand, and was discarded. Gentle but firm massage "strokes" were then applied by the thumb and forefinger of the right hand to the caudal area of the bird with parallel movements directed down the sides of the cloaca. After three to five strokes, a final firmer squeeze to the sides of the cloaca naturally everted the phallus and caused an ejaculation. If the creamy white semen did not appear in the groove of the phallus, the process was repeated; if no semen appeared after this second attempt, the male was replaced and a different male was obtained for semen collection. The semen obtained was removed from the male with a micropipette (Drummond Digital Microdispenser) for immediate transfer into capillary tubes. For a microscope slide study of quail sperm motility (see page 41), the semen was removed with a small vinyl spatula.

2. Experimental procedure.

a) Sperm penetration and motility in capillary tubes.

The volume of semen per ejaculate in quail is low compared with the volume of chicken semen (3.87-6.90 µl *vs* 0.41-2.25 ml, respectively, Buxton and Orcutt, 1975). An ejaculate from one male furnished enough semen for at most two experimental-control pairs of capillary tubes. Because of the longer time needed to train quail males to respond to semen collection, only five males were fully trained and used. Not all males were used to the same extent because of inconsistency in semen production. A total of 12 experimental-control pairs of capillary tubes were examined between 1 January and 17 February, 1983.

Because quail sperm lose motility quickly *in vitro* (Ogasawara and Huang, 1963; Schein *et al.*, 1972), the incubation time for the capillary tubes after semen deposition was reduced to five minutes after which the maximum distance travelled by sperm was scored. The capillary tubes were incubated only at room temperature (21-22°C) to reduce variations in temperature by short periods in an incubating oven. Motility of sperm was also scored at the 5 minute observation and thereafter checked periodically to determine in which tubes (experimental or control) sperm motility lasted longest. The same criteria as in Experiment 3A were used for scoring sperm concentration and motility.

b) Sperm motility on microscope slides.

In addition to examining penetration and motility of quail sperm in capillary tubes, I compared sperm motility with and without foam on microscope slides. Four comparisons were made. In two of these, semen from a male was mixed with its own foam and in the remaining two comparisons, semen from a cauterized male was mixed with foam from a different male. I mixed semen with foam from the same and from different males to check if the foam had an immune or suppressant response against sperm from other males. Thus, comparisons of sperm motility could be made both between foam vs no foam and between foam and semen from the same male vs foam and semen from different males. Slides were prepared just prior to semen collection: approximately 20-25 µl of thin albumin from a fresh quail egg was placed on both slides and approximately 20-25 µl of freshly obtained foam was added to one slide. A small quantity (around 5 µl) of freshly obtained quail semen was mixed with the contents on each slide, a cover slip was applied, and the slides were placed on separate microscopes at 400x magnification. The slides were periodically checked for up to 55 minutes after the addition of semen and the motility of the sperm was observed and scored.

B) ANALYSIS

The same criteria used to determine maximum distance travelled by chicken sperm in Experiment 3A were applied to determine quail sperm penetration in capillary tubes. However, due to the smaller number of samples observed, only a comparison of sperm travel in tubes with or without foam was made using the Student's t-test for paired samples (Steel and Torrie, 1980, p. 102). Motility data from both the capillary tube and microscope slide studies were summarized, but no analyses were performed.

C) RESULTS

1. Effect of foam on quail sperm penetration.

As with chicken sperm, quail sperm were able to penetrate further through the capillary tubes when there was no foam to contend with. Without foam, sperm travelled an average of 7.90 mm while with foam, sperm only averaged 2.80 mm ($t = 4.64$, $df = 11$, $p<0.01$).

2. Effect of foam on quail sperm motility.

The effect of foam on quail sperm motility was observed both in capillary tubes and on microscope slides. In the capillary tube experiment, sperm motility was more vigorous in tubes with foam

TABLE 14: Duration of quail sperm motility on microscope slides[1].

TREATMENT	TIME OF FINAL EXAMINATION (minutes)	MOTILITY[2]
Foam and sperm from same male:		
Sample 1	16	vigorous
Sample 2	55	vigorous
No foam:		
Sample 1	4	ceased
Sample 2	10	poor
Foam and sperm from different males:		
Sample 3	45	vigorous
Sample 4	45	good
No foam:		
Sample 3	45	poor
Sample 4	11	poor

1 Samples with the same number indicate sperm from a single male.

2 On slides without foam, sperm remained motile only when associated with air bubbles.

compared to sperm motility in tubes without foam at the 5 minute observation. In capillary tubes without foam, sperm motility was poor or had ceased within an average of 8.5 minutes of incubation whereas in capillary tubes with foam, sperm motility was often still vigorous after 10.6 minutes.

The ability of foam to prolong sperm motility in vitro became apparent when observed on the microscope slides (Table 14). Sperm associated with foam remained motile for more than 55 minutes while sperm not associated with foam usually ceased motility within 4 to 11 minutes. Occasionally, where air bubbles were trapped under the cover slips on microscope slides without foam, sperm associated with these air bubbles remained motile for much longer than sperm not associated with bubbles on the same slides. Whether or not foam was from the same male whose semen was used on the microscope slide apparently did not differentially affect the duration of sperm motility.

DISCUSSION

This research was conducted to study the function of foam secreted by the foam gland of the male Japanese quail. The first question asked was, "Does foam play a role in affecting the male's

reproductive success during natural matings?" Results from Experiment 1 clearly indicate that it does. Sham-operated (foam-producing) males sired significantly more progeny than cauterized (foamless) males (Tables 1 and 2). Behavioural observations during Experiment 1 indicated that cauterized males attempted and completed copulations at least as often as sham-operated males (Tables 4 and 6). The difference in fertility, therefore, was not due to differences in mating frequencies between the foamless and foam-producing males.

Ogawa et al. (1974) noted that the volume of artificially ejaculated semen from males whose foam glands (along with part of the cloaca) were surgically removed was only about a third of that obtained from normal males, although semen from the glandless males had a higher sperm concentration. Never-the-less, the total number of sperm per ejaculate was 40 % higher in normal males. In the present experiments, the proctodeal gland of some males was rendered non-functional by cautery and was not surgically removed. Macroscopically, no reduction in semen volume per ejaculate was noticed in these males compared to sham-operated or normal birds. However, I did not measure semen volume from the males. Also, I noticed no gross differences in sperm motility when semen was collected from cauterized males, mixed with foam, examined microscopically, and compared with semen of normal males. In a separate experiment (see Appendix, p. 80), examinations of cloacal samples and expelled foam from females post-copula revealed that 45 out of 99 (45.5%) samples contained sperm after copulation with cauterized males, while 16 out of 27 (61.5%) samples contained sperm

after copulation with normal males. The difference in fertility between males with and without foam in Experiment 1, therefore, was not due to semen quality or quantity, although further experiments are needed to confirm this observation.

I then asked, "How did foam increase the reproductive success of males?" Two possible answers to this question are: 1) foam could increase a male's reproductive success by aiding the fertilizing ability of its own sperm. 2) foam could also increase a male's reproductive success by hindering the fertilizing ability of sperm from any other males copulating with the same female.

In the Introduction, a hypothesis on the function of foam was developed based on differences in oviposition time between the quail and other domestic birds such as chickens and ducks (see page 4). In this hypothesis, foam is suspected to be an agent which suspends sperm near the oviduct opening for slow release of sperm into the vagina while blocking the travel of sperm from any subsequent copulations. Results from the present experiments not only provide support but also a refinement for this hypothesis.

Experiment 2 revealed that after a successful copulation, foam is deposited into the coprodeum of the female. When examined immediately after a successful copulation, sperm was well mixed with foam. Sperm was also always found in the oviduct; foam was found only once in the oviduct, and then only as a few stray bubbles. Experiment 3 indicated that <u>in vitro</u>, foam prolonged the motility of quail sperm to a great extent; sperm were either attracted to or trapped in the bubbles of the foam. The above observations are consistent with the "sperm

block" hypothesis.

The fact that foam was found in the coprodeum of the female and not in the oviduct provides an excellent demonstration that sperm can be suspended in a "pocket" out of the way of an egg as it moves down the oviduct. Foam may even buffer sperm against the adverse environment of the cloaca as fecal material is harmful to the viability of sperm (Boone and Hughes, 1970). Foam is occasionally found on feces excreted by the female and on the shells of freshly laid eggs (personal observation), but never in the amount originally deposited into the female. Therefore, even if copulation occurs before oviposition, enough sperm (suspended in foam) will remain in the female after the egg is laid to fertilize the next egg, or to be stored in her uterovaginal sperm-host glands. Unless copulation occurs late in the evening (after oviposition), without such a mechanism the ejaculate of the male, being small in quantity and viscous in constitution, could easily be eliminated by the female along with the egg (Bobr et al., 1964b) without leaving enough sperm to maintain good fertility.

In nature, this hypothesized function of foam may not be crucial as wild Japanese quail are mostly monogamous (Whetherbee, 1961; Moreau, 1951). Wild males maintain territories and form pair-bonds with one female. Males can copulate with their mate more frequently and/or at appropriate times to optimize fertilization. The foam gland of the wild quail is not well developed compared with the gland of domestic quail and volume of foam produced is only a fraction of that from domestic quail (Schleidt and Shalter, 1972). Under domestic

conditions, Japanese quail are promiscuous; a larger number of females than males are usually maintained in a given cage. Competition for fertilization among males under these conditions is intense and would exert a strong selection pressure for development of the males' foam gland if the foam aided the males' reproductive success.

The Chinese Painted Quail, (Excalfactoria chinensis) is closely related to the Japanese quail. Females of this species also lay eggs late in the evening (Nichols, unpublished data). This species is domesticated but has seldom been raised in high densities as with Japanese quail. Chinese Painted quail have remained monogamous even after domestication and the males of this species have only rudimentary glandular tissue in their proctodeum.

In 2-male mating groups similar to the ones in Experiment 1, Cheng (unpublished data) has observed that unless one male completely dominates the other male, each will form a small territory in an opposite corner of the pen and associate closely with one or two females, while some females will "float" freely between the two males. Occasionally, a male in one corner will rush into the territory of the other male and steal copulations with females associated with that male.

Experiment 1 showed that when both males were foam-producing, these occasional copulations were successful in fertilization as all females in the pen had progeny from both males. On the other hand, foamless males may not be able to achieve fertilization even when successful in stealing copulations. In pens where one male was foamless and one male foam-producing, females either produced progeny

from both males or produced progeny sired exclusively by the foam-producing male. Thus, the foam-producing males were successful in fertilizing eggs laid by females associated with foamless males, but the reverse did not occur. This becomes apparent in the results from pens where both males were foamless. In these pens, some females produced progeny sired exclusively by one male, while some females produced progeny sired exclusively by the other male. Some females produced progeny sired by both males; presumably, these were the females which "floated" between the two males.

Behavioural observations in Experiment 1 showed that foamless UBC-A males attempted and completed more copulations than foam-producing UBC-A males. The even proportion of progeny found when two foam-producing males were competing (Table 1), however, indicates a similar frequency of copulation between the foam-producing UBC-W and UBC-A males. This implies that foamless UBC-A males were copulating more often than the foamless UBC-W males, and may be an explanation why a higher proportion of progeny from Treatment 3 (Experiment 1) were sired by the UBC-A males. Furthermore, in pens where both males were foamless, not only total fertility but also hatchability of fertile eggs was significantly lowered. In chickens and other domestic birds it has been shown that aged sperm (from the UV sperm-host glands) will significantly lower egg hatchability (Nalbandov and Card, 1943). All these results indicate that without foam, not enough sperm from the male survive to be stored in the sperm-host glands. This may be due to the harsh conditions of the cloaca or to difficulties in sperm reaching the oviduct. With small amounts of stored sperm being

only periodically sparcely replenished, stale sperm will account for more fertilizations.

The question of whether foam can also block sperm from subsequent copulations, however, remains unsettled. Experiment 3 shows that foam does interfere with sperm travel *in vitro*, but since foam is deposited into the coprodeum and not in the oviduct, the significance of this result is not clear. However, the stability of the foam (Fujihara and Nishiyama, 1984), and the difficulty sperm has travelling through foam, may indicate that sperm from two copulations will not become mixed. Females often evacuate foam with defecation and oviposition. These observations lead Cheng (personal communication) to hypothesize that although foam does not directly block the entrance to the oviduct, it occupies space in the coprodeum so that foam from subsequent copulations is deposited closer to the cloacal opening and thus has a better chance of being eliminated by the female before any foam from the first male. Thus, sperm from the male that mates first has a competitive edge over sperm from any subsequent copulations. The testing of this hypothesis, however, is beyond the scope of this thesis.

In summary, this reasearch provides helpful insights into how natural selection can shape sperm competition mechanisms, even in domestic environments, and raised interesting questions concerning sperm competition in Japanese quail.

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APPENDICES

EXPERIMENT 4

Results from Experiments 2 and 3 indicate that:

1) during natural copulations, sperm and foam are mixed yet sperm can precede the foam from the coprodeum into the oviduct, and
2) foam from the male Japanese quail hinders sperm travel *in vitro*.

This experiment was an attempt to determine whether *in vivo*, foam can act as a blocking agent to sperm from subsequent inseminations, by studying its effects on the onset of fertility. If foam acts as a blocking agent to sperm travel, then its presence in the female's cloaca before insemination should increase the latency of the first fertile egg laid after the insemination. The experiment also allowed for determination of the effect of foam on fertility and duration of fertility in individual matings. However, due to poor fertility among experimental females, insufficient data were collected to allow for analyses and meaningful interpretation. The experimental procedure, however, may be of interest to researchers in this area of study. Thus, the experiment is described here with a brief discussion.

A) MATERIALS AND METHODS

The birds used in this experiment were from the same two strains (UBC-A "wild-type" and UBC-W "white phenotype") as birds used in the other experiments, and were reared under similar conditions. Again,

UBC-W females and males from both strains were used because of the recessive white plumage genetic marker. Twenty males of each strain underwent cauterizations of their foam glands (see page 10) to provide sexually mature, foamless males. A fertility test was again performed with 12 cauterized males from each strain; a total of nine males were thus excluded from the experiment. Six foam-producing males from both strains were also maintained for the experiment.

Thirty-two "virgin" females were placed in individual cages (30cm x 50cm x 25cm) four days prior to the start of the experiment. Experimental males were also individually caged as were 20 non-experimental males (10 UBC-W and 10 UBC-A) for the use of their foam. Males were kept separate because of reports that male-male copulations often include sperm transfer (Adkins, 1974). During the experiment, females were subjected to one of the following four treatments within 25 minutes after oviposition:

1) The female was allowed to copulate with a foamless male and then foam was artificially deposited into her cloaca
2) Foam was artificially deposited into the female's cloaca and then she was allowed to copulate with a foamless male.
3) The female was allowed to copulate with a foamless male.
4) The female was allowed to copulate with a normal male.

During copulations, the male was placed into the female's cage for 15 minutes and observations were made to detect completed copulations using the same criteria as in Experiment 1. Foam used in the experiment (0.30-0.45 ml foam deposited with a 1cc tuberculin syringe) was always freshly collected from the non-experimental males

phenotypically different from the copulating male. Offspring phenotypes were later checked for paternity in case any sperm had been mixed with the foam. After a completed copulation, a cloacal sample from the partially everted female was obtained with a small vinyl spatula, transferred to a microscope slide, and examined (400x) to determine whether sperm transfer had occurred.

Poor fertility was noticed in the first three hatches which I thought might be due to the physical manipulation involved in obtaining the post-copula cloacal samples from the females. Thus, starting with the fifth replication, cloacal samples were no longer taken. Instead, heavy cardboard "foam catchers" were suspended under the female's cage for 15 to 30 minutes after removal of the male in the treatments involving foam (1,2 and 4). Foam found on the suspended board was examined microscopically for the presence of sperm. Results would reflect the frequency of post-copula foam excretion by the female as well as provide a conservative estimate of the frequency of sperm transfer.

Because of occasional non-laying days for individual females and periods where females would lay within a few minutes of each other, three or four evenings per replication were required to stage a minimum of 20 copulations--five females for each of the four treatments. Eggs from these females were then collected for at least 10 days, identified by female and date, and artificially incubated and hatched as in the other experiments to determine fertility and paternity of offspring. Females were reused, but were allowed at least 10 days between copulations and the genotype of the male partner

was changed in each replication to detect any fertilization from previous matings. There were a total of eight replications involving 190 staged copulations--50 with Treatment 1, 48 with Treatment 2, 47 with Treatment 3, and 45 with Treatment 4.

B) RESULTS AND DISCUSSION

The instances of females laying fertile eggs after a single staged copulation were so low that statistical analyses for testing the differences between treatments in the latency of laying the first fertile egg were not applied (Table 15). Instead, the data are presented in tables and the trends are discussed. Data resulting from examinations of both the post-copula female cloacal samples and the "foam catcher" foam drops are also presented and discussed.

1. Effect of foam on fertility.

The first three treatments involved copulations with cauterized, foamless males. When no foam was added (Treatment 3), the total fertility (among females that laid at least one fertile egg) for a 10-day period after the copulation was 18.52% (5 fertile eggs out of 27 incubated). The addition of foam, either before or after allowing copulation, resulted in 34.69% fertility (34 fertile out of 98), which is comparable to the fertility level achieved with copulations by normal males (37.59% ; 53 fertile out of 141) (Table 16). Thus, these

TABLE 15: Number of completed copulations and number of females producing fertile eggs by treatment.

FACTORS		TREATMENTS			
		1	2	3	4
	Totals	copula, then foam	foam, then copula	copula by foamless male	copula by normal male
Number of completed copulations	155	40	40	40	35
Number of females laying at least one fertile egg	34	7	5	4	18
Number of females[1] where onset could be determined	25	6	3	3	13

1 Females that did not have a cracked egg or non-laying day between copulation and first fertile egg.

TABLE 16: Total fertility and duration of fertility by females that laid at least one fertile egg*.

	TREATMENTS			
	1	2	3	4
DAYS AFTER COPULATION	copula, then foam	foam, then copula	copula by foamless male	copula by normal male
1	4/7	1/4	0/4	3/14
2	4/5	4/4	3/3	12/14
3	2/6	3/4	1/2	11/15
4	3/6	2/4	0/2	8/14
5	2/7	0/5	1/4	10/16
6	2/6	3/5	0/4	3/17
7	2/6	1/5	0/2	3/14
8	1/5	0/4	0/2	3/13
9	0/5	0/3	0/2	0/11
10	0/5	0/2	0/2	0/13
TOTALS:	20/58	14/40	5/27	53/141
PERCENTAGES:	34.48%	35.00%	18.52%	37.59%

* Number of fertile eggs / number of eggs laid (by females that laid at least one fertile egg).

results provide additional support for a conclusion of Experiment 1, that foam aids a male in fertilization.

I was hoping to show in this experiment that in Treatment 2, where foam was added before allowing copulation, oviposition of the first fertile egg would be delayed compared to oviposition of the first fertile eggs from Treatment 1, where foam was added after copulation, and in control Treatment 4. Examining data from Table 2 for fertility on the first day after copulation shows the trend expected, but there are too few fertile eggs for statistical certainty. However, the data show that lack of foam caused a decrease not only in total fertility, but also in duration of fertility with respect to all other treatments.

2. Frequency of sperm transfer by cauterized and normal males.

The occurrence of sperm transfer during apparently successful copulations was recorded both by examining samples from the female's cloaca post-copula and by examining foam excreted by the female post-copula. Foamless males facilitated sperm transfer in 45.5% of the copulations checked (45 out of 99), while 61.5% (16 out of 26) of the copulations by normal foam-producing males involved sperm transfer (Table 17). These results are consistent with Adkins (1974), who found the frequency of ejaculatory and non-ejaculatory copulations to be not significantly different.

Foam was found excreted by the female within 30 minutes post-copula in 43 out of 89 (48 %) copulations where a foam catcher

TABLE 17: Occurrences of sperm transfer during copulations by foamless and foam-producing males.

METHOD OF SAMPLING		SPERM	NO SPERM
1. Microscope slide of cloacal sample from female post-copula:			
Copula, then foam	(TR 1)	8	11
Foam, then copula	(TR 2)	8	14
Copula, no foam	(TR 3)	11	12
Normal copula	(TR 4)	9	9
2. Examination of foam excreted by female post-copula:			
Copula, then foam	(TR 1)	8	12
Foam, then copula	(TR 2)	10	5
Normal copula	(TR 4)	7	1
TOTALS: Foamless males		45	54
Normal males		16	10

was applied. Sperm was found mixed with the excreted foam in 58% of the examined foam droppings.

The poor fertility seen in this experiment was unexpected. Sperm transfer was known to be occurring enough to provide better fertility. In similar staged matings where the major difference was in placing the female into the male's cage for copulation, a much better fertility level was achieved (Cheng, unpublished data). Indeed, females were sometimes reluctant to copulate, whereas, in the study by Cheng (personal communication), copulations were normally immediate.

An important conclusion of this thesis was given additional support by results obtained in this experiment: foam can apparently aid a male in fertilization even when not in a competitive situation.

TABLE 18: Analysis of Variance for total fertility[1] of all incubated eggs in Experiment 1.

Source	df	SS	MS	F
Replication	2	74.70	37.35	0.217
Treatment	3	18,891.71	6297.24	36.550***
Error	54	9303.48	172.29	
Total	59	28,269.89		

[1] Arcsine transformation applied to data before analysis.

*** $p<0.005$

TABLE 19: Analysis of Variance for percent hatchability[1] of all fertile eggs in Experiment 1.

Source	df	SS	MS	F
Replication	2	355.47	177.74	1.40
Treatment	3	1702.17	567.39	4.48**
Error	54	6833.05	126.54	
Total	59	8890.69		

1 Arcsine transformation applied to data before analysis.

** $p<0.01$

TABLE 20: Analysis of Variance for number of white progeny typed out of all progeny typed[1] in Experiment 1.

Source	df	SS	MS	F
Replication	2	908.36	454.18	1.587
Operation (O)	1	33,569.07	33,569.07	117.329***
Condition[2] (C)	1	15,152.71	15,152.71	52.961***
O x C	1	512.99	512.99	1.793
Error	54	15,449.99	286.11	
Total	59	65,593.12		

1 Arcsine transformation applied to data before analysis.

2 Condition of foam gland of the competing UBC-A male.

*** $p<0.005$

TABLE 21: Analysis of Variance for percent hatchability[1] of white progeny out of all fertile eggs in Experiment 1.

Source	df	SS	MS	F
Replication	2	570.61	285.30	1.655
Operation (O)	1	18,403.71	18,403.71	106.756***
Condition[2] (C)	1	5,039.46	5,039.46	29.233***
O x C	1	270.09	270.09	1.567
Error	54	9,309.16	172.39	
Total	59	33,593.03		

1 Arcsine transformation applied to data before analysis.

2 Condition of foam gland of the competing UBC-A male.

*** $p<0.005$

TABLE 22: Analysis of Variance for number of mating attempts per observation[1] by UBC-W males in Experiment 1.

Source		df	SS	MS	F
Replication		1	0.091	0.091	1.083
Operation	(O)	1	0.001	0.001	0.005
Condition[2]	(C)	1	0.400	0.400	4.762
O x C		1	0.277	0.277	3.298
Error 1		3	0.252	0.084	
Time	(T)	2	0.051	0.026	0.513
T x O		2	0.022	0.011	0.217
T x C		2	0.146	0.073	1.440
T x O x C		2	0.451	0.226	4.459*
Error 2		128	6.487	0.051	
Total		143	8.177		

1 Square root transformation applied to data before analysis.

2 Condition of foam gland of the competing UBC-A male.

* $p<0.05$

TABLE 23: Analysis of Variance for number of completed copulations per observation[1] by UBC-W males in Experiment 1.

Source		df	SS	MS	F
Replication		1	0.008	0.008	0.10
Operation	(O)	1	0.013	0.013	0.17
Condition[2]	(C)	1	0.333	0.333	4.27
O x C		1	0.143	0.143	1.83
Error 1		3	0.234	0.078	
Time	(T)	2	0.011	0.006	0.13
T x O		2	0.010	0.005	0.11
T x C		2	0.013	0.006	0.13
T x O x C		2	0.179	0.090	2.00
Error 2		128	5.799	0.045	
Total		143	6.743		

1 Square root transformation applied to data before analysis.

2 Condition of foam gland of the competing UBC-A male.

TABLE 24: Analysis of Variance for number of mating attempts per observation[1] by UBC-A males in Experiment 1.

Source	df	SS	MS	F
Replication	1	0.018	0.018	0.38
Operation (O)	1	0.595	0.595	12.39*
Condition[2] (C)	1	0.033	0.033	0.69
O x C	1	0.025	0.025	0.52
Error 1	3	0.144	0.048	
Time (T)	2	0.002	0.001	0.01
T x O	2	0.883	0.442	3.69*
T x C	2	0.374	0.187	1.56
T x O x C	2	0.769	0.384	3.21*
Error 2	128	15.325	0.120	
Total	143	18.168		

1 Square root transformation applied to data before analysis.

2 Condition of foam gland of the competing UBC-W male.

* $p<0.05$

TABLE 25: Analysis of Variance for number of completed copulations per observation[1] by UBC-A males in Experiment 1.

Source	df	SS	MS	F
Replication	1	0.082	0.082	2.03
Operation (O)	1	0.515	0.515	12.77*
Condition[2] (C)	1	0.003	0.003	0.07
O x C	1	0.001	0.001	0.01
Error 1	3	0.121	0.040	
Time (T)	2	0.125	0.062	0.85
T x O	2	0.120	0.060	0.83
T x C	2	0.242	0.121	1.67
T x O x C	2	0.460	0.230	3.17*
Error 2	128	9.288	0.072	
Total	143	10.957		

1 Square root transformation applied to data before analysis.

2 Condition of foam gland of the competing UBC-W male.

* $p<0.05$

TABLE 26: Analysis of Variance for rooster sperm penetration through capillary tubes.

Source		df	SS	MS	F
Replication		4	10.99	2.75	5.62***
Observer	(O)	1	2.41	2.41	4.93*
Contents	(C)	1	56.32	56.32	115.19***
Temperature	(T)	1	6.26	6.26	12.80***
O x C		1	1.10	1.10	2.45
O x T		1	0.36	0.36	0.74
C x T		1	0.04	0.04	0.08
O x C x T		1	0.12	0.12	0.24
Error 1		120	58.67	0.49	
Time	(S)	1	38.33	38.33	465.45***
S x O		1	0.46	0.46	5.65*
S x C		1	2.77	2.77	33.64***
S x T		1	1.16	1.16	14.09***
S x O x C		1	0.03	0.03	0.36
S x O x T		1	0.07	0.07	0.82
S x C x T		1	0.00	0.00	0.00
S x O x C x T		1	1.81	1.81	22.03***
Error 2		180	14.82	0.08	
Total		319	195.72		

* $p<0.05$ ** $p<0.005$