

## Contribution of Nesting Experience to Progesterone-Induced Incubation in Ring Doves (*Streptopelia risoria*)

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Michel, GF & Moore, CL. Contribution of nesting experience to progesterone-induced incubation in ring doves (*Streptopelia risoria*). Journal of Comparative Psychology. 1985; 99:259-265. Digital Object Identifier: 10.1037/0735-7036.99.3.259

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### **Abstract:**

When a reproductively experienced dove is removed from visual isolation and placed in a breeding cage with an unfamiliar mate and a nest containing eggs, it takes about 5–7 days before it will exhibit incubation behavior. However, similarly experienced doves will exhibit incubation within a few minutes of being placed in a breeding cage if they receive a series of progesterone injections during the last 7 days that they are in isolation (Lehrman, 1958). No other hormone produces such a rapid expression of incubation behavior (Lehrman & Brody, 1961). However, the capacity of progesterone to facilitate incubation requires the joint action of other factors, including other hormones and certain external stimuli. Female doves must have a certain level of circulating estrogen (Cheng, 1979; Cheng & Silver, 1975), and male doves must have appropriate levels of testosterone (Komisaruk, 1967; Stern & Lehrman, 1969). Also, both the mate and the nest with eggs provide stimuli that are required before progesterone will induce incubation (Bruder & Lehrman, 1967; Lehrman, Brody, & Wortis, 1961).

### **Article:**

Lehrman and Wortis (1960) reported that the rapid expression of incubation by gonadally intact ring doves in response to progesterone injections was not present in reproductively naive doves. Subsequently, Michel (1977) found that the salient difference between naive and experienced doves, with respect to progesterone-induced incubation, was some effect of experience attained during the nest building phase of a breeding cycle. Previous breeding experience with only the activities of courtship and nest-site solicitation was not sufficient to make the dove behave like a reproductively experienced dove when injected with progesterone, and experience with phases of a breeding cycle subsequent to nest building, including egg laying and incubation, was not necessary for expression of progesterone-induced incubation. Progesterone-treated doves that had previously completed the nest building phase of a cycle incubated as rapidly as doves that had completed an entire cycle.

One explanation of why experience with the nest building phase of a breeding cycle is sufficient to facilitate the expression of progesterone-induced incubation might be that progesterone secreted during the nest-building phase of a breeding cycle prepares or “primes” the dove's nervous system so that the dove is more sensitive to progesterone that is administered at a later time. Alternatively, the experiences either of nesting behavior (attachment to a nest site and/or sitting in the nest site while building a nest) or of the sensorimotor actions involved in nest building might have some other effects on the dove's nervous system that prepare it to respond to progesterone and the stimuli from the incubation situation with rapid expressions of nest-oriented behavior, including incubation.

Endogenous progesterone is present in significant amounts in female doves during the nest building phase of the breeding cycle (Silver, Reboulleau, Lehrman, & Feder, 1974). Further, Cheng and Silver (1975) showed that the

behavioral patterns exhibited by female doves during the later part of the nest building phase are dependent upon increases in endogenous progesterone. Although similar behavioral changes in the male are not accompanied by increased absolute levels of progesterone (Silver et al., 1974), they may depend on changes in the ratio of progesterone to androgens (Cheng, 1975, 1979) or on androgen-induced changes in neural sensitivity to progesterone (Balthazart, Blaustein, Cheng, & Feder, 1980). Thus, naive and experienced doves differ in their previous exposure and sensitivity to progesterone as a result of differences in their participation in the nest building phase of the breeding cycle. Therefore, differences in their latencies to incubate after progesterone treatment could reflect the consequences of progesterone priming during the nest-building phase of their first cycle.

The present study was designed to determine whether previous exposure to progesterone, or progesterone priming, can account for rapid induction of incubation behavior by progesterone at a later time or whether the experiences attained during the nest-building phase have a facilitating effect on later incubation apart from their stimulating effect on progesterone secretion. Second, the study was designed to compare the effects of different aspects of the experience that can be obtained during the nest building phase of a cycle.

## **Method**

### **Subjects**

The subjects were 60 pairs of sexually mature ring doves (*Streptopelia risoria*) bred in the psychology laboratory colony at the University of Massachusetts, Boston, from stock originally obtained from the Institute of Animal Behavior, Rutgers University.

### **Caging and Maintenance**

The testing cages were breeding cages of wood, measuring 78 × 40 × 38 cm (with dispensers for food, water, and grit) and having wire mesh front doors. Visual isolation cages were double-width rat cages, hanging in racks. Stock cages were 85 × 79 × 91 cm, with wooden frames and wire mesh sides. Breeding, stock, and isolation cages were kept in separate rooms. Fourteen hours of light, beginning at 0700 each day, were controlled by Tork clocks. Temperature usually remained between 21 and 24 °C. Doves were transferred to stock cages 3 weeks after hatching and sexed by exploratory laparotomy at about 4 months of age. After sexing, they were placed in individual isolation cages until they were 8–9 months old, at the time of the study. This isolation period was chosen so as to separate doves before they began to court and to allow them to reach an age at which the first breeding cycle typically is completed successfully and without delay. We have found no effect of 4–5 months of isolation on the breeding behavior or success of doves.

### **Procedure**

Each male and each female was randomly assigned to one of six groups (10 pairs/group) and treated as follows.

#### **Progesterone in isolation (IP)**

These doves remained in isolation cages and were injected with 100 µg of progesterone in 0.1 ml of sesame oil on each of 4 consecutive days at approximately the time that other doves were obtaining their experience. This group allows examination of the effects of simple progesterone priming.

#### **Oil in isolation (IO)**

These doves were treated like IP doves except that they did not get progesterone during their 4 days of injections. This group of doves was both reproductively naive and not primed with progesterone.

#### **Mate and progesterone (MP)**

These doves were placed in heterosexual-pairs in breeding cages without nesting materials (nest bowl and hay) for 4 consecutive days and injected each day with 100 µg of progesterone. These birds were able to engage in courting behavior and nest-site solicitation while being primed with progesterone.

### **Mate and oil (MO)**

These doves were treated like Group MP doves except that they were injected with sesame oil. This group engaged in courtship behavior but were unlikely to have the increased levels of progesterone characteristic of the later part of the nest-building phase of a breeding cycle.

### **Mate, nest material, and progesterone (MNP)**

These doves were treated like those in Group MP except that a nest bowl (11-cm-diameter glass bowl) and hay were available in the breeding cage. These doves experienced all of the activities of the nest building phase of a cycle, including nesting behavior and nest building (each had built at least ½ to ⅔ of a completed nest in 4 days) as well as the progesterone priming.

### **Mate, completed nest, and progesterone (MCP)**

These doves were treated like those in Group MP except that they were provided with a complete nest that had been coated with shellac to prevent nest-material manipulation. These doves could engage in nest-site solicitation and nesting behavior but not in nest building behavior.

The groups were selected so as to provide a comparison between doves that had had equivalently high exposure to progesterone but that differed in experience during the progesterone priming (Groups IP, MP, MNP, and MCP). In addition, two oil-treated control groups (IO, MO) were used to determine whether progesterone priming itself, either alone or in combination with courtship experience, had an effect. At least in female doves, nest building and progesterone secretion are intimately related: Nest building requires the presence of progesterone (Cheng & Silver, 1975), and significant endogenous progesterone secretion is likely during 4 days of exposure to a mate and nest (Silver et al., 1974). Thus, oil-treated groups having experience with nest building or a completed nest were not used.

On the fifth day, all were removed to individual isolation cages where they remained for the next 5–6 weeks. During their last week in isolation, each received seven daily injections, in their pectoral muscles, of 100 µg of progesterone in 0.1 ml of sesame oil per injection.

Between 1 and 4 hr after lights-on the morning after their last injection, the doves were tested for incubation by placing them in a breeding cage containing a nest with two eggs and a similarly treated and experienced, but unfamiliar, mate. During the following hour, their behavior was continuously recorded by hand on sheets of paper divided into 15-s intervals. An electronic timer signaled the 15-s intervals through an earphone.

Data were collected on the following: latency to get on nest (i.e., spend at least 30 s continuously on the nest); time spent on the nest (number of 15-s intervals in which dove was on the nest); time spent nest soliciting (number of intervals in which wing flipping and nest cooing occurred); time spent manipulating nest materials (number of intervals in which carrying or nest building occurred); establishment of incubation (erecting the ventral feathers and bringing the ventral apterium into contact with the eggs for 2 consecutive min).

Two-minute spot checks were made at 20-min intervals during the 2 hr following the initial observation period and a 10-min check was made at 24 hr after testing. If one member of the pair exhibited incubation at the 24-hr test, it was removed, and the remaining bird was observed until incubation was exhibited or 10 min had elapsed.

### **Data Analysis**

Data from male and female members of a pair were analyzed separately. Differences in numbers establishing incubation across the six groups were assessed for significance by chi-square contingency analysis. Multiple pairwise comparison of numbers incubating for each pair of groups was assessed by Fisher's exact probability test only if the chi-square contingency analysis across groups was significant. Latencies and frequencies of behavioral performance during the first hour of incubation testing were examined across groups by the Kruskal-Wallis test. Multiple pairwise comparisons of specific differences among the groups were assessed with Wilcoxon tests only if the Kruskal-Wallis test revealed a significant difference among the groups. A conservative alpha level ( $p = .003$ ) was selected for the multiple Wilcoxon tests so as to be equivalent to an alpha of  $p = .05$  had the tests been independent (Leach, 1979).

### Results

Figure 1 shows the percentage of males and females in each group that established incubation during the first hour, by 3 hr after, and by 24 hr after introduction. The distribution of incubating birds during the first hour was significantly different between the groups for both males,  $\chi^2 (5, N = 60) = 23.75, p < .001$ , and females,  $\chi^2 (5, N = 60) = 16.6, p < .01$ . Significantly more birds with previous nesting experience (Groups MCP and MNP) exhibited progesterone-induced incubation within 1 hr of testing. Progesterone priming alone (Group IP) or in combination with experience in courtship and nest-site solicitation activities (Group MP) did not result in rapid expression of progesterone-induced incubation.

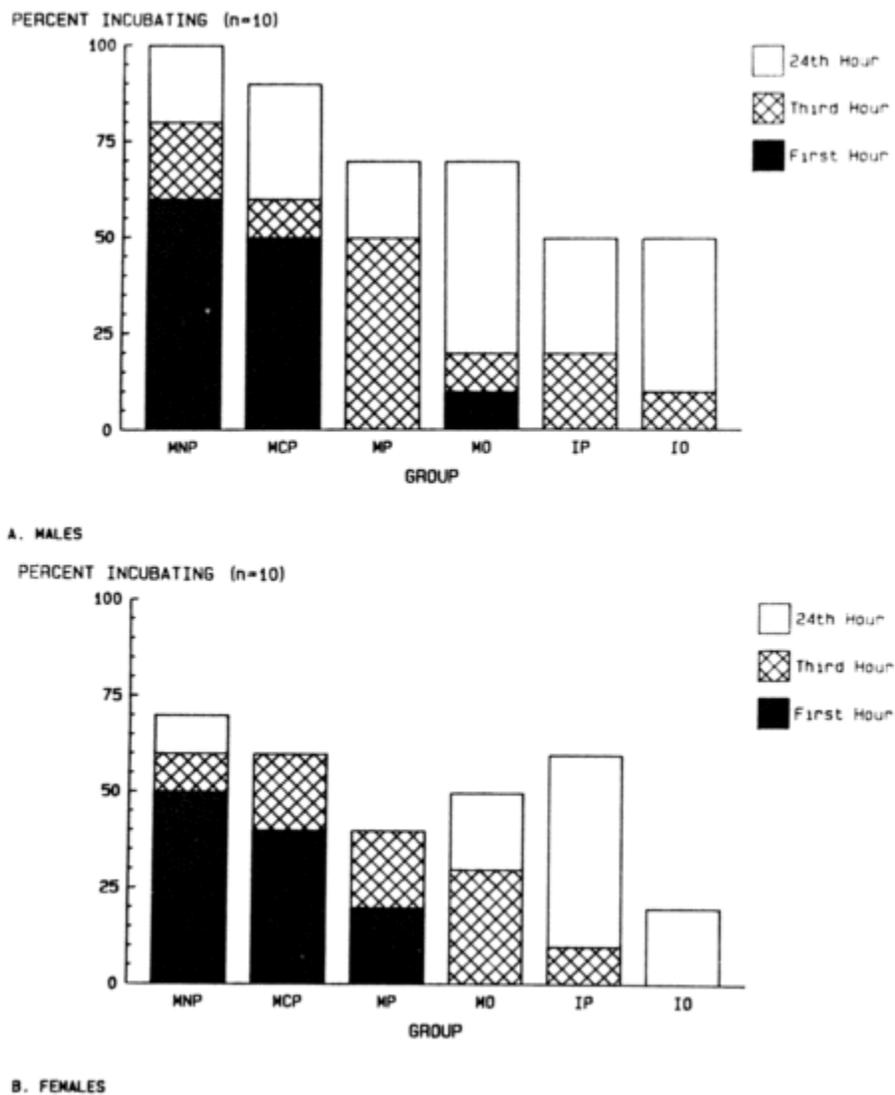


Figure 1. Percentage of (A) males and (B) females in each group establishing incubation during the 1st hour, by the 3rd hour, and by the 24th hour of testing

By 3 hr after introduction, the distribution of incubation between groups was still significantly different for both males,  $\chi^2 (5, N = 60) = 15.8, p < .01$ , and females,  $\chi^2 (5, N = 60) = 14.1, p < .02$ . For females, more nesting-experienced doves (Groups MCP and MNP) exhibited incubation than did reproductively naive doves with (Group IP) or without (Group IO) progesterone priming ( $p = .05$  and  $.025$ , respectively, Fisher's exact probability test). However, more females established incubation,  $\chi^2 (1, N = 20) = 5.0, p < .05$ , when they were progesterone primed during courtship (Group MP) than when they were neither progesterone primed nor courtship experienced (Group IO). For males, significantly more nest-building-experienced doves (Group MNP) established incubation than did naive doves either with (IP group,  $p = .05$ , Fisher's test) or without (IO group,  $p = .025$ , Fisher's test) progesterone priming. Significantly ( $p = .05$ , Fisher's test) more doves with nesting experience (MCP group) incubated than did naive doves (IO group).

By 24 hr after introduction, the differences in number of birds incubating between groups was no longer significant for either males,  $\chi^2 (5, N = 60) = 10.26, p > .05$ , or females,  $\chi^2 (5, N = 60) = 6.8, p > .05$ .

During the first hour of testing, there was a significant difference between groups in latency to get on the nest for both males, Kruskal-Wallis  $H (5) = 36.8, p < .001$ , and females,  $H (5) = 36.5, p < .001$ . Table 1 shows median latencies for males and females in each group. Post hoc multiple comparisons ( $p = .05$ ) revealed that both male and female doves with nesting experience (MNP and MCP groups) have significantly shorter latencies to get on the nest than doves in the other groups. It is interesting that progesterone priming of females during courtship (MP group) significantly reduced their latency to get on the nest compared with MO, IO, and IP females. Neither progesterone priming alone (IP) nor courtship alone (MO) reduced the latency of females to get on the nest when compared with completely naive females (IO). This joint action of progesterone priming and courtship was not evident in males.

**Table 1**  
*Median (and Range) Frequencies of Nest-Related Activities During the First Hour of Testing*

Group	Manipulate nest material		Nest soliciting		Latency to go on nest		Time on nest	
	Male	Female	Male	Female	Male	Female	Male	Female
MNP								
<i>Mdn</i>	16	16	87	86	32	26	137	174
Range	31	39	81	155	175	104	170	144
MCP								
<i>Mdn</i>	22	27	84	46	14	14	178	122
Range	65	58	165	173	22	106	140	210
MP								
<i>Mdn</i>	0	0	103	51	>240	>240	0	0
Range	32	31	148	138	225	223	225	223
MO								
<i>Mdn</i>	0	0	110	62	>240	>240	0	0
Range	9	15	118	132	103	99	103	99
IP								
<i>Mdn</i>	2	2	82	18	>240	>240	0	0
Range	33	40	158	89	162	159	162	159
IO								
<i>Mdn</i>	0	0	57	1	>240	>240	0	0
Range	25	14	121	113	92	95	93	6

*Note.* Maximum possible frequency is 240 fifteen-second time-sampling intervals. The range values were derived by subtracting the lowest from the highest frequency and adding 1. MNP = mate, nest material, and progesterone; MCP = mate, completed nest, and progesterone; MP = mate and progesterone; MO = mate and oil; IP = progesterone in isolation; IO = oil in isolation.

Time spent on the nest also differed significantly between the groups for both males,  $H (5) = 30.8, p < .001$ , and females,  $H (5) = 31.2, p < .001$ . Post hoc multiple comparisons again revealed that for both males and females

nesting experience significantly increased, over all other groups, the time spent on the nest during the first hour (Table 1).

Time spent nest soliciting (Table 1) was significantly different among the groups for females,  $H(5) = 20.5$ ,  $p < .001$ , but not for males,  $H(5) = 7.6$ ,  $p > .10$ . Post hoc comparisons for females showed that both MNP and MO females did more nest soliciting than IP and IO females. Except for IP females, females in the IO group did less nest soliciting than females in all other groups.

Time spent manipulating nest material also differed significantly among the groups (Table 1) for both males,  $H(5) = 19.5$ ,  $p < .005$ , and females,  $H(5) = 29.4$ ,  $p < .001$ . Differences between MNP and MCP males were not significant, but each group was significantly different from the other groups. As with males, manipulation of nest material did not differ between females in Group MNP and those in Group MCP; yet manipulation in each group was greater than that in all other groups. No other differences were significant.

## Discussion

Progesterone priming does not seem to be an adequate explanation for the differences in performance between reproductively naive and experienced doves when tested for progesterone-induced incubation. Previous nesting experience must provide some other lasting change in the dove's nervous system that leads to the rapid expression of incubation during testing because among groups that were equated for previous progesterone priming, only those that had had nesting experience rapidly established incubation. However, progesterone priming, particularly when obtained in combination with courtship experience, can facilitate the expression of some nest-site-related behavior. Because progesterone priming during courtship can subsequently shorten the latency of females to get on the nest during testing, some portion of these birds and their mates may also incubate with a somewhat shorter latency than that of completely naive doves. Although only a shorter latency of females to get on the nest was observed in the present study, some incubation of such females and their mates might become apparent with a larger sample.

Because progesterone requires the previous or concurrent presence of estrogen or testosterone to affect the behavioral performance of breeding doves (Cheng & Silver, 1975; Stern & Lehrman, 1969), it is conceivable that a progesterone priming effect also requires the presence of these hormones. There are increases during a normal cycle in circulating levels of testosterone in males and estradiol in females that begin on the first day of courtship and peak before the beginning of nest building and before the rise in endogenous progesterone of females (Feder, Storey, Goodwin, Reboulleau, & Silver, 1977; Korenbrot, Schomberg, & Erickson, 1974; Silver, 1978). Therefore, progesterone priming during isolation might have had different effects on progesterone-induced behavior if the doves had been provided as well with different regimens of testosterone or estrogen priming. Although this is unlikely to account for differences in incubation, it may account for other behavioral differences among the groups. Doves in Group MP, like those in Group IP and unlike nesting-experienced doves (Groups MNP and MCP), did not incubate, even though it is likely that endogenous estradiol and testosterone levels were elevated during their initial courtship and progesterone priming. However, doves in Group MP did show more nest-related behavior than those in Group IP, and it is possible that this can be accounted for by a joint action of endogenous estrogen or testosterone, with the progesterone provided during priming.

It is especially interesting that the actions of nest building were not necessary for progesterone-induced incubation. Apparently, settling in a nest, nest-site soliciting in a nest, and/or forming an attachment to a nesting place prepare the dove's nervous system, perhaps in combination with progesterone, to be responsive to subsequent exogenous or endogenous progesterone in a manner that leads to the rapid expression of incubation. The process by which nesting experience produces this effect is unknown, but it is conceivable that the process operates within a breeding cycle as well as between cycles (Lehrman & Wortis, 1967).

Although there were few behavioral differences between males and females, progesterone priming may have affected certain behaviors more in females than in males. Because the sexes were tested together, the present study cannot provide evidence for or against sex differences in treatment effects which is not confounded by the

consequences of social interaction. However, in a previous study in which pairs of doves that differed in previous experience were used, it was found that the actions of a mate can affect nest-site-related behaviors without affecting the expression of incubation (Michel, 1976). Therefore, the similarity between males and females in expressing incubation likely reflects the similarity of their treatment rather than the result of their interaction during testing.

These results demonstrate that nesting experience is an important contributor to the effectiveness of progesterone in inducing incubation behavior. They do not rule out a possible role of progesterone priming, nor do they lead one to assume that the contributions of nesting experience and previous exposure to progesterone are independent. In the normal course of a breeding cycle, social, environmental, and hormonal changes complement and reinforce one another in controlling the dove's behavioral expression and exposure to stimulation. The analysis of incubation reported here and in previous work (Lehrman & Wortis, 1960; Michel, 1976, 1977) suggests a similar complementarity of social, environmental, and hormonal factors that operate between cycles.

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