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Effect of DDE on Circulating Luteinizing Hormone Levels in Ring Doves During Courtship and Nesting

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Delayed breeding and reproduction linked to organochlorine pesticides are reported for raptors (RATCLIFFE 1963, HICKEY 1969), wood-pigeons (*Columba palumbus*) (MURTON & VIZOSO 1963, LOFTS & MURTON 1966), song thrushes (*Turdus ericetorum*) and blackbirds (*T. merula*) (MAYER-GROSS 1964).

Low level DDT exposure induced delays between pair formation and egg laying in Bengalese finches (*Lonchura striata*) (JEFFERIES 1967) and ovulatory delays in ring doves (*Streptopelia risoria*) PEAKALL 1970, HAEGELE & HUDSON 1973, 1977). Two different mechanisms were proposed to explain the delay phenomena. PEAKALL (1970, 1970a) indicated lowered estradiol levels, probably the result of liver hydroxylating enzyme induction, caused the delay; since estrogen is involved in gonad maintenance and breeding behavior in the female. JEFFERIES (1967) proposed a possible hypothalamic-hypophyseal influence by DDT, inhibiting gonadotropin secretion. Based on subsequent work showing hypo- and hyperthyroidism by varying DDT dosage levels, he also suggested a possible thryoid effect (JEFFERIES 1969, 1975; JEFFERIES & FRENCH 1971).

DDE interference with normal gonadotropic secretion and function, as indicated by radioimmunoassay of circulating levels of LH during the courtship and nesting phases of the ring dove reproductive cycle, was examined as a contributing factor in DDE-induced ovulatory delays in the dove.

MATERIALS AND METHODS

Maintenance of Experimental Animals: Sexed ring doves, obtained from the Denver Wildlife Research Center, Denver, Colorado, were housed in stainless steel cages (60 x 30 x 60 cm) and supplied Purina Pigeon Checkers® and water ad libitum. Six pairs each of experimental and control birds were paired and allowed to raise at least one clutch to maturity prior to experimentation.

Two dosage levels (10 and 40 ppm) of pp'-DDE (99.9% purity, Patuxent Wildlife Research Center, Laurel, Md.) were used. During administration of the treated diet, approximately 90 days, all birds were maintained on an 8L:16D photoperiod, in visual but not auditory isolation. Upon pairing, all birds received "clean"

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food and were maintained on a 15L:9D photoperiod.

Following completion of the 10 ppm trial, the experimental group received a ration containing 40 ppm DDE, the control group continued to receive clean food. The second trial was run with the same control birds, and the experimental birds now having been exposed to the 40 ppm DDE ration. All pairings were between birds never before together.

Blood Samples: Blood samples (50-100 uL) were taken 3 times daily (2 h after onset of the photoperiod, midpoint of the photoperiod and 2 h before termination of the photoperiod) on alternate days over the first week following pairing. The blood was collected in heparinized capillary tubes following direct puncture of a wing vein with a 25 gauge needle, centrifuged, and the plasma stored frozen at -20°C in flame-sealed hematocrit tubes.

Iodination and Radioimmunoassay: Materials for the radioimmunoassay procedures which were generously supplied by Dr. B. K. Follett (University College of North Wales), included purified chicken luteinizing hormone (AE-1) and rabbit anti-chicken LH, previously shown specific in ring doves (CHENG & FOLLETT 1976); and sheep anti-rabbit precipitating antibody was provided by J. Powell (The Ohio State University). The procedures for the assays and iodination were basically those of FOLLETT et al. (1972, 1975) with the modifications indicated in RICHIE (1978).

RESULTS

The LH radioimmunoassay system utilized gave a 14.5% intrassay coefficient of variation, determined by the mean coefficient of variation for all duplicates in the assay system. Interassay variation assessed by Snedecor's formula C. V. = $\sqrt{\frac{d^2}{2n}} \times 100$ (ABRAHAM et al. 1971), was 17.1 for a pooled plasma sample included in eash assay.

Treatment with 10 ppm DDE in the diet caused a small increase in the latency to oviposition following pairing, relative to control birds. Birds receiving the diet containing 40 ppm DDE showed a marked delay ($P = 0.002$) in achieving first oviposition following pairing (19.5 days) relative to control birds (6.5 days) (Table 1).

No significant differences were found in circulating LH levels between control and experimental males in the 10 ppm trial ($P > 0.33$), nor doves of either sex in the 40 ppm trial ($P > 0.12$ for females and $P > 0.18$ for males; two way analysis of variance for each day, based on Kruskal-Wallis rank sums). During the 10 ppm trial, day 2 LH levels in control birds were significantly different from treated birds, being lower in both

TABLE 1

Median number of days to first oviposition following pairing in control ring doves and those maintained on diets containing 10 ppm and 40 ppm DDE.

Reproductive Trial	Group (N)	Days	
I	Control (6)	12.5	
			(0.047 ≤ P ≤ 0.066)
	10 ppm DDE (6)	15.5	
II	Control (6)	6.5	
			(P = 0.002)
	40 ppm DDE (6)	19.5	

N = number of birds
P = lowest significance level at which the null hypothesis may be rejected. Wilcoxon Rank Sum.

the first and second sampling periods and higher than experimental birds from the third daily sampling period (Fig. 1).

Control birds of both sexes and females in the DDE-treated groups attained LH levels significantly above baseline (Multiple comparisons experimentwise error rate ($P \leq 0.05$) following a significant ($P \leq 0.05$) ANOVA). Experimental males in both the 10 and 40 ppm treatment regimes failed to show significant differences in their LH levels over the sampling period (one-way ANOVA based on Kruskall-Wallis rank sums, $P > 0.05$).

Only control females (days 2 and 6) in the 10 ppm trial demonstrated significant variation (one-way ANOVA over the 3 sampling periods, within a sex and treatment group $P < 0.05$) over the 3 sampling periods each day. In the 40 ppm trial both control males and females showed a significant change in LH within day 4. All changes were toward increasing levels during the day. Reduction of the data to a single point for each day, except where significant differences were indicated within a day, illustrated the general LH response to pairing within each group (Fig. 1 and 2).

Discussions of the statistical treatments are available in HOLLANDER & WOLFE (1973).

DISCUSSION

Previous studies with DDT and its metabolites have demonstrated significant effects on reproduction. Reduction of both courtship time and bow-coo frequency in male ring doves fed diets containing 10 and 40 ppm DDE was found (HAEGELE &

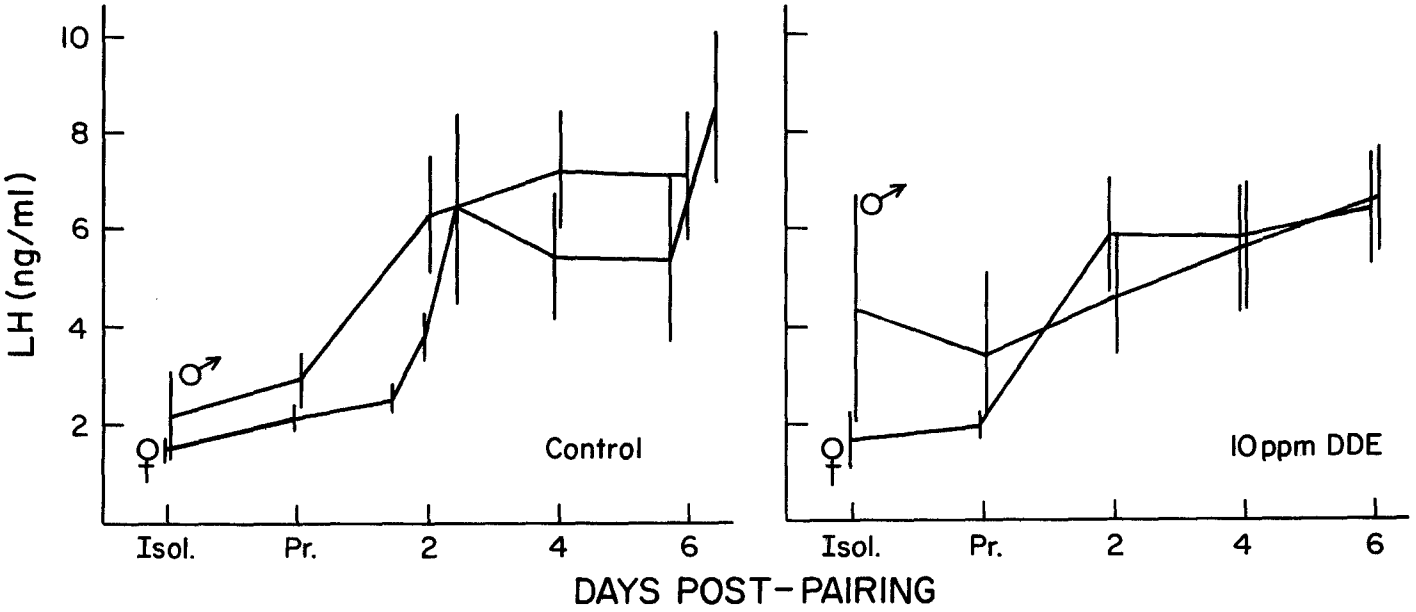

FIGURE 1

Circulating levels of plasma luteinizing hormone in ring doves maintained on diets containing 10 ppm DDE and control birds, during the courtship and nesting phases of the reproductive cycle. Vertical bars locate mean ± 2 SEM, indicating approximate 95% confidence intervals. Individual daily values were obtained by pooling all plasma samples from that day if no significant differences existed over the sampling period.

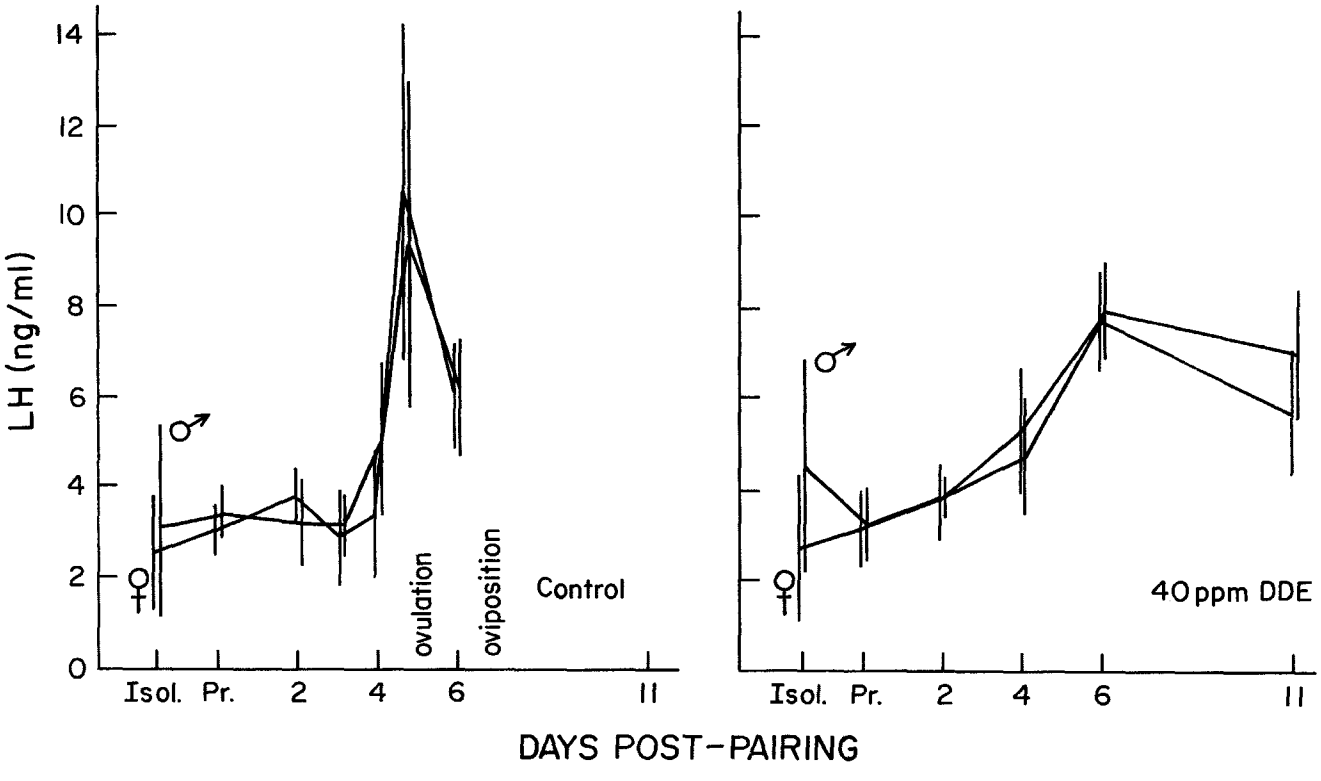

FIGURE 2

Circulating levels of plasma luteinizing hormone in ring doves maintained on diets containing 40 ppm DDE and control birds, during the courtship and nesting phases of the reproductive cycle. Vertical bars locate mean ± 2 SEM, indicating approximate 95% confidence intervals. Individual daily values were obtained by pooling all plasma samples from that day if no significant differences existed over the sampling period.

HUDSON 1977). Increased hepatic enzyme induction and decreased estradiol levels in the female dove followed treatment with 10 ppm DDT (PEAKALL 1970). Enzyme induction patterns for DDE and DDT are also known to be the same (RISEBROUGH et al. 1968). Presumably a similar situation exists for other gonadal steroids in both sexes, in addition to estradiol, and via negative feedback regulation, gonadotropin secretion would increase to return steroid levels to normal.

During the 10 ppm trial, control birds showed a longer latency to oviposition than the normal of 7-10 days. This apparently involved some environmental factor uniformly affecting both control and experimental groups. A repeat trial showed control groups laying within the expected period and a small delay by the experimental group proportionately identical to that reported here.

Luteinizing hormone levels were not significantly different between control and DDE-exposed doves during the isolation period. Birds fed the diet containing 40 ppm DDE failed to show the strong LH surge on day 4 seen in the control group. Instead, a more gradual increase in circulating hormone levels to day 6 was observed, with an apparent plateau to day 11.

A clear cause and effect relationship between DDE, circulating LH levels, and delayed ovulation was not readily apparent. The differences in circulating LH patterns between experimental and control groups indicates a possible DDE effect on normal circulating levels and/or function of the gonadotropin.

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