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The Effect of Progesterone and Estradiol-17 β on the Pigment Accumulation of the Shell Gland in Japanese Quail Pretreated with Aminoglutethimide

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Effects of the combined injection with progesterone and estradiol-17 β i.m. on the accumulation of the superficial pigment of the eggshell in the quail shell gland were investigated. Aminoglutethimide (AG, 20 mg/100 g BW) was administered s.c. to quail hens 10 h before the expected ovulation, followed by the administration of progesterone (0.1 mg/100 g BW) and estradiol-17 β (0.1 mg/100 g BW). Pigment in the shell gland at 18 h after oviposition of the preceding egg was measured. Progesterone had increasing effect on pigment in the shell gland of both hens ovulated and failed to ovulate. The effect of combined injection with progesterone and estradiol-17 β was appeared only if ovulation was induced and estradiol-17 β was injected 1 h after oviposition of the preceding egg. These results suggested that the accumulation of pigment in the shell gland was enhanced by estradiol-17 β closely related to egg formation.

INTRODUCTION

It has been shown that the superficial pigment (porphyrin) of a quail egg shell accumulates in the shell gland (uterus) accompanying with egg formation (Tamura and Fujii, 1966; Poole, 1967; Soh *et al.*, 1989; Soh *et al.*, 1993) and is secreted 2 to 3.5 hrs before the expected oviposition time (Woodard and Mather, 1964; Poole, 1965; Tanaka *et al.*, 1977; Soh *et al.*, 1989; Soh *et al.*, 1993). Similarly as calcium secretion that was associated with occurrence of ovulation (Eastin and Spaziani, 1978; Nakada and Koga, 1990), the pigment were accumulated in the shell gland even if the ovulated ovum failed to enter into oviduct and the shell gland was empty (Soh *et al.*, 1989). Soh and Koga (1994) suggested that progesterone involving with ovulation might affect the accumulation of pigment in the shell gland of laying quail hen. Also, estradiol-17 β could activate δ-aminolevulinic acid, one of the enzymes those synthesize porphyrin, in immature quail's (Yamada, 1972) and chicken's (Tushima and Yamada, 1988) shell gland. However, single injection with estradiol-17 β failed to increase the amount of pigment in the shell gland of laying quail hens, of which ovulation were blockaded by aminoglutethimide (Soh and Koga, 1994) that inhibits steroidgenesis. The present study was performed to investigate the effect of the combined injection with progesterone and estradiol-17 β on the pigment accumulation in the shell gland of quail hen pretreated with aminoglutethimide.

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MATERIALS AND METHODS

Animals

Laying quail hens were maintained in individual cages and exposed to a 16-h photoperiod (lights on from 0500 to 2100 h). The oviposition times were recorded by the same method of Soh *et al.* (1989). Feed (23% CP; 2,800 kcal ME/kg) and water were available at all times. A total of 82 hens laying midsequence eggs at nearly 24-h intervals was selected for the present experiment.

Reagents

Aminoglutethimide (AG, Ciba-Geigy Pharm. Co., Basel, Switzerland) was prepared to adjust the concentrations to 100 mg/ml according to Soh and Koga (1994). Progesterone and estradiol-17 β (Sigma Chemical Co., St. Louis, USA) were dissolved in ethanol and diluted to yield desired concentrations of 1 mg/ml with ethylene glycol.

Experiment

AG (20 mg/100 g BW) were injected s.c. into 75 laying quail hens 10 h before the expected ovulation to blockade ovulation (Soh and Koga, 1994). Hens injected with AG were divided into four experimental groups. First, nothing was injected following AG injection, i.e. control of ovulation blockade (OB group, n=13). Second, progesterone (0.1 mg/100 g BW) was injected i.m. 2 h after AG injection (P group, n=25). Third, progesterone and estradiol-17 β (0.1 mg/100 g BW, respectively) were injected i.m. 2 h after AG injection (PE1 group, n=19). Fourth, progesterone (0.1 mg/100 g BW) was injected i.m. 2 h after AG injection and estradiol-17 β (0.1 mg/100 g BW) was injected i.m. 1 h after oviposition of the preceding egg, which was present in the shell gland at the time of AG injection (PE2 group, n=18). In addition to these experimental groups, hens injected with the vehicle used for AG delivery (HCl solution, pH 3.5) served as ovulated controls (V group, n=7).

All quail hens were decapitated under anesthesia 18 h after oviposition of the preceding egg, and their shell glands were removed and stored at −20 C. Pigment in the shell gland was extracted and measured according to Soh *et al.* (1989).

Statistical Analysis

All of the statistical analyses for the present experiment were carried out according to Snedecor (1956). For comparisons between treatments, Student's t test or Cochran-Cox test were employed after test of the equality of two variances. The χ^2 test was employed for comparison between ovulation rates.

RESULTS

The ovulation rates of P, PE1 and PE2 group were 32% (eight of 25), 32% (six of 19) and 28% (five of 18), respectively, and there were no significant difference among these rates. The amounts of pigment in the shell glands 18 h after oviposition of the preceding egg are shown in Table 1. Both ovulated and not ovulated hens received steroid hormones showed significantly larger amount of pigment ($P<0.05$) comparing with OB

Table 1. The amount of pigment in the shell gland of quail hens treated with aminoglutethimide, progesterone and estradiol-17 β.

	Amount of pigment (μ g/g tissue)				
	V[1]	OB[1]	P[1]	PE1[1]	PE2[1]
ovulated	143 ± 22[a]	· · ·	91 ± 25[b]	126 ± 53[ab]	136 ± 32[a]
	(7)		(8)	(6)	(5)
Non-ovulated	· · ·	58 ± 16[c]	97 ± 29[b]	88 ± 19[b]	81 ± 25[b]
		(13)	(17)	(13)	(13)

[1] See Materials and Methods

[a-c] values (mean±SD) with no common superscripts differ significantly ($P<0.05$). Number of hens sampled are shown in parentheses.

group, all of which failed to ovulate. There were no significant difference among the amounts of pigment in not ovulated hens of P, PE1 and PE2 group. In ovulated hens received steroid hormones, the amount of pigment ascended in order of P, PE1 and PE2 group, and there were significant difference between P and PE2 group ($P<0.05$). The amount of pigment of the ovulated hens in PE2 group was nearly to that of V group, and significantly larger than that of not ovulated hens in the same group ($P<0.05$).

DISCUSSION

However blockade of ovulation by AG injection inhibited following accumulation of pigment in the shell gland, progesterone injection was able to recover the amount of pigment even if ovulation did not occured. These results correspond to Soh and Koga (1994). The significantly effect of combined injection with progesterone and estradiol-17 β beyond the recovery by progesterone injected singly appeared only when ovulation was induced and estradiol-17 β was injected at the initiation of egg formation in the present experiment. Single injection of estradiol-17 β had no effect on the amount of pigment in the shell gland of hens pretreated with AG (Soh and Koga, 1994). These results suggest that the increase in plasma progesterone involving with ovulation was necessary to the accumulation of pigment and estradiol-17 β enhanced the accumulation closely related to egg formation. It has been also suggested that the stimulation of oviduct by descending egg accelerate accumulation of pigment in the shell gland (Soh *et al.*, 1989). Moreover, there are other suggestions that ovulation was very important to eggshell formation (Nakada *et al.* 1976; Eastin and Spaziani, 1978; Nakada *et al.* 1980) and the presence of an egg also may be necessary for eggshell formation or calcium secretion of the shell gland in chicken (Tanaka, 1976; Eastin and Spaziani, 1978; Nakada, 1990; Nakada and Koga, 1990; Ieda *et al.*, 1995). Considering these results, it could be hypothesized that not only ovulation but also the presence of an egg in the oviduct may be one of potential stimulatory factors for total activity of the shell gland.

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