Effects of Exogenous Estrogen on the Differentiation and Development of the Right Oviduct in Female Chickens

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On day 5 of incubation, 0.64 mg of estradiol benzoate (EB) was administered to fertilized eggs to investigate its effects on the differentiation and development of the oviduct in female chickens.

The increases of body and ovary weights of treated females in 0 to 366 days old were similar to those of females not treated with EB (control). However, thirty one % of the mature treated females did not produce any eggs. The egg production rate of the treated females that produced eggs was low, and the first egg production was delayed, although there were large individual variations among the treated females. A right oviduct at various developmental stages was seen in every treated female ; there was a significant negative correlation between egg production rate and the weight of the right oviduct ($p < 0.01$), and a significant positive correlation between egg production rate and the weight of the left oviduct ($p < 0.01$). The number of yellow follicles in the ovary of the treated females was similar to those of the control, but intraperitoneal ovulations were observed in most of the treated females.

These findings suggest that suppression of anti-Müllerian hormone by estrogen in embryogenesis allows the development of the right Müllerian duct, which differentiates and develops into an oviduct. One of the causes of the reduced egg production in the mature treated females could be inhibition of the normal differentiation and development of the left Müllerian duct by exogenous estrogen, thus resulting in a functionally immature left oviduct.

Key words : estrogen, right oviduct, sex differentiation, chicken

Introduction

In chickens, the development of the right Müllerian duct ceases at about day 8 of incubation, and, as is the case with many other bird species, only the left oviduct develops (Aitken, 1971). Factors that play a role in the differentiation of the Müllerian ducts are thought to be estrogen and anti-Müllerian hormone (AMH) (Groenendijk-Huijbers, 1962 ; Burns, 1973 ; Josso, *et al.*, 1977 ; Hutson, *et al.*, 1981 ; Hutson, *et al.*, 1982 ; Ikawa *et al.*, 1982 ; Eusebe *et al.*, 1996). The blood concentration of estrogen in female embryos is always higher than that in male embryos after day 7.5 of incubation (Woods and Brazzill, 1981), and radio-receptor assays have shown estrogen-binding sites in the left and right Müllerian ducts on days 8 and 9 of incubation

(MacLaughlin *et al.*, 1983). Although there are no differences in the number of these binding sites between male and female embryos, it has been documented that there are more intranuclear estrogen-binding sites in the left Müllerian duct than in the right Müllerian duct (MacLaughlin *et al.*, 1983). AMH has been detected by bioassay in the male and female gonads after day 8 of incubation (Hutson *et al.*, 1981). Eusebe *et al.* (1996) have reported that the level of AMH mRNA is high after day 10 of incubation. Abinawant *et al.* (1997) have confirmed the presence of the left oviduct in 10-month-old masculinized female chickens after administration of aromatase inhibitor (AI) to fertilized eggs. They stated that the effects of AI may have been insufficient and that estrogen might therefore have induced the development of the Müllerian ducts. Also, Masuda and Koyanagi (2001) confirmed the presence of left and right oviducts in mature male chickens after administration of estrogen during incubation. Therefore, it is currently thought that estrogen suppresses the actions of AMH on the Müllerian ducts (Hutson *et al.*, 1981 ; Hutson *et al.*, 1982 ; MacLaughlin *et al.*, 1983). However, there have been contradicting reports on the effects of estrogen administration during incubation on the development of the right Müllerian duct in male embryos : Hamilton (1961) reported that estrogen could induce the development of the left and right Müllerian ducts ; Wolff (1979) reported that estrogen could not induce the development of the right Müllerian duct. Hamilton (1961) reported that estrogen could not affect the development of the right Müllerian duct in female embryos. Therefore, the effects of estrogen and AMH on the differentiation of the Müllerian ducts, in particular the differentiation and development of the right Müllerian duct, have not been fully elucidated.

In the present study, a large amount of estrogen was administered during incubation, and the morphology of the oviduct in mature female chickens was then assessed to ascertain the effects of estrogen on the differentiation and development of the oviducts.

Materials and Methods

Fertilized eggs, obtained by mating Rhode Island Red males and Barred Plymouth Rock females using artificial insemination, were incubated. On day 5 of incubation, estradiol benzoate (EB), suspended in 0.1 ml of sesame oil, was injected into the egg albumen near the embryo using the previous procedure (Koyanagi *et al.*, 1986). The dosage of EB was set at 0.64 mg according to the results of Koyanagi *et al.* (1986). In the untreated group, only sesame oil (0.1 ml) was injected, as a control. Among day-old chicks, females were identified based on color of down feathers. In the ages of 0 to 366 days, 5 to 13 female chickens at a time were killed at intervals to measure body and ovary weights. At 366 days, the oviducts were weighted.

All data were analysed using Duncan's multiple range test or Tukey's test at $P < 0.05$ (Duncan, 1955).

Results

The increases in body weight of treated female chicks after hatching were comparable to those of the control group (Table 1). Also, the increases in ovary weight of

Table 1. Body and ovary weights of the female chickens after hatching injected with estradiol benzoate (EB) on 5th day of incubation

		Age in days after hatching				
		0	30	60	104	366
Body weight	EB	42.2±1.3 ¹⁾ (5)	292.8±26.0(5)	648.3± 56.7(6)	1.4± 0.1(5)	2.6± 0.5(13)
	Control	41.9±1.7 (5)	288.8±12.1(5)	758.0±116.7(5)	1.3± 0.1(5)	2.4± 0.5(3)
Left ovary weight	EB	3.9±1.0 (5)	68.0±13.0(5)	144.4± 25.3(6)	387.6±71.9(5)	49.4±24.0(13)
	Control	9.3±3.4 (5)	79.1± 8.6(5)	175.8± 21.2(5)	371.5±13.7(5)	46.1± 8.1(3)

¹⁾ Mean±SD.

Body weight : 0~60 days old g, 104~366 days old Kg.

Ovary weight : 0~104 days old mg, 366 days old g.

() No. of birds

the treated females were nearly the same as those of the control group.

However, thirty one % of the mature treated females did not produce any eggs. Also, treated females that produced eggs tended to have delayed first egg production, and their egg production rate was low, although there were large individual variations among the treated females (Table 2). Also, fifty four % of the treated females produced eggs with a diameter of about 3 cm containing only egg albumen throughout the study period. Four to eight yellow follicles were seen in the ovary of 85% of the treated females at 366 days, and only white follicles were seen in the ovary of the other 15% of the treated females. Furthermore, intraperitoneal ovulations were seen in 62% of the treated females. On the other hand, control females laid normal eggs throughout the study period. Intraperitoneal ovulations were not seen in them. Number of

Table 2. Age at first egg production, egg production rate and oviduct weight of the female chickens injected with estradiol benzoate (EB) on 5th day of incubation

	No. of birds	Age at first egg production	Egg production rate ¹⁾	Oviduct weight (g) ²⁾		Left oviduct weight/Total oviduct weight (%)
				Left	Right	
EB	9	182.2±26.7 ³⁾	61.1±31.2	1.79±0.46 ^a	0.14±0.21 ^b	92.6±10.7 ^a
	4 (nonlaying)	—	0	0.76±0.42 ^b	0.63±0.41 ^a	54.9± 8.9 ^b
Control	3	167.3± 6.5	90.1± 4.8	2.04±0.18 ^a	0.03±0.02 ^b	98.7± 1.1 ^a

¹⁾ Rate of egg production during the 84 days after first egg production (%/day/bird).²⁾ Oviduct weight per 100 g of body weight.³⁾ Mean±SD.^{a, b} Means with the different superscripts within the same column are significantly different (P<0.05).

Fig. 1. The oviduct of a mature female injected with estradiol benzoate on 5th day of incubation.

In the well-developed right oviduct (RO), infundibulum (In), magnum (Ma) isthmus (Is) and uterus (Ut) were distinguishable. Cl cloaca, LO light oviduct, Va, vagina

the yellow follicles in their ovaries was similar to that of the treated females.

Every treated female was found to have a developed right oviduct. The weight of right oviduct of the treated females was greater than that of control group (Table 2). In 31% of the treated females, the infundibulum, magnum, isthmus, uterus and vagina of the right oviduct were distinguishable (Fig. 1) ; in 7% of the treated females, the individual parts of the right oviduct could not be distinguished by appearance ; and, in 62% of the treated females, the right oviduct was slightly more developed than that of the control group. There was a significant positive correlation between egg production rate and the weight of the left oviduct ($p < 0.01$), and a significant negative correlation between egg production rate and the weight of the right oviduct ($p < 0.01$) (Fig. 2).

Discussion

Masuda and Koyanagi (2001) reported that the degree of post-hatching body weight increase of male chickens treated with EB during incubation was comparable to that of male chickens not treated with EB. In the present study, the degree of post-hatching body weight increase of female chickens treated with EB was comparable

Fig. 2. The relationship between egg production rate and oviduct weight in the female chickens injected with estradiol benzoate on 5th day of incubation.

Females with well-developed right oviduct and smaller left oviduct produced none or very few eggs and females with well-developed left oviduct produced many eggs.

to that of the females not treated with EB. These findings indicate that the administration of estrogen during incubation does not affect the body weight increase of chicks after hatching.

In the present study, the degree of increase in ovary weight of the female chickens treated with EB was comparable to that of the females not treated with EB, suggesting that estrogen administration during incubation does not affect the increase in ovary weight. However, some of the treated females produced none or very few eggs. These females had well-developed right oviduct and smaller left oviduct. Furthermore, intraperitoneal ovulations were seen in most of the females. Therefore, one of the causes of reduced egg production rate in the treated females may be that exogenous estrogen inhibited the normal differentiation and development of the left Müllerian duct, resulting in a functionally underdeveloped left oviduct. However, even treated females with a functional well-developed left oviduct tended to have lower egg production rates than untreated females, and most of them ovulated intraperitoneally. In addition, 15% of treated females, only white follicles were seen in the ovary. Masuda and Koyanagi (1990, 1998) investigated the ganglions in the central nervous system of

mature male chickens treated with EB during incubation, and found that the medial preoptic area (a mating behavior center in male chickens) was histologically feminized; thus altered male chickens did not exhibit any mating behaviors. Therefore, it is possible that estrogen could interfere with the normal development of a female's central nervous system, which is involved in follicular growth and egg transport.

The cause of the variations in the degree of development of the left and right oviducts could be individual differences in sensitivity of the Müllerian ducts to estrogen. Also, EB was not necessarily administered to the same location of the albumen in every egg. Thus the timing and amount of EB uptake by embryos could have varied depending on the route of uptake (yolk sac circulation or allantois circulation).

Hamilton (1961) reported that development of the right Müllerian duct could not be induced by administering estradiol-17 β into the chorioallantoic sac of female chicken embryos. However, both left and right oviducts have been observed in male chickens treated with EB during incubation (Masuda and Koyanagi, 2001). In the present study, a morphologically developed right oviduct was observed in the treated females. The differences among the results of these studies may be due to differences in kinds of estrogen used and treatment methods.

We therefore conclude that the presence of a large amount of estrogen, which could inhibit the actions of AMH during incubation, induces the development of the right Müllerian duct in both male and female chickens.

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References

- Abinawanto, Shimada K and Saito N. Sex-Reversal effects of non-steroidal aromatase inhibitor on aromatase (P450_{arom}) mRNA expression in adult chicken gonads. *Japanese Poultry Science*, 34 : 158-168, 1997.
- Aitken RNC. The oviduct. In : *Physiology and Biochemistry of the Domestic Fowl* (Bell DJ and Freeman BM eds.). Vol. 3. pp. 1237-1289. Academic Press London, New York. 1971.
- Burns RKB. Role of hormones in the differentiation of sex. In : *Sex and Internal Secretions* (William CY ed.) Vol. 1. pp. 76-158. Robert E. Krieger Publishing Company. Huntington, New York. 1973.
- Duncan DB. Multiple range and multiple F test. *Biometrics*, 11 : 1-42. 1955.
- Eusebe DC, Clemente N, Rey R, Pieau C, Vigier B, Josso N and Picard J-Y. Cloning and expression of the chick anti-müllerian hormone gene. *The Journal of Biological Chemistry*. 271 : 4798-4804. 1996.
- Groenendijk-Huijbers MM. The cranio-caudal regression of the right müllerian duct in the chick embryo as studied by castration experiments and estrogen treatment. *Anatomical Record*, 142 : 9-19. 1962.
- Hamilton TH. Studies on the physiology of urogenital differentiation in the chick embryo. *The Journal of Experimental Zoology*, 146 : 265-274. 1961.
- Hutson J, Ikawa H and Donahoe PK. The ontogeny of mullerian inhibiting substance in the gonads of the chicken. *Journal of Pediatric Surgery*, 16 : 822-827. 1981.
- Hutson JM, Ikawa H and Donahoe PK. Estrogen inhibition of mullerian inhibiting substance in

- the chick embryo. *Journal of Pediatric Surgery*, 17 : 953-959. 1982.
- Ikawa H, Hutson JM, Budzik GP, MacLaughlin DT and Donahoe PK. Steroid enhancement of mullerian duct regression. *Journal of Pediatric Surgery*, 17 : 453-458. 1982.
- Josso N, Picard J-Y and Tran D. The antimüllerian Hormone. *Recent Progress in Hormone Research*, 33 : 117-167. 1977.
- Koyanagi F, Masuda S and Nisiyama H. Feminization of the male left gonad of chicks by injection of estrogen during incubation, especially on the interrelations between doses and morphological and histological changes. Reprinted from *Proceeding of the Faculty of Agriculture, Kyushu Tokai University*, 5 : 61-69, 1986.
- MacLaughlin DT, Hutson JM and Donahoe PK. Specific estradiol binding in embryonic mullerian ducts : A potential modulator of regression in the male and female chick. *Endocrinology*, 113 : 141-145. 1983.
- Masuda S and Koyanagi F. Sexual behavior of cocks hatched from eggs treated with estrogen during incubation. *Japanese Journal of Zootechnical Science*, 61 : 661-662. 1990.
- Masuda S and Koyanagi F. Effect of estrogen on sexual differentiation of the medial preoptic area in chicken. *Japanese Poultry Science*, 35 : 245-248. 1998.
- Masuda S and Koyanagi F. Changes in the ovotestes transformed by exogenous estrogen during incubation in the male chicken after Hatching. *Japanese Poultry Science*, 38 : 28-35. 2001.
- Wolff E. Old experiments and new trends in avian sex differentiation. *In Vitro*, 15 : 6-10. 1979.
- Woods JE and Brazzill DM. Plasma 17β - estradiol levels in the chick embryo. *General and Comparative Endocrinology*, 44 : 37-43. 1981.