ONTOGENETIC ASPECTS OF STEROIDOGENESIS BY GONADS OF DUCKS AND ITS ROLE IN SEX DIFFERENTIATION

O. Doi[1], A. Iwasawa, T. Nakamura and Y. Tanabe[2]

Department of Animal Science and Technology, Faculty of Agriculture
Gifu University, Gifu 501-11, Japan

Summary

To elucidate the relationship between steroidogenesis and sex differentiation in the duck, plasma, testicular and ovarian testosterone, estradiol-17β and progesterone concentrations in male and female embryos of day 11 to 27 (just before hatching) of incubation and in 1- to 7-day old male and female ducklings were investigated by radioimmunoassays. Plasma estradiol-17β concentrations in female embryos declined from very high at days 11 and 15 of incubation and remained at low levels after hatching. Male plasma estradiol-17β concentrations were always lower than those of the female throughout this period. Plasma testosterone and progesterone concentrations in both sexes were low during the embryonic stage, but then increased to peaks 3 days and 1 day after hatching, respectively. Estradiol-17β contents were much higher in the left ovary than the right ovary or testes throughout the experimental period. The estradiol-17β content of the left ovary was very high at day 15 of incubation, and decreased gradually thereafter. Both in right ovary and testes, estradiol-17β contents were always low. Testosterone and progesterone contents in the left ovary were low from day 11 to 23 of incubation, and reached a peak 1 day after hatching. Progesterone contents in the right ovary and testes were low levels over the time period examined. Testosterone and progesterone contents were much higher in the left ovary than the right ovary and testes. The present results clearly demonstrate that the capacity of the embryonic left ovary of duck to synthesize estradiol-17β and testosterone is much higher than that of the embryonic testis. It is suggested that estrogen secreted from the embryonic ovary earlier than day 15 of incubation has an important role in female sexual differentiation in the duck, and the sex of the avian species is basically male with homozygous sex chromosomes (ZZ).

(Key Words: Sex Differentiation, Ontogeny, Steroidogenesis, Gonad, Duck)

Introduction

Numerous attempts have been made to demonstrate the ontogenetic steroidogenesis of mammals and birds. In mammals, it is generally agreed that the onset of production of the steroid hormones takes place simultaneously in the fetal ovary and testis, and that the fetal testis secretes much more testosterone than the fetal ovary (George et al., 1978; Rigaudière, 1977). In contrast with mammals, the embryonic left ovary in the chicken and the duck secretes much more estradiol-17β than the embryonic testes (Guichard et al., 1977; Tanabe et al., 1983, 1986). Estradiol-17β secreted during the embryonic stage especially has an important role in female sex differentiation of avian species (Guichard et al., 1977; Tanabe et al., 1983, 1986), but it is not always understood why this should be so. Moreover, estradiol-17β produced in the embryonic ovary protects the left Müllerian duct against Müllerian inhibiting substance (MacLaughlin et al., 1983), but in the male, the Müllerian duct regresses under the effect of Müllerian inhibiting substance (Groenendijk-Huijbers, 1962).

The present work undertaken to elucidate the relationship between ontogenetic steroidogenesis and sex differentiation in the duck by measuring the levels of plasma and gonadal testosterone, estradiol-17β and progesterone in the embryonic and postembryonic male and female ducks. In this paper, we would like to emphasize the endocrine functional differences between the left and right ovaries and testes.

[1]Address reprint requests to Dr. O. Doi, Department of Animal Science and Technology, Faculty of Agriculture, Gifu University, Gifu 501-11, Japan.

[2]Present address: Azabu University, School of Veterinary Medicine, Sagamihara 229, Japan

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Materials and Methods

Eggs were obtained from Khaki Campbell ducks and were incubated in an incubator at 38 ± 0.5℃ and 72 ± 0.5% relative humidity in constant darkness. The eggs were hatched on day 28 of incubation. The ducklings were maintained in an electric brooder at 28-30℃, with feed and water *ad libitum* on a 15L:9D photoperiod (lights on at 05:00 and off at 20:00). Blood samples were collected with a heparinized syringe by heart puncture in 10 male and female embryos at days 11, 15, 19, 23 and 27 of incubation and in 10 ducklings of both sexes at 1, 3 and 7 days of age. The blood samples were centrifuged for the separation of plasma, which was frozen and kept at −20℃ until assay. One-tenth to one half milliliter plasma for the determination of testosterone, estradiol-17β and progesterone were used. For the assay of these plasma steroid hormones, plasma samples were extracted with ethyl ether and repeated three times. The extracts were evaporated to dryness.

After blood samples were taken, the embryos or ducklings were killed, and testes or left and right ovaries were removed. The tissue was triturated with 0.25 ml of 0.9% NaCl using a loose-fitted Teflon-glass homogenizer. The homogenate was extracted four-times with ethyl ether and the extracts were evaporated to dryness. The dry extracts of plasma and the tissues were redissolved in 0.2 ml of 1% bovine serum albumin-phosphate buffer saline. The radioimmunoassay of testosterone, estradiol-17β and progesterone were carried out as described previously (Tanabe et al., 1983). All antisera and antibodies used in the radioimmunoassays for steroid hormones and the cross-reactivities of these antisera in this study are the same as those also described in the previous paper (Tanabe et al., 1983). The radioactivity was measured with a liquid scintillation spectrometer (LS9000, Beckman, Calif.).

These data were analyzed using Student's t test, Duncan's new multiple range test and Mann-Whitney U test (Yoshida, 1983).

Results

Changes in plasma concentrations of estradiol-17β, testosterone and progesterone of embryonic and postembryonic ducks are illustrated in figures 1, 2 and 3, respectively. These data were analyzed between sexes using Student's t test. Plasma estradiol-17β concentrations in female embryos were very high at days 11 and 15 of incubation (figure 1). There was a rapid drop at day 19 of incubation, which continued to decrease until day 27 of incubation. The concentration dropped to below the detectable level, and remained low up to 7 days of age. Male plasma estradiol-17β was steady at low levels, being less than about 20 pg/ml throughout the experimental period. Much higher estradiol-17β concentrations in the plasma of female embryos than those of the male embryos were observed in this study. Plasma samples in days 11, 15 and 19 of incubation

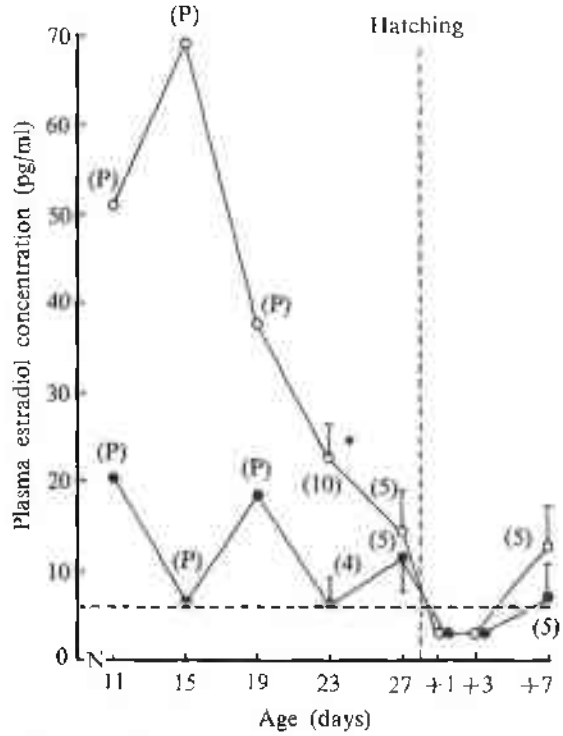

Figure 1. Plasma estradiol-17β concentrations for male (• — •) and female (○ — ○) embryonic and postembryonic ducks. (P) represents pooled samples from 10-15 embryos. Figures in parentheses show number of embryos or birds. Vertical lines represent SEM and horizontal dotted line indicate not detectable level (6.0 pg / ml). * Difference between the sexes is statistically significant at 5% level by Student's t test.

were pooled and each concentration of the steroid hormone was measured, so that statistically significant sex difference was observed only at day 23 of incubation ($p < 0.05$).

Plasma testosterone concentrations were low during the embryonic stage in the male and the female, rapidly increased after hatching, and rose to the peak from 1 to 3 days of age in both sexes (figure 2). No significant sex difference ($p > 0.05$) was observed in testosterone concentration of the plasma, but higher concentration of the hormone in the female than the male was observed throughout the experimental period of this study.

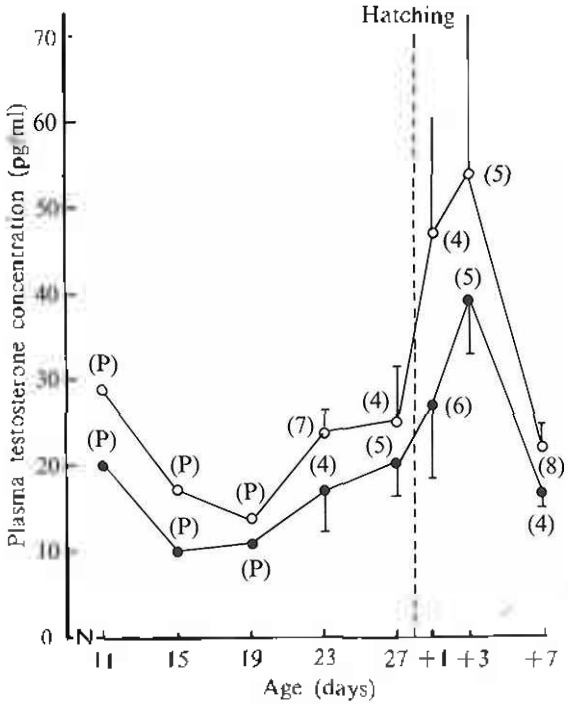

Figure 2. Plasma testosterone concentrations for male (• – •) and female (o – o) embryonic and postembryonic ducks. (P) represents pooled samples from 10-15 embryos. Figures in parentheses show numbers of embryos or birds. Vertical lines represent SEM.

Plasma progesterone concentrations remained at low levels from day 15 to 23 of incubation, but increased rapidly and reached a peak 1 day after hatching, and remained at high levels there after. No significant sex difference was observed in plasma progesterone concentrations (figure 3).

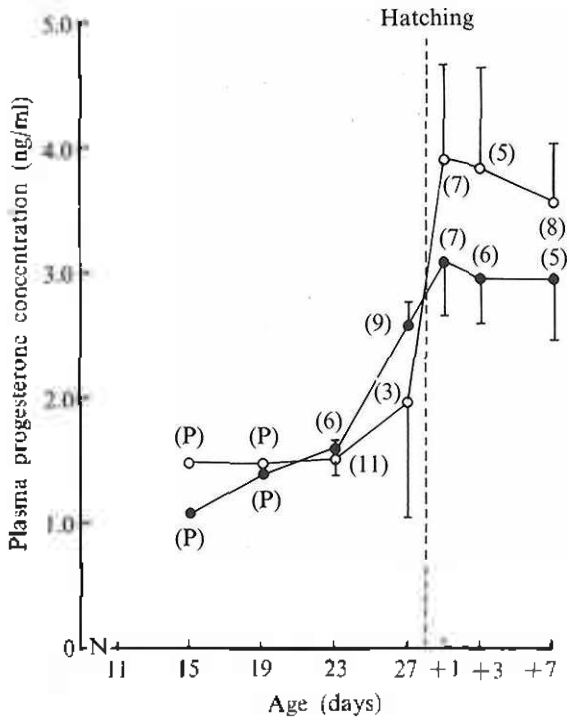

Figure 3. Plasma progesterone concentrations for male (• – •) and female (o – o) embryonic and postembryonic ducks. (P) represents pooled samples from 10-15 embryos. Figures in parentheses show numbers of embryos or birds. Vertical lines represent SEM.

Testicular and ovarian contents of estradiol-17 β, testosterone and progesterone of embryonic and postembryonic ducks are given in table 1, 2 and 3, respectively. These data were analyzed among same ages using Duncan's new multiple range test and Mann-Whitney U test. Estradiol-17 β, testosterone and progesterone contents were almost much higher in the left ovaries of female embryo and duckling than in the right ovary and testes during the embryonic and postembryonic stages. Differences between both sexes were statistically significant ($p < 0.05$) in most cases, except for progesterone content until day 23 of incubation. Estradiol-17 β content of the left ovary increased about 3-fold from day 11 to reach a peak at day 15 of incubation, and decreased gradually after the peak (table 1). The fall con-

tinued until 7 days of age after hatching. In contrast with the left ovary, testicular and right ovarian contents remained at low levels over the time period measured and were less than detectable level (<6 pg/gland) after day 23 of incubation.

TABLE 1. ESTRADIOL-17β CONTENT IN TESTES, LEFT OVARY AND RIGHT OVARY OF EMBRYONIC AND POSTEMBRYONIC DUCKS

Age in days	Estradiol-17β (pg/gland) Testes		Left ovary		Right ovary	
Embryo						
11	138.72 ± 34.01	(18)[a]	362.19 ± 37.61	(15)[b]	58.47 ± 6.13	(15)[a]
15	19.81 ± 1.79	(13)[a]	1,061.23 ± 39.02	(15)[c]	140.75 ± 12.85	(15)[b]
19	16.46 ± 1.00	(17)[a]	742.57 ± 51.43	(14)[b]	68.20 ± 6.49	(14)[a]
23	11.92 ± 1.85	(15)[a]	771.56 ± 59.06	(19)[b]	21.16 ± 3.44	(19)[a]
27	< 6.0*	(8)[a]	507.58 ± 114.25	(6)[b]	< 6.0*	(6)[a]
Duckling						
1	< 6.0*	(8)[a]	251.94 ± 32.02	(7)[b]	< 6.0*	(7)[a]
3	< 6.0*	(7)[a]	94.26 ± 27.43	(8)[b]	< 6.0*	(8)[a]
7	< 6.0*	(5)[a]	107.76 ± 25.64	(7)[b]	< 6.0*	(7)[a]

Values are mean ± SEM. Numbers in parentheses are shown for the samples collected in this study. Within rows (at same age), values with different superscript letters are significantly different ($p < 0.05$) by Duncan's new multiple range test and Mann-Whitney U test.

* Not detectable, being less than 6.0 pg/gland.

Testosterone content of the left ovary remained at low levels until day 23 of incubation (table 2). The levels were higher in the left ovary than in testes and the right ovary. At day 27 of incubation, just before hatching, testosterone content increased markedly, and reached a peak 1 day after hatching. Testosterone contents of the right ovary were low and was less than detectable level (<7.5 pg/gland) until hatching. The level of the right ovary increased a little after

TABLE 2. TESTOSTERONE CONTENT IN TESTES, LEFT OVARY AND RIGHT OVARY OF EMBRYONIC AND POSTEMBRYONIC DUCKS

Age in days	Testosterone (pg/gland) Testes		Left ovary		Right ovary	
Embryo						
11	10.44 ± 1.06	(18)[b]	8.62 ± 0.90	(15)[a]	< 7.5*	(15)[a]
15	11.71 ± 1.51	(13)[b]	16.30 ± 0.76	(15)[c]	< 7.5*	(15)[a]
19	7.75 ± 0.76	(17)[b]	11.32 ± 0.98	(14)[c]	< 7.5*	(14)[a]
23	8.93 ± 1.36	(15)[b]	12.64 ± 0.84	(19)[c]	< 7.5*	(19)[a]
27	9.69 ± 1.37	(8)[b]	20.91 ± 1.77	(6)[c]	< 7.5*	(6)[a]
Duckling						
1	17.54 ± 2.19	(8)[a]	26.64 ± 3.24	(7)[b]	11.24 ± 2.73	(7)[a]
3	8.76 ± 1.58	(7)[a]	19.04 ± 4.55	(8)[a]	12.95 ± 2.95	(8)[a]
7	14.21 ± 3.39	(5)[ab]	17.68 ± 4.38	(7)[b]	< 7.5*	(7)[a]

Values are mean ± SEM. Numbers in parentheses are shown for the samples collected in this study. Within rows (at same age), values with different superscript letters are significantly different ($p < 0.05$) by Duncan's new multiple range test and Mann-Whitney U test.

* Not detectable, being less than 7.5 pg/gland.

hatching, but returned into undetectable levels. In contrast with the ovary, testicular testosterone content was much lower than the left ovary, but was slightly higher than the right ovary. Testicular content was low during the embryonic stage and tended to increase slightly from 1 to 7 day after hatching.

Progesterone contents of the left ovary was low from day 11 to 23 of incubations, but rapidly increased at day 27 of incubation and remained at peak values until 3 days of age. This value decreased markedly at 7 days of age (table 3). In the right ovary, progesterone contents remained at low levels throughout the experimental period, and were much lower compared with the left ovary after hatching ($p < 0.05$). Progesterone contents were higher in the testes than in the ovary at day 11 of incubation. The testicular content decreased gradually until the time of hatching, but rose to a peak at 1 day of age.

TABLE 3. PROGESTERONE CONTENT IN TESTES, LEFT OVARY AND RIGHT OVARY OF EMBRYONIC AND POSTEMBRYONIC DUCKS

Age in days	Progesterone (ng/gland)					
	Testes		Left ovary		Right ovary	
Embryo						
11	1.57 ± 0.06	(18)[b]	0.99 ± 0.03	(15)[a]	1.11 ± 0.12	(15)[a]
15	0.84 ± 0.12	(13)[a]	0.68 ± 0.07	(15)[a]	0.74 ± 0.10	(15)[a]
19	0.58 ± 0.06	(17)[b]	0.39 ± 0.04	(14)[a]	0.46 ± 0.06	(14)[a]
23	0.55 ± 0.12	(15)[a]	0.60 ± 0.10	(19)[a]	0.65 ± 0.07	(19)[a]
27	0.36 ± 0.04	(8)[a]	2.42 ± 0.91	(6)[b]	1.32 ± 0.24	(6)[ab]
Duckling						
1	1.33 ± 0.22	(8)[b]	1.53 ± 0.51	(7)[b]	0.31 ± 0.09	(7)[a]
3	0.32 ± 0.08	(7)[a]	3.04 ± 0.89	(8)[b]	0.77 ± 0.02	(8)[a]
7	0.19 ± 0.04	(5)[a]	1.10 ± 0.30	(7)[b]	0.30 ± 0.12	(7)[ab]

Values are mean ± SEM. Numbers in parentheses are shown for the samples collected in this study. Within rows (at same age), values with different superscript letters are significantly different ($p < 0.05$) by Duncan's new multiple range test and Mann-Whitney U test.

Discussion

The present results demonstrate that the left ovary of embryonic and postembryonic (until 7 days of age) ducks is much more active than the right ovary or the testes in the biosynthesis of sex steroid hormones, such as estradiol-17β, testosterone and progesterone. Previous reports also show that the embryonic ovary of the chicken and the duck are more active than the embryonic testis (Guichard et al., 1977, 1979; Tanabe et al., 1979, 1983). These studies, however, were limited to comparisons between ontogenetic steroidogenesis in the male testis and the female ovary. The present study demonstrates that the capacity of the embryonic left ovary of the duck to synthesis estradiol-17β and testosterone is much higher than the embryonic right ovary and testis. Gonzalez et al. (1987) reported the responsiveness of the two ovaries in the chick embryos from day 13 to 21 of incubation to ovine LH *in vitro*, and suggested that LH stimulation to the left ovary *in vitro* leads to a predominant secretion of progesterone at all times during the embryonic development, and of estradiol-17β except in the last period of incubation, while the right ovary exhibits low sensitivity to LH. Therefore, the report of Gonzalez et al. (1987) strongly supports the view the embryonic left ovary is more active than embryonic right ovary either in chickens or ducks. The present study supports the hypothesis that estradiol-17β secreted from the embryonic ovary at early embryonic stage, such as at day 11 of incubation, has an important role in the female sexual differentiation in the duck. Furthermore, plasma estradiol-17β

concentrations in female embryos at days 11 and 15 of incubation were very high compared with male, whereas differences between the sexes in plasma progesterone and testosterone concentrations. The present study shows that a higher progesterone concentrations in the right embryonic ovary compared to the left ovary or the testes may be due to the low activities of several steroidogenetic enzymes in the right ovary. These results can also be compared to those of Imataka et al. (1988), who reported on the steroidogenesis in the left ovary and the testis of the chick embryo. Tanabe et al. (1986) showed that the male chicken testis after hatching secreted more testosterone than the female chick ovary. This observation in the immature chicken is different from that in the duckling after hatching observed in this study. There is a functional difference in the genital gland of the chicken and the duck after hatching.

The ontogenetic aspects of steroidogenesis in the avian species are quite different from those in the mammalian species. In the guinea pig (Rigaudière, 1977) and the rabbit (George et al., 1978), it has been reported that the fetal testis is more active than the fetal ovary. In the fetal rabbit, the onset of estradiol-17β synthesis by the ovary occurs simultaneously with the onset of testosterone synthesis by the testis, but elevated concentrations of plasma estradiol-17β are not observed in the female embryo. These findings indicate that ovarian estradiol-17β would probably affect locally within the developing ovary and that the endocrine function of the fetal ovary seems to be not essential to the formation of the female phenotype (George et al., 1978). The induction of the development of the female phenotype in the rabbit by the removal of genital glands in both sexes prior to the onset of the phenotypic differentiation was demonstrated by Jost (1972). These reports strongly suggest that the sex of the mammals with male heterozygosity (XX/XY) is basically female which has homozygous sex chromosomes (XX), and the male is the induced sex by androgen secretion in the fetal testis.

In the avian species, it has been known that hens could be masculinized by some experimental procedures. Testicular grafts into the extraembryonic coelom of 3-day-old chicken embryos induces atrophy of the ovaries in both sides and regression of the Müllerian ducts (Sugiyama et al., 1989). Removal of the left ovary within 14 days after hatching causes the masculinization of the right gonad in the chicken (Kagami and Tomita, 1990). When estrogen is administrated to the male embryo of Japanese quail at day 4 of incubation, the left gonad develops an ovary-like structure, but it become testicular tissue showing spermatogenesis in a good many of genital gonad in these adult birds (Ro and Kondo, 1977, 1979). Furthermore, the present results in the duck embryos demonstrate that the capacity of the embryonic left ovary to synthesize estradiol-17β on the day 15 of incubation is more than 100 times that of the embryonic testis. It is strongly suggested that the sex of the avian species is basically male having homozygous sex chromosomes (ZZ).

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