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# Expression of five steroidogenic genes including aromatase gene at early developmental stages of chicken male and female embryos

Osamu Nomura[a, b,*], Osamu Nakabayashi[c], Katsuhiko Nishimori[c], Hiroshi Yasue[a], Shigeki Mizuno[c]

[a]*Department of Animal Breeding and Genetics, National Institute of Animal Industry, Norin Kenkyu-Danchi-Kyoku, Tsukuba, Ibaraki 305-0901, Japan*

[b]*Science and Technology Corporation, Hon-machi 4-1-8-12F, Kawaguchi, Saitama 332, Japan*

[c]*Tohoku University Agricultural Department, Tsutsumidori Amamiya-machi 1-1, Aoba-ku, Sendai 981, Japan*

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**Abstract**

In the course of avian embryo development, estrogen has been indicated to play a key role in gonadal differentiation by the inhibition of aromatase (P-450arom) that synthesizes estrogen from androgen. Biosynthesis of estrogen requires not only P-450arom but also other enzymes for a steroidogenic pathway. To elucidate gonadal differentiation, the steroidogenic pathway should be studied comprehensively in the early developmental stages including that of sex differentiation. Therefore, in the present study, the expressions of the steroidogenic genes, P-450scc, 3β-HSD, P-450c17, 17β-HSD and P-450arom, were measured at the developmental stages (days 2–9 of incubation) of chicken embryos by quantitative RT-PCR. Transcripts for all the genes studied, except for P-450arom were detected in all the developmental stages examined, indicating that mRNAs for the steroidogenic enzymes required to convert cholesterol to androgens are present in the avian embryo before gonadal differentiation. In contrast, P-450arom mRNA was detected in female embryos during days 5–9 of incubation but not in male embryos throughout incubation. The onset of P-450arom gene expression at day 5 coincides with the stage of gonadal differentiation, corroborating the role of estrogen in the process of gonadal differentiation in chicken. 

## 1. Introduction

During bird and mammal development, the genotype of the zygote determines the nature of the gonad, ovary or testis, which then presumably determine the male or female phenotype through secretion of the sex-steroid hormones, estrogen and androgen. In mammals, the male is the heterogametic sex (XY) and the female is homogametic (XX). A gene to initiate male development was isolated in humans and mice, and localized on the Y chromosome [1–3]. The gene was named SRY in humans and *Sry* in mice. In contrast, in birds, the female is the heterogametic sex (ZW) and the male is homogametic (ZZ). Although the W chromosome is presumed to be equivalent to the Y chromosome, it remains unclear whether or not a homologous gene to Sry for sexual differentiation exists.

There are observations suggesting that the mechanism of sex-differentiation in birds is different from that in mammals. For instance, testis grafting to chicken female embryos on day 3 of incubation resulted in sex reversal, though those individuals were infertile [4]. In mammals, to the best of our knowledge, such a phenomenon has not yet been reported. As an another example of the difference between avian and mamma-

* Corresponding author. Tel.: +81-298-388-664; fax: +81-298-388-674.

*E-mail address:* onomura@niai.affrc.go.jp (O. Nomura).

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lian species, treatment of embryos with an inhibitor of aromatase (P-450arom), which synthesizes estrogen from androgen, on days 0, 5 and 7 of incubation resulted in development of the male-type vent sex characteristic in almost all genetic females [5]. The differentiation of testis was observed in some of the treated genetic females. On the other hand, in mice, the disruption of a functional P-450arom gene by gene targeting did not affect ovary differentiation [6]. Observations of the P-450arom inhibitor on chicken embryos indicated that an early exposure to estrogen is crucial for the sexual differentiation of the gonad in avian species, and that P-450arom is a key enzyme in that process.

Since estrogen synthesis requires not only P-450arom but also the other steroidogenic enzymes, it is important to clarify the expression of a steroidogenic pathway in embryos for elucidation of the roles of sex-steroids in the gonad. Although various steroids and steroidogenic enzymes have been measured in avian embryos [7,8], a quantitative and comprehensive analysis of the expression of the steroidogenic genes in the course of gonad differentiation in avian embryos has not yet been reported.

In the present study, we examined the expression of the genes for five steroidogenic enzymes, i.e. cholesterol side chain cleavage (P-450scc)[9], 3β-hydroxysteroid dehydrogenase (3β-HSD)[10], steroid 17α-hydroxylase/$C_{17-20}$ lyase (P-450c17)[11], 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD)[12] and aromatase (P-450arom)[13] in male and female chicken embryos on days 2–9 of incubation using a quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) [14]. It was suggested that male and female embryos had the capacity for androgen synthesis as early as day 2 of incubation, which is much earlier than the onset of the differentiation of gonads (days 5–7 of incubation [15–17]). In addition, female embryos at a stage as early as day 5 were indicated to be capable of estrogen production by P-450arom, whereas male embryos appeared to be incapable of estrogen production in any stage examined.

## 2. Materials and methods

### 2.1. Sexing of chicken embryos

Extra-embryonic membranes were separated from individual embryos and used for the preparation of genomic DNA. The genomic DNAs were prepared with proteinase K/phenol–chloroform treatment, following the procedure described by Maniatis [18]. The DNAs thus obtained were slot-blotted in duplicate on a nylon membrane (GeneScreen, NEN, USA), then hybridized with W chromosome-specific probe DNA.

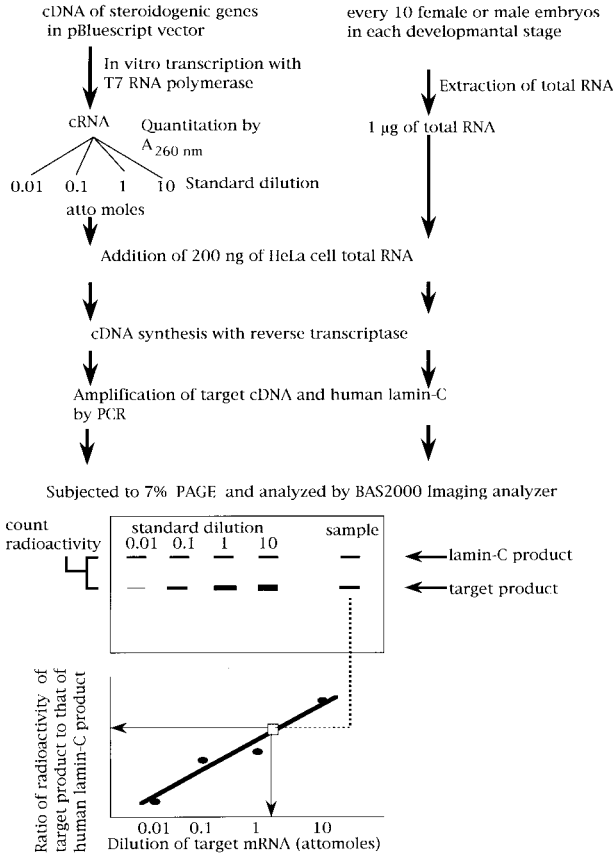


Fig. 1. Schematic presentation of quantitative RT-PCR for stoichiometric analysis. PCR primers used in this analysis were described in our previous study [14].

For the probe DNA, a 0.7 kbp fragment of the *Xho*I-family derived from the chicken W chromosome [19] was labeled with digoxigenin using a nonradioactive DNA Labeling and Detection Kit (Boehringer Mannheim, Germany). The resulting membrane was treated, following the instructions of the manufacturer, to visualize the probe DNA hybridized to DNA slot blots on the membrane.

### 2.2. Preparation of total RNA from embryos and cultured cells for RT-PCR assay

After the sexing of each embryo using extra-embryonic membrane, 10 embryos of the same gender were pooled to make one sample, and three samples were prepared for each gender at each developmental stage, i.e. from days 2 to 9 of incubation. Ten embryos in a pool were homogenized together in the presence of 4 M guanidinium thiocyanate and subjected to CsCl centrifugation to prepare an RNA sample [18]. For controls in the stoichiometric analysis described below, RNAs of HeLa cells and MSB-1 cells (a Marek virus-transformed chicken cell line) [20] were prepared exactly as noted above. In order to check the degra-

dation of RNA in the course of RNA preparation, the RNA samples thus prepared were subjected to Northern blot analysis using a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) [21] cDNA fragment as a probe (data not shown).

### 2.3. RT-PCR system for the stoichiometric analysis of gene expression

The RT-PCR system developed in our previous study [14] was employed to determine the amount of transcript from genes in RNA samples. The flow of the system was summarized in Fig. 1. Briefly, reverse transcription was first performed using a random hexamer (Pharmacia Biotech, Uppsala, Sweden) in all the samples including the controls that contained predetermined amounts of respective transcripts (standard cRNA). The samples were then supplemented with primer-sets of the respective genes, and subjected to PCR for appropriate cycles in the presence of dNTP containing [$\alpha$–$^{32}$P] dCTP (Amersham, Bucks., UK). Subsequently, the primer-set for amplification of lamin-C [22] cDNA (derived from the HeLa RNA) was added to all the samples, which were then subjected to additional PCR under the same conditions as above. After PCR, the samples were electrophoresed in polyacrylamide gel, and the radioactivity of PCR products in the gel was determined using a BioImage analyzer (BAS 2000; Fuji Film, Tokyo, Japan). For each gene transcript, the ratios of radioactivity derived from standard cRNAs to those derived from lamin-C RNA were plotted against the amount of cRNA in the standard samples (see Fig. 1). The ratios of experimental samples were interpolated into the plots of corresponding standard samples to determine the amounts of respective transcripts in the experimental samples.

## 3. Results

In an earlier study, P-450arom, one of the steroidogenic enzymes, has been strongly indicated to be involved in gonad differentiation from the fact that P-450arom inhibitor interfered with the gonad differentiation in female chicken embryos [5]. This fact further indicated that steroidogenic enzymes other than P-450arom were also involved, though no expression of steroidogenic genes nor any activity of steroidogenic enzymes have been studied comprehensively in avian embryos. In our previous study, we developed a sensitive method to measure the level of steroidogenic gene expression [14]. Therefore, in the present study, employing this method (Fig. 1), we attempted to detect the transcripts from the steroidogenic genes in the early stages of chicken embryonic development.

For the present analysis, we first determined the gender of chicken embryos by slot-blot hybridization using a fragment of the *Xho*I family which is specific for the W chromosome, as a probe. Based on the results shown in Fig. 2, embryos presenting hybridization signals were judged as female; and those presenting no signals as male. To make sure that slot-blots showing no signals contained the same amount of DNA as those showing signals, the slot-blots were hybridized with $^{32}$P-labeled chicken whole genomic DNA as a probe. The hybridization revealed that all the slot-blots contained almost equal amount of DNA, supporting the above judgement (data not shown). Then, 10 embryos of the same gender at the same developmental stage were pooled to make one sample,

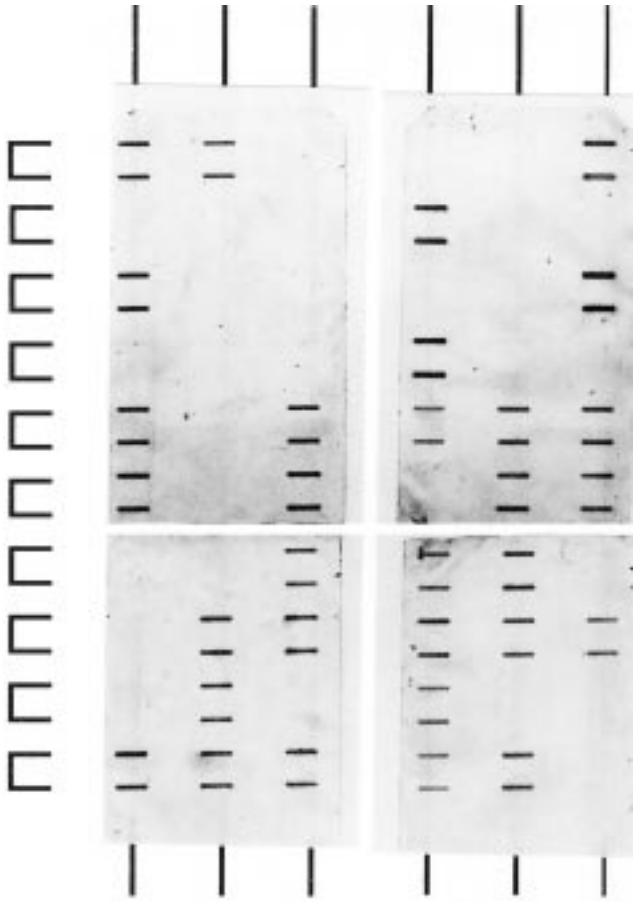

Fig. 2. Slot-blot analysis of embryo sexing using *Xho*I family sequence as a probe. Extra-embryonic membranes were separated from individual embryos and used for preparation of genomic DNA. Approximately 100 ng of the genomic DNAs prepared as described in Materials and methods were slot-blotted in duplicate on a nylon membrane (GeneScreen, NEN, USA), then hybridized with a digoxigenin-labeled 0.7-kbp fragment of the *Xho*I-family specific for chicken W chromosome [19]. After hybridization, the probe DNA hybridized to the DNAs on the membrane was visualized using mouse anti-digoxigenin/alkaline phosphatase-labeled goat anti-mouse IgG system (non-radioactive DNA labeling and detection kit; Boehringer Mannheim, Germany). The vertical lines show the positions of DNA blotting; and the brackets denote duplicate of the samples in the blotting.

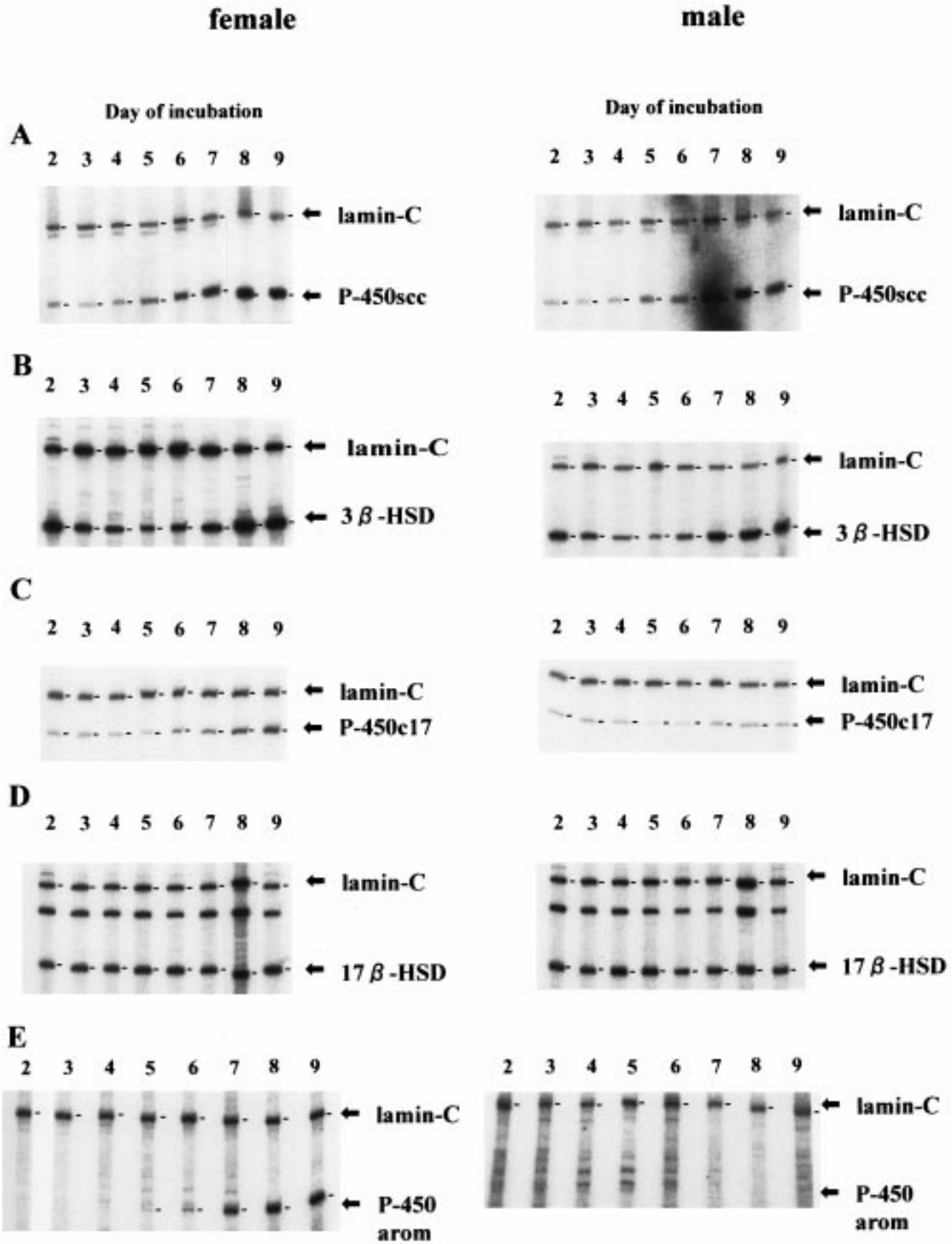


Fig. 3. Polyacrylamide gel electrophoresis of PCR products amplified from mRNAs of human lamin-C and five steroidogenic genes. The radioactivities of PCR products were visualized by BAS2000. Panel A was for the transcript of P-450scc gene; panel B, 3β-HSD gene; panel C, P-450c17 gene; panel D, 17β-HSD gene and panel E, P-450arom gene.

Table 1
The mRNA amounts of five steroidogenic genes in chicken embryos (ND: not detected)

| | | Day of incubation (atto mol/μg total RNA) | | | | | | | | Quantitation range (atto mol) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | |
| P450scc | ♀ | 1.1 ± 0.17 | 0.90 ± 0.30 | 2.2 ± 0.67 | 3.9 ± 0.26 | 7.0 ± 2.3 | 15 ± 1.8 | 25 ± 2.2 | 30 ± 6.7 | 0.05–10 |
| | ♂ | 1.1 ± 0.058 | 1.3 ± 0.37 | 2.3 ± 0.84 | 4.4 ± 0.72 | 10.1 ± 3.3 | 15.2 ± 2.6 | 20 ± 2.8 | 19 ± 4.7 | |
| 3β-HSD | ♀ | 41 ± 5.2 | 9.4 ± 2.0 | 4.2 ± 1.8 | 5.5 ± 0.91 | 13 ± 3.6 | 21 ± 7.3 | 53 ± 28 | 57 ± 22 | 0.05–10 |
| | ♂ | 40 ± 7.3 | 10.8 ± 3.8 | 6.9 ± 3.3 | 5.0 ± 0.52 | 19 ± 0.8 | 37 ± 23 | 58 ± 31 | 45 ± 16 | |
| P-450c17 | ♀ | 0.30 ± 0.05 | 0.24 ± 0.08 | 0.42 ± 0.15 | 0.11 ± 0.02 | 0.60 ± 0.19 | 0.87 ± 0.16 | 1.4 ± 0.31 | 3.0 ± 0.38 | 0.001–10 |
| | ♂ | 0.32 ± 0.02 | 0.21 ± 0.02 | 0.13 ± 0.02 | 0.30 ± 0.14 | 1.1 ± 0.42 | 0.62 ± 0.21 | 0.38 ± 0.04 | 0.33 ± 0.042 | |
| 17β-HSD | ♀ | 3.2 ± 1.2 | 2.5 ± 0.073 | 2.3 ± 0.99 | 2.6 ± 0.75 | 2.5 ± 1.1 | 3.4 ± 1.3 | 4.2 ± 0.94 | 5.6 ± 1.1 | 0.05–10 |
| | ♂ | 2.3 ± 0.12 | 1.9 ± 0.52 | 2.0 ± 0.80 | 2.7 ± 0.1 | 2.4 ± 0.24 | 2.8 ± 1.4 | 3.0 ± 0.73 | 3.3 ± 0.70 | |
| P-450arom | ♀ | ND | ND | ND | 0.007 ± 0.003 | 0.19 ± 0.10 | 3.1 ± 0.33 | 8.9 ± 0.93 | 9.8 ± 0.43 | 0.01–5 |
| | ♂ | ND | ND | ND | ND | ND | ND | ND | ND | |

and three samples were prepared for each gender at each developmental stage, i.e. from days 2 to 9 of incubation. All the samples were processed as described in Materials and methods, to determine the amounts of transcripts from five steroidogenic genes, i.e. P-450scc, 3β-HSD, P-450c17, 17β-HSD, and P-450arom, in the samples. As a control, we first examined whether or not fragments were produced in the PCR using the primer-set for human lamin-C cDNA and using chicken embryo samples as a template. The examination revealed that no DNA fragments were produced in the system (data not shown), indicating that the procedure was capable of measuring the amounts of each transcript accurately.

The PCR-amplified fragments were electrophoresed, and the radioactivities of the fragments were detected by the BAS2000 at positions corresponding to those from respective transcripts of the genes [14] (Fig. 3), indicating that those fragments were derived from those transcripts. The amplified fragments in day 9 samples were eluted from the gel, cloned, and sequenced to confirm the gene identity. The sequence analysis demonstrated that they were derived from transcripts of the respective genes (data not shown).

As shown in Fig. 3 (panels A, B, C and D), all the steroidogenic genes except P-450arom were expressed in all stages examined in both male and female embryos. The P-450arom transcript was detected from day 5 through day 9 of incubation in females, whereas no transcript was found in males throughout the incubation period (Fig. 3 panel E). The amounts of transcripts from the respective genes were calculated as depicted in Fig. 1. When the amount value obtained was higher than the upper limit of the stoichiometric range (see Table 1), the sample was diluted to present a value within that range. In Table 1, the mean value of three samples of either gender at the same stage is presented with the standard error as a value of atto mol per 1 μg of total RNA. For P-450scc gene, the amount of the transcript increased in the course of incubation with no difference between the sexes. For 3β-HSD gene, the amount of the transcript decreased from days 2 to 5 of incubation, then increased after day 6 with no difference between the sexes. For P-450c17 gene, the amount of the transcript increased sharply after day 6 of incubation in female but not in male embryos. For 17β-HSD gene, the amount of the transcript appeared to show no significant difference either in the course of incubation or between the sexes. No transcript was found for P450arom gene throughout the incubation period in male embryos, whereas in female embryos the transcript was first detected on day 5 of incubation and sharply increased thereafter.

## 4. Discussion

In the present study, using the quantitative RT-PCR system developed in our previous study [14], we examined whether the five steroidogenic genes essential to sex differentiation were expressed at the stages before gonadal differentiation during embryonic development in chicken. The present study first revealed that P-450scc, 3β-HSD, P-450c17 and 17β-HSD genes were expressed in male and female embryos as early as day 2 to the end of incubation on day 9. The P-450arom transcript was found as early as day 5 through day 9 in females, whereas no transcript was found in male throughout the incubation period. These findings indicate that male and female embryos are capable of androgen synthesis as early as day 2 of incubation, which is much earlier than the onset of the differentiation of gonads (days 5–7 of incubation [15–17]); and that estrogen production by P-450arom may occur in female embryos as early as day 5 of incubation but not in male embryos at any stage examined.

Treatment of chicken embryos with an inhibitor of P-450arom on days 0, 5 and 7 of incubation resulted

in development of the male-type vent sex characteristic in almost all genetic females, revealing that P-450arom plays a key role in avian gonadal differentiation [5]. Since the morphological differentiation of the gonad into ovary or testis was shown to begin between days 5 and 7 of incubation [15–17], it was examined in subsequent studies whether P-450arom and esterogen receptor genes were expressed before the stage of gonad differentiation [23–25]. Those studies revealed that expressions of P-450arom and estrogen receptor genes were detected as early as days $6\frac{1}{2}$ and $3\frac{1}{2}$ of chicken embryo incubation, respectively. These findings together with our findings in the present study provide strong supporting evidence for the fact that the P-450arom and estrogen play a key role in gonad differentiation in avian embryo development.

In previous studies [23–26], RT-PCR and in situ hybridization on gonads detected the transcripts from P-450scc, 3β-HSD, P-450c17 and P-450arom genes at 5–7 days of incubation in chicken embryos. Although expression of the genes was first detected in earlier developmental stages in the present study, the present observations are essentially consistent with those in the previous studies. The detection of the gene expressions at an earlier stage in the present study may be due to the high detection sensitivity of the method used and/or to production of the transcripts in the tissues other than the gonad [14]. Nakabayashi et al. [25] detected 17β-HSD transcript in the ovary but not in the testis on day 7 of incubation. When this finding is compared to the amount of 17β-HSD transcript detected in the present study, it may indicate that 17β-HSD gene is expressed in tissues other than the testis [15].

The transcripts from P-450scc, 3β-HSD, P-450c17 and 17β-HSD genes were detected much earlier than gonadal differentiation, i.e. day 2 of incubation, though the activities of the gene products have not yet been confirmed in the early developmental stages. However, one can infer that these enzymes may play an additional role in gonadal differentiation during chicken embryonic development.

In mammals, there are at least seven types of 17β-HSD with different substrate specificities [27–33], and 17β-HSD type 3 is indicated to be involved in gonadal differentiation based on its expression site [29]. The chicken 17β-HSD type 1 showing the substrate specificity as same as that of mammalian type 1 was reported to express in the chicken ovary on day 7 of incubation [25 and unpublished data]. In addition, as pointed out earlier, the mechanism of sex-differentiation in avian species is different from that in mammalian. These facts taken together, the data presented here suggest that the 17β-HSD type 1 is involved in the process of the sex-differentiation. Alternatively, the hypothetical 17β-HSD type 3 may also be involved in the process of gonadal differentiation in chicken.

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## References

[1] A.H. Sinclair, P. Berta, M.S. Palmer, J.R. Hawkins, B.L. Griffiths, M.J. Smith, J.W. Foster, A.M. Frischauf, R. Lovell-Badge, P.N. Goodfellow, A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif, Nature 346 (1990) 240–244.
[2] J. Gubbay, J. Collignan, P. Koopman, B. Capal, A. Economou, A. Münsterberg, N. Vivian, P. Goodfellow, R. Lovell-Badge, A gene mapping to the sex-determining region of the mouse Y chromosome is a member of a novel family of embryonically expressed genes, Nature 346 (1990) 245–250.
[3] P. Koopman, J. Gubbay, N. Vivian, P.N. Goodfellow, R. Lovell-Badge, Male development of chromosomally female mice transgenic for *Sry*, Nature 351 (1991) 117–121.
[4] M. Rashedi, R. Maraud, R. Stoll, Development of the testis in female domestic fowls submitted to an experimental sex reversal during embryonic life, Biol. Reprod. 29 (1983) 1221–1227.
[5] A. Elbrecht, R.G. Smith, Aromatase enzyme activity and sex determination in chickens, Science 255 (1992) 464–470.
[6] C.R. Fisher, K.H. Graves, A.F. Parlow, E.R. Simpson, Characterization of mice deficient in aromatase (ArKO) because of targeted disruption of the cyp19 gene, Proc. Natl. Acad. Sci. USA 95 (1998) 6965–6970.
[7] Y. Tanabe, N. Saito, T. Nakamura, Ontogenetic steroidogenesis by testis, ovary and adrenals of embryonic and postembryonic chickens (*Gallus domesticus*), Gen. Comp. Endocrinol. 63 (1986) 456–463.
[8] H. Imataka, K. Suzuki, H. Inano, K. Kohmoto, B. Tamaoki, Sexual differences of steroidogenic enzymes in embryonic gonads of the chicken (*Gallus domesticus*), Gen. Comp. Endocrinol. 69 (1988) 153–162.
[9] O. Nomura, O. Nakabayashi, K. Nishimori, S. Mizuno, The cDNA cloning and its transient expression of a chicken gene encoding cytochrome P-450scc, Gene 185 (1997) 217–222.
[10] O. Nakabayashi, O. Nomura, K. Nishimori, S. Mizuno, The cDNA cloning and transient expression of a chicken gene encoding a 3β-hydroxysteroid dehydrogenase/$\Delta^5$-$\Delta^4$ isomerase unique to major steroidogenic tissues, Gene 162 (1995) 261–265.
[11] H. Ono, M. Iwasaki, N. Sakamoto, S. Mizuno, cDNA cloning and sequence analysis of a chicken gene expressed during the gonadal development and homologous to mammalian cytochrome P-450c17, Gene 66 (1988) 77–85.
[12] Y. Wajima, S. Kawauchi, T. Furusawa, N. Wakabayashi, O. Nakabayashi, K. Nishimori, S. Mizuno, DDBJ accession No. AB002410.
[13] M.J. McPhaul, J.F. Noble, E.R. Simpson, C.R. Mendelson, J.D. Wilson, The expression of a functional cDNA encoding the chicken cytochrome P-450arom (aromatase) that catalyzes the formation of estrogen from androgen, J. Biol. Chem. 31 (1988) 16358–16363.
[14] O. Nomura, K. Nishimori, O. Nakabayashi, H. Yasue, S. Mizuno, Determination by modified RT-PCR of transcript amounts from genes involved in sex-steroid synthesis in chicken organs including brain. J. Steroid Biochem. Mol. Biol. (1998).
[15] A.L. Romanoff, The avian embryo, The Macmillan Company, New York, 1960.

[16] M.H. Thorne, Genetics of poultry reproduction, in: P. Hunton (Ed.), Poultry production, Elsevier, Amsterdam, 1995.
[17] K. Haffen, Sex differentiation of avian gonads in vitro, Am. Zool. 15 (1975) 257–272.
[18] J. Sambrook, E.F. Fritsch, T. Maniatis, Molecular cloning: a laboratory manual, Cold Spring Harbor Laboratory Press, 1989.
[19] M. Tone, N. Nakano, E. Takao, S. Narisawa, S. Mizuno, Demonstration of W chromosome-specific repetitive DNA sequences in the domestic fowl, Gallus g. domesticus, Chromosoma 86 (1982) 551–569.
[20] Y. Akiyama, S. Koto, Two cell lines from lymphomas of Marek's disease, Biken J. 17 (1974) 105–116.
[21] F. Panabieres, M. Piechaczyk, B. Rainer, C. Dani, P. Fort, S. Riaad, L. Marty, L. Imbach, P. Jeanteur, J.-M.M. Blanchard, Complete nucleotide sequence of the messenger RNA coding for chicken muscle glyceraldehyde-3-phosphate dehydrogenase, Biochem. Biophys. Res. Commun. 118 (1984) 767–773.
[22] Z.D. Fisher, N. Chaudhary, G. Blobel, cDNA sequencing of nuclear lamins A and C reveals primary and secondary structural homology to intermediate filament proteins, Proc. Natl. Acad. Sci. USA. 83 (1986) 6450–6454.
[23] J.E. Andrews, C.A. Smith, A.H. Sinclair, Sites of estrogen receptor and aromatase expression in the chicken embryo, Gen. Comp. Endocrinol. 108 (1997) 182–190.
[24] C.A. Smith, J.E. Andrews, A.H. Sinclair, Gonadal sex differentiation in chicken embryos: expression of estrogen receptor and aromatase genes, J. Steroid Biochem. Mol. Biol. 60 (1997) 295–302.
[25] O. Nakabayashi, H. Kikuchi, T. Kikuchi, S. Mizuno, Differential expression of genes for aromatase and estrogen receptor during the gonadal development in chicken embryos, J. Mol. Endocrinol. 20 (1998) 193–202.
[26] K. Yoshida, K. Shimada, N. Saito, Expression of P-450$_{17\alpha}$ hydroxylase and P-450 aromatase genes in the chicken gonad before and after sexual differentiation, Gen. Comp. Endocrinol. 102 (1996) 233–240.
[27] H. Peltoketo, V. Isomaa, O. Mäentausta, R. Vihko, Complete amini acid sequence of human placental 17β-hydroxysteriud dehydrogenase deduced from cDNA, FEBS Lett. 239 (1988) 73–77.
[28] C. Martel, E. Rheaume, M. Takahashi, C. Trudel, J. Couét, V. Luu-The, J. Simard, F. Labrie, Distibution of 17β-hydroxysteriud dehydrogenase gene expression and activity in rat and uman tissues, J. Steroid Biochem. Molec. Biol. 41 (1992) 597–603.
[29] W.M. Geissler, D.L. Davis, L. Wu, K.D. Bradshaw, S. Patel, B.B. Mendonca, K.K. Elliston, J.D. Wilson, D.W. Russel, S. Andersson, Male pseudohermaphroditism caused by mutaions of testicular 17β-hydrixysteriud dehydrigenase 3, Nat. Gen. 7 (1994) 34–39.
[30] J. Adamski, T. Normand, F. Leenders, D. Monte, A. Begue, D. Stéhelin, P. Jungblut, Y. Launoit, Molecular cloning of a novel widely expressed human 80 kDa 17β-hydroxysteriud dehydrogenase IV, Biochem. J. 311 (1995) 437–443.
[31] Y. Deyashiki, K. Ohshima, M. Nakanishi, K. Sato, K. Matsuura, A. Hara, Molecular cloning and characterization of mouse estradiol 17β-dehydrogenase (A-specific), a member of the aldoketoreductase family, J. Biol. Chem. 270 (1995) 10461–10467.
[32] M.G. Biswas, D.W. Russel, Expression cloning and characterization of oxidative 17beta- and 3alpha-hydroxysteroid dehydrogenase from rat and human prostate, J. Biol. Chem. 272 (1997) 15959–15966.
[33] P. Nokelainen, H. Peltoketo, R. Vihko, P. Vihko, Expression cloning of a novel estrogenic mouse 17beta-hydroxysteroid dehydrogenase/17-ketosteroid reductase (m17HSD7), previously described as a prolactin receptor-associated protein (PRAP) in rat, Mol. Endocrinol. 12 (1998) 1048–1059.