**Research Note**

Expression of P450arom, AMH and ERα mRNA in Gonads of Turkey, Duck and Goose within One Week of Age

Naomi Koba, Toshimichi Ohfuji, Yonju Ha, Shusei Mizushima, Akira Tsukada, Noboru Saito and Kiyoshi Shimada

Laboratory of Animal Physiology, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Aichi 464-8601, Japan

Although many studies have shown that P450aromatase (P450arom), anti-Müllerian hormone (AMH) and estrogen receptor α (ERα) play pivotal roles in sexual differentiation of the gonads during early embryonic development in chickens and quail, few studies have been reported for other domestic birds. Furthermore, little information is available in relation to the mRNA expression in gonads after sexual differentiation in posthatching birds. The present study was conducted to assay mRNA expression of P450arom, AMH and ERα in gonads at day of hatch in turkey and duck and 2 days after hatching in goose using the real-time PCR. The mRNA expression was also determined at one week of age in gonads of these birds. At the time of collection of the left gonads for total RNA extraction, the external appearance of the left and right gonads of male and female was documented by digital camera. Clear asymmetry was observed in the female ovary in which the right ovary was regressed completely by days in posthatching turkey, duck and goose. Although gonadal asymmetry was as remarkable as in females, the left testis was larger than the right one in males. Remarkable expression of P450arom mRNA was observed only in females in all the species but substantially no expression was detected in males. Significantly higher expression of AMH mRNA was detected in males than females only in goose but there was no sex difference in turkey and duck at 1–2 days posthatching and one week of age. Weak ERα mRNA expression without a sex difference was detected in the 3 birds. These results suggest that estrogen plays a key role for ovarian development via P450arom mRNA expression after hatching, whereas absence of its expression in males leads to testis development in turkey, duck and goose.

**Key words:** AMH, duck gonad, goose, P450arom mRNA, turkey


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**Introduction**

Although details of the mechanism of sexual differentiation in birds still remains unclear, downstream gene expression has been elucidated (Shimada, 2002; Shimada et al., 2007; Smith et al., 2007). Namely, in females, P450 aromatase (P450arom) may be involved because of its exclusive mRNA expression in the female gonad in association with the beginning of ovary formation around at 6–7 days of incubation but no expression in male gonad in the chicken (Yoshida et al., 1996; Andrews et al., 1997; Smith et al., 1997; Nakabayashi et al., 1998; Shimada, 1998; Nomura et al., 1999; Nishikimi et al., 2000; Vaillant et al., 2001; Akazome et al., 2002; Yamamoto et al., 2003). In contrast, in males AMH may be involved because of its higher mRNA expression in the male gonad in association with the beginning of testis formation but lower expression in female gonad (Carre-Eusebe et al., 1996; Oreá et al., 1998; Nishikimi et al., 2000).

The above information has been obtained from studies in relation to embryogenesis during the incubation period in the chicken but may not be necessarily true for quail and duck because of the lack of sex-specific mRNA expression of these genes (Koba et al., 2008b). Furthermore, little is known about gene expression in gonadal development after hatching in birds including these poultry species except for chicken. In the chicken, high levels P450arom mRNA were maintained at 7 days after hatching and about 10 months of age (Yoshida et al., 1996; Abinawanto et al., 1997a, b) and AMH persists in the sexually mature gonads of both laying hens and cockerels long after the Müllerian duct has regressed, suggesting a wider role for this substance in reproductive physiology (Hutson et al., 1981).

Therefore, the present study was conducted to elucidate mRNA expression of P450arom, AMH and ERα in turkey, duck and goose at 1 or 2 days after hatching and 1 week of age to compare the expression of mRNA.
between these birds.

**Materials and Methods**

**Birds and Tissue Collection**

All procedures for the use and the care of animals were conducted after approval by the Institutional Animal Care and Use Committee of Nagoya University.

Commercial turkey poults, ducklings and goslings were shipped at hatching day after sex sorting from hatchery companies. The strains of these birds were Haydn and Bronze for turkey (Fujisaki Hatchery, Aomori, Japan), Osaka duck for duck (Takahashi Hatchery, Osaka, Japan) and Toulouse for goose (Japan Foie gras, Inc., Aomori, Japan), respectively. Twenty six of 45 turkey poults, 17 of 35 ducklings and 10 of 22 goslings were sacrificed on the arrival day. Goslings’ arrival was delayed due to an unexpected transportation incidence by one day. The left gonads were collected for RNA extraction. The remaining birds were raised up to 1 week of age with water and feed *ad libitum* at 35°C in the brooder and sacrificed to collect the left gonads. Digital images of the external appearance of the gonads were taken at the time of collection (Olympus Co., Tokyo, Japan). The areas of the left and right gonads were determined using the NIH-Image analysis program for assessment of asymmetry or symmetry of the gonad.

**Sequencing of Partial cDNA**

Total RNA was extracted from the individual left gonads of respective bird species. Reverse transcription was performed with each amount of the total RNA, 0.08, 0.1 and 0.05 μg for turkey, duck and goose, respectively using Power Script™ Reverse Transcriptase (BD Biosciences Clontech, Tokyo, Japan) in 10 μl mixture according to the manufacturer’s protocol. Based on the nucleotide sequence of the chicken cDNA previously registered with the GenBank, primer pairs were designed to amplify each cDNA as a single PCR product. The sequence of the PCR primers were listed in Table 1. The methods for PCR amplification, cloning and sequencing the cDNA fragments of P450arom, AMH and ERα were essentially same as the previously reported (Koba et al., 2008a, b).

**Real-Time PCR for mRNA Expression Level**

As stated above, the cloned cDNA was used for the assay of the mRNA expression of P450arom, AMH and ERα in the individual gonads by relative quantitative RT-PCR, using real-time PCR. The real-time PCR was carried out as described previously (Koba et al., 2008b).

**Statistical Analysis**

Data were compared using two-way ANOVA and Bonferroni post-test. *P* < 0.05 was considered statistically significant. Statistical analysis was performed using GraphPad Prism 4 (GraphPad Software Inc., San Diego, USA).

**Results**

**External Appearance of Gonads of Turkey, Duck and Goose**

Figure 1 shows external appearance of the gonads of turkey, duck and goose at 1–2 days and 1 week after hatching. At 1–2 days after hatching, the morphology of male gonads showed bilateral testes with a larger size for the left one, whereas in females only the left ovaries were observed in turkey, duck and goose. At one week of age, males and females had gonads as large as about 1.5 times in comparison to those at 1–2 days after hatching in each species, respectively. Figure 2 shows size of male gonads as area (mm²) and Table 2 shows ratio (%) of right gonad area relative to the left one. The ratios of male gonads in turkey, duck and goose were 69, 76 and 52% at 1–2 days after hatch, and then 57, 68 and 48% at 1 week of age, respectively. In brief, asymmetry of the testes similar to that at 1–2 days posthatching was also observed at 1 week of age in each bird. Again, only the left ovaries were observed in female turkey, duck and goose at 1 week of age.

**Sequence of cDNA Fragments of P450arom, AMH and ERα of Turkey, Duck and Goose**

Figures 3A, B, C and D show the nucleotide sequences

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**Table 1. Primer pairs used for RT-PCR**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Location</th>
<th>Sequence (5’→3’)</th>
<th>GenBank accession number</th>
</tr>
</thead>
<tbody>
<tr>
<td>P450arom (A)</td>
<td>1195–1214, 1477–1496</td>
<td>CAG CCA GTT GTG GAC TTA AT CCT CTTC TCC TTC ATT GTC TG</td>
<td>NM00100176</td>
</tr>
<tr>
<td>P450arom (B)</td>
<td>260–290, 501–530</td>
<td>AAT TGG GCC TCT CAT TTC ACA TGG GAG ATT CGG ATC TCC TTC CAG TGT GCT GGG TTG TTA</td>
<td>NM00100176</td>
</tr>
<tr>
<td>AMH</td>
<td>1034–1053, 1321–1340</td>
<td>GTG GAT GTG GCT CCC TAC CC GCA GCA CCG AGG GCT CCT CC</td>
<td>NM205030</td>
</tr>
<tr>
<td>ERα</td>
<td>1522–1542, 1801–1821</td>
<td>GTG CCT TAA GTC CAT CAT CCT GCG TCC AGC ATC TCC AGT AAG</td>
<td>X03805</td>
</tr>
<tr>
<td>S17</td>
<td>18–38, 145–170</td>
<td>GCC GCG GGT GAT CAT CGA GAA CGC TGG ATG CGC TTC ATC AGG TGG GT</td>
<td>X07257</td>
</tr>
</tbody>
</table>
Fig. 1. Gonads of male and female turkey, duck and goose at 1–2 days and 1 week after hatching. Upper photos of (A): Male gonads of turkey, duck and goose at 1–2 days after hatching. Lower photos of (A): Female gonads of turkey, duck and goose at 1–2 days after hatching. Upper photos of (B): Male gonads of turkey, duck and goose at 1 week of age. Lower photos of (B): Female gonads of turkey, duck and goose at 1 week of age. Arrows indicate right (R) and left (L) gonads in each photo. Scale bar = 2 mm.
of the amplified regions of P450arom (A, 1215–1476, Table 1), P450arom (B, 291–500, Table 1), AMH (C) and ERα (D) in gonads of turkey, duck, and goose. The predicted sizes of PCR products of individual genes were 262, 210, 267 and 258 bp, respectively. Due to different efficiencies of amplification of P450arom cDNA, P450arom (A) was not used but P450arom (B) was used for duck P450arom mRNA assay. The homology of nucleotide sequences of turkey and goose P450arom (A) was 97% and 95%, and duck P450arom (B) was 95% in relation to that of chicken. The nucleotide sequences of turkey, duck, and goose AMH exhibits 97%, 84% and 85% homology in relation to that of chicken. The homology of nucleotide sequences of turkey, duck, and goose ERα was 96%, 93% and 92% in relation to that of chicken.

**P450arom mRNA Expression**

Marked expression of P450arom mRNA at day 1–2 and 1 week of age was observed in only female gonads of turkey, duck, and goose (Fig. 4), but the expression was not detected in males of any species.

**AMH mRNA Expression**

Figure 5 shows AMH mRNA levels at day 1–2 and 1 week of age in male and female gonads of turkey, duck, and goose. There was no significant difference between males and females in turkey and duck at day 1. In

**Fig. 2.** Size measurements in mm² of left (L) and right (R) gonads of males at 1–2 days after hatching and at 1 week of age. Data are represented as mean ± SEM (3–5 turkeys, 3–4 ducks, and 4 geese, respectively). Different superscripts indicate statistically significant difference each other (P < 0.05).

**Table 2.** Ratio of areas between left and right of male gonads

<table>
<thead>
<tr>
<th></th>
<th>Turkey</th>
<th>Duck</th>
<th>Goose</th>
</tr>
</thead>
<tbody>
<tr>
<td>After hatch</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1–2</td>
<td>69 ± 9</td>
<td>76 ± 3</td>
<td>52 ± 7</td>
</tr>
<tr>
<td>Week 1</td>
<td>57 ± 4</td>
<td>68 ± 4</td>
<td>48 ± 8</td>
</tr>
</tbody>
</table>

Each value is shown as a ratio of right/left (%). Data are represented as mean ± SEM (3–5 turkeys, 3–4 ducks and 4 geese, respectively).

**Fig. 3.** Alignment of partial cDNA sequence of P450arom (A), P450arom (B), AMH (C) and ERα (D) in gonads of turkey, duck, and goose compared to chicken. Partial sequences of chicken used for comparison with sequences of each gene of 3 species are follows: P450arom (A): GenBank accession no. NM001000176, Position 1215–1476. P450arom (B): NM001000176, 291–500. AMH (C): NM205030, 1054–1320. ERα (D): X03805, 1543–1800. Dark shading indicate identical nucleotides among chicken, turkey, duck and goose.
Each value was shown as relative expression of RT-PCR products compared with S17. Data are represented as mean ±SEM (9–13 turkeys, 8–10 ducks and 4–6 geese, respectively). Different superscripts indicate statistically significant difference each other ($P<0.05$).

contrast, in goose the level of AMH mRNA was significantly higher in males than that in females.

**ERα mRNA Expression**

There was no significant difference of ERα mRNA expression between males and females at 1–2 days after hatching in turkey, duck and goose. No sex difference in expression was observed at 1 week of age (Fig. 6B).

**Discussion**

The present study demonstrated that the left testis is larger than the right one in turkey, duck and goose at the age of hatching day to 1 week. Normally asymmetry is not so marked in the testes of chicken (Ha et al., 2004) and quail (Koba et al., 2008a), although it is well known that females have asymmetrical ovaries and oviducts due to a complete regression of the right side. This asymmetrical formation has been claimed to be attributable to the localized expression ERα in the left ovary at days of incubation (Nakabayashi et al., 1998; Ha et al., 2004). More recently, Guioli and Lovell-Badge (2007) proposed that PITX2 plays the key role for asymmetrical formation of gonads in the chicken and expression of PITX2 influences downstream mechanisms of gonad sex-differentiation, i.e. P450arom mRNA expression. It is interesting to test if this hypothesis is applied to asymmetrical formation of the testis in turkey, duck and goose.

The present study demonstrated marked expression of P450arom mRNA in females without expression in males of turkey, duck and goose at 1–2 days and 1 week after hatching. The results are consistent with those in female chicken and quail gonads. These findings may indicate that P450arom expression in female birds is critical for ovary formation and functions (i.e. estrogen synthesis) not only during the embryonic development but also after hatching.

It is known that AMH mRNA expression is detected in
both male and female embryos of chickens although the expression in males was higher than in females (Smith et al., 1999; Nishikimi et al., 2000; Yamamoto et al., 2003). In the present study, significantly higher expression of AMH mRNA was detected in males than females only in goose, but there was no sex difference in turkey and duck at 1–2 days posthatching and one week of age. Oreáel et al. (2002) reported, as in mammals, AMH were expressed in chicken follicular cell layer at day 7 after hatch. But, unlike in mammals, AMH expression was also found in abundance in unorganized cortical interstitial cells of female gonads. Therefore, it is considered that AMH expression of different type than male is performed and accumulated in female gonads for the late embryonic development stage and after hatching, consequently there is no apparent difference between males and females in turkey and duck. By the way, AMH may play an important role in ovarian folliculogenesis in mammals, but the beginning time of the AMH expression varies by species. For example, female pigs showed AMH expression at one-month after birth, and neonatal female goats immediately showed low expression of AMH mRNA (Parma et al., 1999; Pailloux et al., 2001, 2002). Female mice showed expression of AMH mRNA at day 6 after birth (Münsterberg and Lovell-Badge, 1991). Similarly, AMH mRNA expression in female goose gonads may be delayed compared with that in female turkey and duck gonads at day 1 after hatch and 1 week of age.

The previous studies reported that the estrogen concentration in the chicken gonad was significantly higher in females than in males. Although no sex difference was observed in plasma testosterone concentrations during the incubation periods and after hatching, higher testosterone levels were observed in the testes than in the ovary (Tanabe et al., 1986). On the other hand, in testes and ovary of duck, testosterone and estrogen concentrations were much higher in females than in males before and after hatching (Tanabe et al., 1983). Although no sex difference of ERα mRNA expression was observed in this study, it does not mean a no role for ERα in ovarian formation. As long as estrogen is available as a ligand for ERα, expression of ERα should not be neglected.

The present results suggest that estrogen plays a key role for ovary development via P450arom mRNA expression after hatching in turkey, duck and goose, whereas absence of its expression in males leads to testis development.

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References


