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Plasma 17β-Estradiol Levels and Ovarian Interstitial Cell Structure in Embryonic Japanese Quail

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ABSTRACT Plasma concentrations of 17β-estradiol (E_2) and left ovarian histology were investigated by light and electron microscopy in female Japanese quail from Day 10 of embryonic development through Day 7, posthatch. Plasma E_2 levels remained relatively constant (102 to 140 pg/mL) in the embryo followed by a sharp decrease posthatch (47 to 70 pg/mL).

Beginning on Day 10 of incubation, cells in the medullary portion (medullary cell; MC) of the left ovaries exhibited ultrastructural evidence of steroidogenic capability. The MC had numerous lipid droplets in close proximity to the smooth endoplasmic reticulum (SER). Mitochondria were also observed in the vicinity of the lipid droplets and SER. On Days 10 and 12, the cristae of the inner mitochondrial membranes were of a lamellar configuration; the cristae of some mitochondria in MC had a tubular appearance by Day 14. These data document relative ontogenic changes in ovarian morphology and plasma E_2 levels during the early developmental period in female Japanese quail. These data further support the role of this steroid in sexual differentiation.

(*Key words*: embryo, 17β-estradiol, ovary, quail, female)

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INTRODUCTION

Testosterone and 17β-estradiol (E_2), which are synthesized and secreted by the gonads and adrenal glands during avian embryonic development, regulate growth and differentiation of the sex accessory structures (Teng and Teng, 1979; Huston et al., 1985). In addition, these steroids are thought to organize those areas of the hypothalamus that regulate the neuroendocrine and behavioral components of reproduction (Adkins, 1979, 1985; Schumacher et al., 1989). Left ovaries of chick (Guichard et al., 1977, 1979, 1980; Tanabe et al., 1979, 1986) and quail (Guichard et al., 1973; Scheib et al., 1974, 1981) embryos synthesize and secrete steroid hormones. In the undifferentiated gonads of the chick embryo, the sex hormone E_2 has been detected immunohistochemically as early as Day 3.5 of incubation (Woods and Erton, 1978); the E_2 receptor has also been measured in the hypothalamus and adenohypophyseal pars distalis of the chick embryo (Woods et al., 1995). In female embryos, plasma E_2 levels rise from the time of ovarian differentiation (Day 6.5) until time of hatch, whereas in males the E_2 levels remain relatively low throughout embryonic development (Woods and Brazzill, 1981; for review, see Ottinger and Abdelnabi, 1997).

The purpose of the present study was to describe the sequence of ontogenic events that contribute to sexual differentiation in female Japanese quail. Ovarian histology, studied by light and electron microscopy, and plasma E_2 concentrations were measured during the embryonic and early posthatch periods.

MATERIALS AND METHODS

Experimental Animals and Sampling

Fertilized eggs were obtained from the Japanese quail colony of the University of Maryland Poultry Science Department. Eggs were incubated at 36.9 C and 65% relative humidity in a forced-draft incubator. The day that the eggs were placed in the incubator was counted as incubation Day 0. Newly hatched quail chicks were brooded in heated cages and consumed feed (Purina Game Bird Startina[3]) and water ad libitum.

Two hundred females were sampled at 2-d intervals between Day 10 of incubation and Day 7 posthatch. Blood

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Abbreviation Key: E_2 = 17β-estradiol; MC = medullary cells; SER = smooth endoplasmic reticulum.

samples from embryos were collected from the chorioallantoic vein in heparinized capillary tubes; after making a window in the shell above the air sac, the vein was lifted out, punctured, and blood was collected (Ottinger and Bakst, 1981). Blood from posthatch quail was obtained by decapitation. Samples were then centrifuged, and the plasma was stored in glass vials at –20 C until the E_2 assay. Gonads were identified in situ as testes or ovary by means of a dissecting microscope.

For assay of E_2 in embryonic blood, plasma samples were pooled in equal amounts by age to obtain a total volume of 100 μL. Samples were taken at Days 10, 12, 14, and 16 of incubation (n = 10, 16, 10, and 10, respectively), on the day of hatching (n = 16), and Days 1, 3, 5, and 7 posthatch (n = 11, 10, 16, and 14, respectively).

17β-Estradiol RIA

Concentrations of E_2 were measured in 100-μL plasma samples by a modification of the RIA method of Woods and Brazzill (1981); samples were extracted with methylene chloride, air-dried, and reconstituted in PBS. The assay was validated for quail serum for sensitivity (10 pg/mL), parallelism in serial dilutions of plasma, inter- and intraassay variability (below 5%), and specificity; no significant cross-reaction was found with other steroids (Woods and Brazzill, 1981). The E_2 antibody was diluted to 1:50,000 to yield 35 and 50% total binding in assays.

Histological Analysis

Ovarian tissue was prepared for transmission electron microscopy as described previously (Ottinger and Bakst, 1981). For light microscopy, 2 μm thick plastic sections were stained with toluidine blue and examined with a Zeiss Universal microscope[4] and were photographed at 400× magnification.

Experimental Design and Statistical Analysis

Samples were collected from two replicate hatches. Statistical analysis showed nonsignificant differences between replicates. Therefore, the results were combined to increase the sample size. Completely randomized design with unequal replicates per age, as given above, was used in this experiment. Statistical analyses done by ANOVA were followed by Student Newman Keul's test.

RESULTS AND DISCUSSION

Plasma E_2 Concentrations

Plasma E_2 concentrations were higher ($P \leq 0.05$) during the embryonic period from Days 10 to 16 than during the immediate posthatch period (Figure 1). On Days 10, 12, 14, and 16 of incubation E_2 levels were 135.4, 108.8, 140.0, and 102.6 pg/mL, respectively. On the day of hatching, E_2 levels decreased to 69.8 pg/mL and remained at a relatively constant level (68.5, 49.6, 67.6, and 46.7 pg/mL on Days 1, 3, 5, and 7, respectively). These hormonal changes are similar to embryonic and posthatch patterns observed in chickens (Woods and Bazzill, 1981; Tanabe et al., 1986).

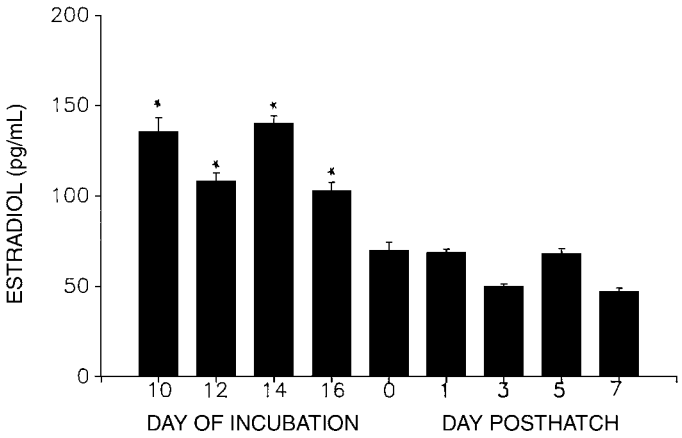

FIGURE 1. Mean plasma estradiol (pg/mL ± SEM) in embryonic and posthatch females. Lc = 10, 16, 10, and 10 for Days 10, 12, 14, and 16 of incubation, respectively; lc = 16 on day of hatch; and lc = 11, 10, 16, and 14 for Days 1, 3, 5, and 7 posthatch. Prehatch levels have significantly higher ($P < 0.05$) means and are identified by an asterisk.

Ovarian Morphology

The light micrographs (Figures 2 and 3) show the ovarian cortical and medullary region from 10- and 14-d-old embryos. Cells found in the medullary region of the ovary (medullary cells; MC) were grouped in clusters and were characterized by numerous lipid droplets. Ovarian sections examined by transmission electron microscopy at

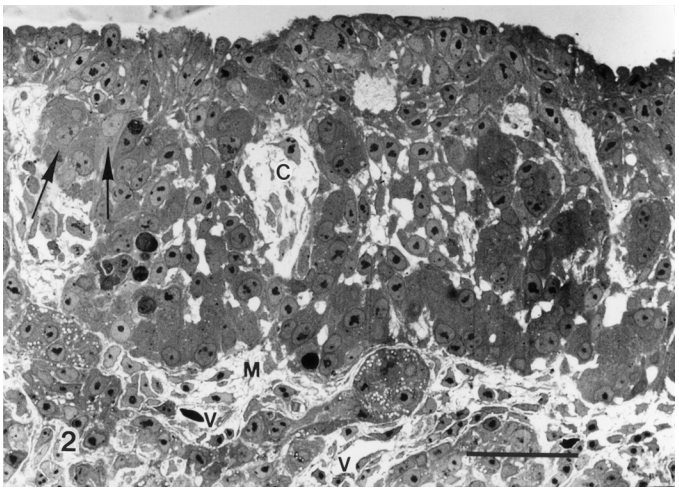

FIGURE 2. Ovarian cortical (c) and medullary (M) regions from embryos on Day 10. Blood vessels (v) and two primary oocytes (arrows) are observed. (Bar = 50 μm).

[4]Zeiss, Thornwood, NY 10594.

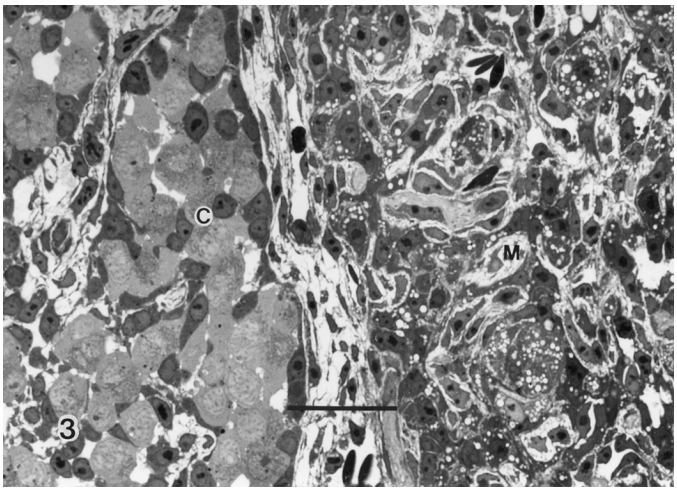

FIGURE 3. Ovarian cortical (c) and medullary (M) regions from embryos on Day 14. (Bar = 0.5 μm).

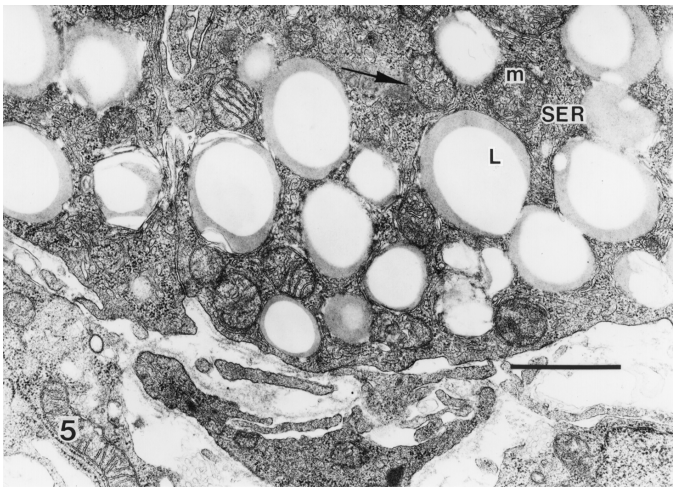

FIGURE 5. This transmission electron micrograph shows portions of ovarian medullary cells from a 14-d-old embryo. The steroidogenic cell is characterized by numerous lipid droplets (L) in close association with smooth endoplasmic reticulum (SER). Some of the mitochondria (m) contain tubular cristae (arrow). (Bar = 1 μm).

10 (Figure 4) and 14 (Figure 5) d of incubation show MC with the morphological features of steroidogenic cells. Typically such cells had numerous lipid droplets associated with the smooth endoplasmic reticulum (SER). Mitochondria were also found adjacent to the lipid droplets and SER; mitochondrial cristae were of a lamellar configuration on Days 10 and 12; by Day 14 the cristae of some mitochrondria had a tubular appearance.

In the present study, MC with the morphological features characteristic of steroidogenic cells were observed in the quail ovary on Day 10 of incubation, the first day of examination. This finding is in accordance with the electron microscopy observations of Scheib (1970), who demonstrated their initial presence on Day 7.0. In contrast, the ultrastructure of the interstitial cells of the quail testes does not possess characteristics of steroidogenic cell until after hatching, in spite of substantial plasma androgen levels (Scheib, 1970; Ottinger and Bakst, 1981). However, in the case of ovarian development, the present investigation provides evidence that changes in morphology of MC in the left ovary associated with steroidogenesis parallel the profile of plasma E_2 levels observed during the embryonic period as well as posthatch. Because the ovaries (Guichard et al., 1973; Scheib et al., 1974, 1981) and the adrenals of embryos of Japanese quail and domestic fowl produce steroids, overall plasma E_2 reflects contributions from both (Guichard et al., 1977, 1979, 1980; Tanabe et al., 1979).

Plasma E_2 is essential in the differentiation of the sex accessory structures, such as the oviducts (Teng and Teng, 1979), as well as for brain sexual differentiation (Shumacher et al., 1989; Adkins-Regan et al., 1994; Balthazart et al., 1996; Ottinger and Abdelnabi, 1997). The actions of gonadal steroids on brain differentiation have been demonstrated by injections of steroid hormones into embryonated eggs and by hormone replacement after posthatch castration. Injection of quail eggs with 17β-estradiol benzoate before Day 12 of embryonic development demasculinized posthatch males (Adkins, 1979; Schumacher et al., 1989). Blocking endogenous E_2 action by embryonic exposure to an antiestrogen in female quail embryos on Day 9 of incubation prevented feminization (Adkins, 1976). When these observations are examined in the light of the present findings of increasing plasma E_2 on Days 10 through 14 of embryonic development, it becomes apparent that endogenous E_2 is available during the time of sexual differentiation of neuroendocrine systems that modulate behavior. In conclusion, it is clear from these data that the steroidogenic capability, as seen by morphology and the timing of changes in plasma E_2 levels, clearly supports the role of this steroid in sexual differentiation of female quail.

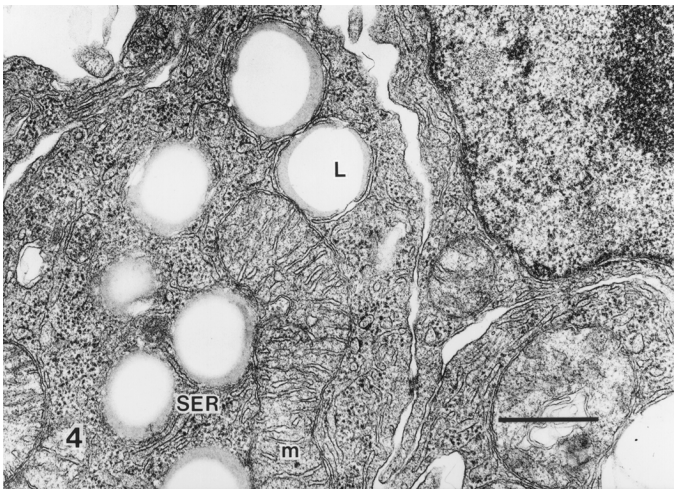

FIGURE 4. This transmission electron micrograph shows ovarian medullary cells from a 10-d-old embryo. The close association of the cisternae of smooth endoplasmic reticulum (SER) and the lipid (L) droplets as well as mitochondria (m) indicate that such cells are capable of steroidogenesis. (Bar = 0.5 μm).

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