

Research Notes

Comparison of Gonadal Hormone Levels in Turkey Embryos Incubated in Long-Term Shell-Less Culture and In Ovo

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ABSTRACT Changes in concentrations of 17β -estradiol (E_2) and androgenic hormones were measured in turkey embryos incubated in long-term, shell-less culture (ex ovo) and in ovo. Blood samples were obtained from both sets of embryos on Days 14, 16, 18, 20, and 22 and from embryos incubated in ovo on Days 24, 26, and 28. Ex ovo and in ovo embryos showed no differences in either hormone within sexes, with one exception. On Day 14 of incubation, the ex ovo females had higher ($P < 0.05$) E_2 levels (55.6 ± 5.1 pg/mL) than the in ovo females (32.2 ± 2.3 pg/mL); however, this result might have been due to

the small sample size ($n = 3$) for ex ovo females. No significant differences were found in androgen concentrations between sexes in ovo on Days 24, 26, and 28 of incubation. However, on Days 24, 26, and 28, in ovo females showed highly significant differences ($P < 0.01$) in E_2 compared with males of the same age. These results indicate a similar developmental pattern for the endocrine system in ovo and ex ovo through Day 22 of incubation. Further, there were sex differences in E_2 that are likely to be critical for sexual differentiation that emerges late in embryonic development.

(Key words: turkey, cultured embryo, estradiol, androgen)

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INTRODUCTION

During the last few decades, cultivation of avian embryos outside of the eggshell has been developed and used for several different species including chick (Auerbach et al., 1974; Dunn and Bonne, 1976, 1977, 1978; Barnett, 1982;), turkey (Richards, 1982), and quail (Ono and Wakasugi, 1983). Utilization of this technique facilitates continual monitoring of development and treatment application directly to the embryo. However, a high mortality rate occurs in the cultured embryos, especially during the second half of incubation (Dunn and Boone, 1977; Richards, 1982; Ono and Wakasugi, 1983). It has been suggested that lack of calcium, or other minerals supplied by the eggshell, and altered endogenous hormones may relate to this problem (Dunn and Boone, 1977; Richards, 1982; Ono and Wakasugi, 1983). Therefore, several studies have been conducted to understand factors affecting embryonic mortality in vitro and to improve hatchability through alternative designs of the culture vessel and the use of surrogate eggshells. (Dunn, et al., 1981; Naito and Perry, 1989; Naito et al., 1990; Kamihira et al., 1998). These experiments have led to successful in vitro culture of the

chick embryo. However, reproductive development and measurement of gonadal steroids in ovo or ex ovo have not been conducted with the turkey embryo.

Several comparisons have been made between embryos incubated in ovo and ex ovo with respect to growth rates (Dunn and Boone, 1976, 1977, 1978; Richards, 1982) and mineral content utilization (Dunn and Boone, 1977; Richards, 1982; Ono and Wakasugi, 1983; Richards, et al., 1984). Comparison of the differences between the embryos incubated in ovo and ex ovo at the level of the endocrine system, however, has not been done. Therefore, the objective of this experiment was to determine the levels of estradiol and androgen in the serum of male and female turkey embryos incubated in ovo or in long-term, shell-less culture (ex ovo).

MATERIALS AND METHODS

Experimental Eggs

Three hundred eggs from heavy medium turkey hens² were used in this experiment. Eggs were incubated in a commercial-type incubator at 37.5 C and 60% RH. At Day 4 of incubation, those eggs to be used for embryo culture (ex ovo) were removed, washed with 70% ethanol, and allowed to air dry. The remaining eggs from the same

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Abbreviation Key: E_2 = 17β -estradiol; 5-DHT = dihydrotestosterone.

²Nicholas strain, Sanoma, CA 95471-1111.

set were returned to the commercial-type incubator and served as in ovo age-matched controls.

Embryo Culture and Sample Collection

Embryos were cultured according to the method of Dunn and Boone (1976), as modified by Richards (1982). The egg contents were carefully transferred to the culture chamber (8 oz. plastic container) with the sides and bottom removed to permit better air circulation and even temperature (Figure 1). A pocket of plastic wrap was held in place by the outer edge of the lid that had the center portion removed. The chamber was sealed with a Petri plate and placed in a forced-draft, humidity-controlled incubator at 37.5 C and 93% RH. By using this technique, Richards (1982) found that the survival (expressed as a percentage of the total remaining culture) was greater than 90% through Day 24. Thereafter, mortality increased rapidly with no cultures surviving to Day 28. Blood samples were collected from both sets of embryos at 2-d intervals beginning on Day 14 and continuing through Day 22 of incubation. As none of the ex ovo embryos survived past 22 d of incubation, only embryos developing in ovo were sampled on Days 24, 26, and 28. Blood samples were collected from the vitelline vessels and from vessels located in the chorioallantoic membrane at early embryonic ages and by cardiac puncture of those embryos incubated in ovo and near to hatch (Day 28). Serum was harvested after centrifugation of clotted blood and stored at -40 C prior to analysis. The sex of the embryo was determined by the examination of the gonads by using a dissecting microscope.

Hormone Analysis

17 β -Estradiol RIA. Concentrations of E₂ were measured in 100- μ L 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 (0.01 mL). The assay was validated for quail plasma with a sensitivity of 1 to 2 pg/tube. Parallelism and specificity were established; no significant cross-reaction was found with other steroids. Inter- and intra-assay coefficients of variability were less than 10 and 5%, respectively. The E₂ antibody was diluted to 1:50,000 to yield 35 to 50% total binding in assays.

Androgen RIA. The androgen assay [testosterone + 5-dihydrotestosterone (5-DHT)]³ was validated for quail plasma (Ottinger and Bakst, 1981; Abdelnabi, 1990). Samples were extracted using hexane-benzene (2:1), air-dried, and reconstituted in PBS (0.01 mL). Serial dilutions of plasma were found to be parallel to the standard curve. The cross-reactivity of the antibody was 100% with testosterone and 46% with 5-DHT. Accuracy was checked by use of known concentrations of steroids. The inter- and

intraassay variabilities were 7 and 5%, respectively. The average sensitivity was 3 pg/tube for testosterone and 6 pg/tube for 5-DHT.

Statistical Analysis

All data were analyzed using general linear models of SAS software. The significance of mean differences was determined using least squares means for the comparison of in ovo vs. ex ovo or male vs. female. Sample numbers at each age are presented in Table 1, and data are presented as means \pm SEM.

RESULTS AND DISCUSSION

Androgen and E₂ concentrations were determined for serum samples collected between Days 14 and 22 of incubation from embryos maintained in ovo or in shell-less culture. This report is the first containing information of endogenous gonadal steroid hormones in the turkey embryo. After Day 22, samples were only collected from embryos incubated in ovo until hatch (Day 28). The results showed that there were no significant differences in serum androgen levels between the embryos incubated in ovo vs. those incubated ex ovo, in males or females. In ovo, androgen levels increased in male embryos during the last third of incubation (Days 21 to 28). This rise was not as pronounced in female embryos; by Day 28 androgen levels reached 4.36 \pm 0.18 in males and 2.84 \pm 1.16 ng/mL in females (Table 1). Serum estradiol levels declined until Day 18 in embryos of both sexes. This decline continued through Day 28 in male embryos incubated in ovo. At Days 24, 26, and 28 of incubation, however, female embryos showed highly significant differences ($P < 0.01$) in estradiol levels compared to male embryos (Table 1). There were no significant differences in estradiol levels between the two groups of female embryos incubated ex ovo and in ovo with the exception of Day 14, on which the ex ovo group showed a higher ($P < 0.05$) level of this hormone than did the in ovo group.

Steroids, especially E₂ and androgen during embryonic development, are considered key hormones in the process

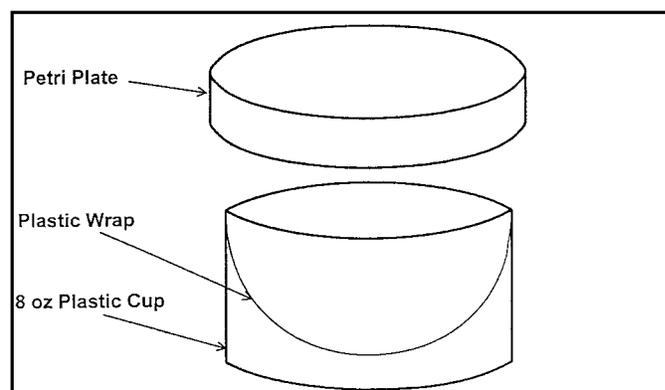


Figure 1. A diagram for the shell-less culture chamber.

³Amersham, UK.

TABLE 1. Serum androgen and estradiol levels in turkey embryos grown in ovo or ex ovo¹

Day	Sex	Androgen (ng/mL)		Estradiol (pg/mL)	
		In ovo	Ex ovo	In ovo	Ex ovo
14	Male	1.44 ± 0.65 (2)	1.35 ± 0.10 (6)	33.50 ± 6.50 (2)	50.50 ± 6.28 (7)
	Female	1.42 ± 0.17 (6)	1.34 ± 0.71 (2)	32.20 ± 2.33 (6)	55.63 ± 5.52 (3)
16	Male	1.37 ± 0.25 (7)	1.54 ± 0.22 (5)	15.48 ± 3.29 (6)	19.54 ± 1.85 (5)
	Female	1.09 ± 0.43 (3)	1.43 ± 0.35 (5)	26.70 ± 9.66 (3)	29.97 ± 8.07 (4)
18	Male	1.30 ± 0.36 (4)	1.22 ± 0.25 (4)	15.90 ± 3.10 (2)	16.10 ± 3.00 (2)
	Female	1.16 ± 0.34 (5)	1.54 ± 0.36 (5)	20.23 ± 3.54 (6)	19.76 ± 6.44 (5)
20	Male	1.13 ± 0.22 (7)	0.88 ± 0.33 (4)	21.85 ± 2.59 (6)	27.32 ± 3.70 (4)
	Female	0.80 ± 0.03 (3)	0.71 ± 0.13 (6)	30.32 ± 7.82 (4)	20.82 ± 4.29 (5)
22	Male	2.23 ± 0.48 (5)	0.79 ± 0.23 (2)	18.48 ± 1.97 (6)	22.00 ± 1.30 (2)
	Female	2.62 ± 0.55 (4)	2.65 ± 1.21 (2)	24.60 ± 5.01 (3)	23.80 ± 1.15 (3)
24	Male	2.57 ± 0.25 (5)	ND	7.43 ± 0.90 (3)	ND
	Female	2.77 ± 0.17 (4)	ND	29.77 ± 7.45 (4)	ND
26	Male	2.80 ± 0.39 (2)	ND	4.80 ± 1.10 (2)	ND
	Female	2.86 ± 0.63 (5)	ND	40.00 ± 1.00 (2)	ND
28	Male	4.36 ± 0.18 (3)	ND	5.60 ± 1.40 (2)	ND
	Female	2.84 ± 1.16 (2)	ND	30.50 ± 1.50 (2)	ND

¹Values represent means ± SEM.

²ND = Not determined.

³Values in parentheses represent sample numbers.

of sexual differentiation (for review see Ottinger and Abdelnabi, 1997). Synthesis and release of these hormones depend on the activity of the gonads and the establishment of hypothalamo-hypophyseal-gonadal axis (Woods and Thommes, 1984). From the present results, it appears that the turkey embryonic ovary has a higher activity in the synthesis and secretion of E₂ than the embryonic testes. The production of androgen from the ovary was similar to that observed for the embryonic testes. This observation is also reflected in the higher levels of plasma E₂ in females ($P < 0.01$) and the lack of difference in androgen levels between sexes, regardless of age and treatment. Similar results have been observed for endogenous hormone levels in chicken embryos (Guichard et al., 1977, 1979; Woods and Brazzill, 1981; Tanabe et al., 1986). The differences in the embryonic gonadal activity for steroid production may be explained by sex differences in steroidogenic enzymes (Imataka et al., 1988) and by sex differences in gene expression at early developmental stages (Nomura et al., 1999).

In summary, the developmental patterns for changes in serum concentrations of E₂ and androgen were similar for male and female turkey embryos incubated through Day 22 in ovo or ex ovo. These data provide information about endogenous gonadal steroid hormones in turkey embryo and suggest that long-term, shell-less culture may be a useful technique to study aspects of steroidogenesis and sexual differentiation during development.

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