Concentrations of androstenedione, testosterone and LH in the blood before and after the onset of spermatogenesis in the cockerel

J. Culbert, P. J. Sharp and J. W. Wells

Agricultural Research Council's Poultry Research Centre, King's Buildings, West Mains Road, Edinburgh EH9 3JS, U.K.

Spermatogenesis begins at about 16 weeks of age in Thornber 909 cockerels when subjected to a lighting schedule of 14 h light/day. Most birds produce semen by 24 weeks. The onset of spermatogenesis is associated with increases in the concentrations of plasma LH and testosterone (Sharp, 1975; Sharp, Culbert & Wells, 1977). In the present study these increases are related to changes in the concentration of circulating androst-4-ene-3,17-dione (androstenedione). This steroid is one of the androgens secreted by the mammalian testis (Eik-Nes & Hall, 1965) but little is known about its secretion in birds. Additionally, concentrations of LH, testosterone and androstenedione were measured for 4 months after the birds began to produce semen.

Blood samples were taken from 15 birds which were allocated to one of four groups such that each bird was bled once every 4 weeks from 9 to 44 weeks of age. The birds were part of a larger group killed at 2-week intervals between 8 and 29 weeks of age to assess the development of their reproductive organs (Sharp *et al.*, 1977). The androgens were extracted from 2 ml aliquots of plasma by 'Florisil' adsorption and purified by paper chromatography to separate testosterone from androstenedione and 5 α -dihydrotestosterone. The mean recoveries of tritium-labelled testosterone and androstenedione (Radiochemical Centre, Amersham) added to plasma were 62.7% (C.V. 19.0; $n = 47$) and 49.9% (C.V. 19.7; $n = 46$), respectively. Testosterone and androstenedione were measured by radioimmunoassay essentially as described by Sharp *et al.* (1977). Paper chromatography was used to eliminate unwanted steroids which cross-react significantly in the assays (i.e. 5 α -dihydrotestosterone in the testosterone assay and androsterone, adrenosterone and 5 α -androstanedione in the androstenedione assay). The minimum amount of testosterone and androstenedione detected in plasma was 125 pg/ml and 250 pg/ml respectively. The coefficients of intra-assay variation were 23.5% ($n = 40$) and 29.8% ($n = 39$) and of inter-assay variation were 22.0% ($n = 54$) and 21.0% ($n = 10$) for the testosterone and androstenedione assays respectively. LH was measured with the radioimmunoassay described by Follett, Scanes & Cunningham (1972). Coefficients of inter- and intra-assay variation were 9% ($n = 8$) and 6% ($n = 10$). Statistical analyses were carried out by using the Student's t test.

The concentration of androstenedione in the plasma fell progressively between 9 and 20 weeks of age ($r = 0.71$, $n = 45$, $P < 0.001$). During the same period, the concentrations of LH and testosterone did not change (Table 1). Plasma LH rose significantly ($P < 0.05$) between 19–20 and 21–22 weeks of age to reach a level characteristic of the adult (Sharp, 1975; Table 1). Significant increases in the mean concentrations of androstenedione ($P < 0.05$) and testosterone ($P < 0.001$) occurred 1–2 weeks after the increase in the concentration of LH. Subsequently, the concentration of testosterone remained at a significantly higher value than in juvenile birds. The androstenedione concentration in adult birds was similar to that found during the early stages of the onset of spermatogenesis (Table 1).

During the 4 months after semen production would have begun there were no consistently significant changes in the mean concentrations of androstenedione and LH. In contrast, the concentration of testosterone was significantly higher ($P < 0.05$) between 37 and 44 weeks of age than between 27 and 34 weeks of age. The mean concentration of testosterone did not increase after the birds had reached 37–38 weeks of age.

These results show that the increase in the concentration of testosterone in the blood when semen production begins is accompanied by a rise in the concentration of androstenedione. The increased secretion of both androgens follows a rise in plasma LH levels. Since injections of chicken LH

Table 1. Mean concentrations ($\pm$ S.E.M.) of androstenedione, testosterone and LH in the blood of cockerels during sexual development (n = 7 or 8).

	Age (weeks inclusive)	Androstenedione (ng/ml)	Testosterone (ng/ml)	LH (ng/ml)
Juvenile	9–10	19.1 $\pm$ 3.80 ^a	2.5 $\pm$ 0.32 ^a	7.0 $\pm$ 0.80 ^{ab}
	11–12	11.1 $\pm$ 1.94 ^a	2.3 $\pm$ 0.42 ^a	5.7 $\pm$ 1.35 ^a
	13–14	4.7 $\pm$ 0.96 ^{bc}	2.9 $\pm$ 0.40 ^a	8.6 $\pm$ 1.26 ^{ab}
	15–16	5.3 $\pm$ 0.47 ^b	1.6 $\pm$ 0.28 ^a	6.7 $\pm$ 0.72 ^a
Spermatogenesis initiated and completed	17–18	3.5 $\pm$ 0.65 ^{bcd}	2.3 $\pm$ 0.35 ^a	7.3 $\pm$ 1.41 ^{ab}
	19–20	2.7 $\pm$ 1.28 ^d	1.5 $\pm$ 0.11 ^a	6.4 $\pm$ 0.89 ^a
	21–22	2.5 $\pm$ 0.95 ^d	2.2 $\pm$ 0.96 ^a	10.8 $\pm$ 1.55 ^{bc}
	23–24	6.7 $\pm$ 0.81 ^b	9.5 $\pm$ 1.64 ^c	8.7 $\pm$ 0.44 ^b
Adult	25–26	5.5 $\pm$ 0.90 ^b	3.4 $\pm$ 1.68 ^{ab}	10.4 $\pm$ 0.98 ^{bc}
	27–28	3.0 $\pm$ 0.92 ^{bc}	5.4 $\pm$ 1.15 ^{bc}	10.3 $\pm$ 0.90 ^{bc}
	29–30	2.6 $\pm$ 1.77 ^{cd}	3.7 $\pm$ 1.28 ^{ab}	11.7 $\pm$ 1.25 ^c
	31–32	1.5 $\pm$ 0.48 ^d	4.3 $\pm$ 1.36 ^{ab}	10.5 $\pm$ 0.81 ^{bc}
	33–34	3.7 $\pm$ 0.26 ^{bc}	5.0 $\pm$ 0.75 ^b	11.6 $\pm$ 1.09 ^c
	35–36	5.0 $\pm$ 0.28 ^b	8.2 $\pm$ 5.60 ^c	12.0 $\pm$ 1.25 ^c
	37–38	4.2 $\pm$ 0.45 ^{bc}	8.4 $\pm$ 1.55 ^c	11.6 $\pm$ 0.98 ^c
	39–40	3.8 $\pm$ 0.51 ^{bc}	7.5 $\pm$ 0.22 ^c	12.1 $\pm$ 0.98 ^c
	41–42	5.7 $\pm$ 0.94 ^b	9.3 $\pm$ 1.30 ^c	12.2 $\pm$ 0.67 ^c
	43–44	3.3 $\pm$ 0.45 ^{bc}	7.3 $\pm$ 0.58 ^{bc}	10.8 $\pm$ 0.79 ^c

Means in a column with a common superscript are not statistically different ($P < 0.05$), unpaired Student's *t* test.

stimulated the differentiation of steroid-synthesizing interstitial cells in the testes of hypophysectomized quail (Brown, Bayle, Scanes & Follett, 1975), it is likely that increased plasma LH stimulates steroid secretion by the testes of the cockerel when spermatogenesis begins. The high concentration of androstenedione in the blood of 9–12-week-old prepubertal cockerels may also be caused by LH, which is present in significant amounts in the blood of juvenile cockerels at a concentration approximately four times that found in the blood of the laying hen (Sharp, 1975). Most of the androstenedione in the blood of juvenile cockerels appears to be of testicular origin because the progressive fall in plasma androstenedione concentration between 9 and 16 weeks of age parallels a fall in the total amount of androstenedione in the testes (Sharp *et al.*, 1977). These observations suggest that there is a maturational change in the steroidogenic component of the testes at least 6 weeks before the onset of rapid testicular growth. The concentration of plasma androstenedione exceeds that of testosterone in the juvenile cockerel while in the adult it is about half that of testosterone.

The continued rise in plasma testosterone concentration after the testes have started to produce spermatozoa suggests that the testes continue to mature after 24 weeks of age. Since plasma testosterone concentrations stabilized after 37–38 weeks of age, this maturation appears to continue for 12 weeks after semen production begins.

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