Steroid hormones in the postovulatory follicle of the domestic fowl (*Gallus domesticus*)

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Summary. Progesterone, androstenedione, testosterone, oestrone and oestradiol were measured in the postovulatory follicle (POF) at various times up to 52 h after ovulation. The 3-fold decrease in progesterone, the major constituent, which occurred over the first 15–20 h resembled the changes previously described for the enzyme, 3 β -hydroxysteroid dehydrogenase.

At 1 h before ovulation the granulosa cells of the anteovulatory follicle (AOF) contained 50 times more progesterone than the POF granulosa fraction collected 2–3 h later. The thecal portion of the AOF had progesterone concentrations 5 times those of the POF theca, but the latter contained higher concentrations of androstenedione and oestrone.

Introduction

Although the avian postovulatory follicle (POF) is not homologous with the mammalian corpus luteum (Gilbert, 1971) recent work has confirmed that the POF contains substances which influence the timing of oviposition (Tanaka, 1976; Tanaka & Goto, 1976). In addition, removal or even damage of the unicellular layer of granulosa cells of the POF leads to delayed oviposition (Gilbert, Davidson & Wells, 1978). The enzyme 3 β -hydroxysteroid dehydrogenase (3 β -HSD) has been demonstrated in the granulosa and theca cells of the POF by histochemical and biochemical procedures (Armstrong, Davidson, Gilbert & Wells, 1977), and steroid hormones may therefore be produced by the POF to modulate oviductal function. The present study was an examination of the amounts of five steroid hormones in the granulosa and thecal fractions of POFs collected at known times after ovulation.

Materials and Methods

Animals

Fifty (50) light-hybrid hens (Babcock 808) were used for the analysis of POF tissue. Husbandry conditions, egg readings, collection and histology of tissue fractions of the POF were as described by Armstrong *et al.* (1977). A further 5 hens were selected 1 h before an expected ovulation (see Gilbert *et al.*, 1978). Immediately after death, samples of blood, kidney, shell gland, and anteovulatory follicles (AOF) were taken for hormone analysis. Details for separation of the granulosa cells from the theca of the AOF are given by Gilbert, Evans, Perry & Davidson (1977). All tissue samples taken for analysis were frozen in liquid nitrogen within 5 min of collection and stored at -20°C .

The viability of the granulosa cells in POFs of different ages was assessed in a separate group of birds by adding a few drops of a solution of Trypan Blue (Flow Laboratories, Irvine; 0.1 % w/v in 0.145 M-NaCl) to the granulosa in the POF immediately after dissection (1–3 min after death). After 10 min the granulosa layer was scraped out and examined microscopically for uptake of the dye: living cells remained unstained.

Measurement of steroid hormones in tissue and plasma

Homogenates of tissue fractions in 0.154 M-NaCl were extracted thrice with dichloromethane (8 vols). Neutral and phenolic steroids in the extract were separated by partitioning between a mixture (1:1 v/v) of light petroleum (b.p. 40–60°C) and toluene and 1 M-NaOH (see Brown, 1955). The non-phenolic steroids were separated by paper chromatography as described previously (Sharp, Culbert & Wells, 1977), but the technique was improved by the use of a dye marker, Sudan Black B, on adjacent strips to locate progesterone and androst-4-ene-3,17-dione (androstenedione). The testosterone region of the chromatogram was defined by location of two non-cross-reacting steroid markers, androst-4-ene-3,11,17-trione and 21-hydroxypregn-4-ene-3,20-dione. Oestrone and oestradiol were separated by paper chromatography in a 3–4 h run with the system of Mayes & Nugent (1970), after equilibration for 12 h. Rhodamine B and *p*-hydroxyazobenzene were used as markers for locating oestradiol and oestrone respectively. All steroids were measured by radioimmunoassay with dextran-coated charcoal to separate the free from the bound radioactivity. The antisera have been characterized previously, namely for the progesterone by Dighe & Hunter (1974), for androstenedione by Baird, Burgher, Heaven-Jones & Scaramuzzi (1974), for testosterone by Sharp *et al.* (1977) and for oestradiol and oestrone by Bolton & Hunter (1973).

For measurement of non-phenolic steroids, blood plasma (2 ml) was extracted as described by Culbert (1976) and the constituents in the extract were separated by paper chromatography. Oestrogens were extracted with dichloromethane (5 vols, twice) and purified by the procedure described above.

The accuracy of the measurements was assessed by recovery of various steroids added to water or 0.154 M-NaCl. For 10 ng progesterone the recovery was $96.6 \pm 21.6\%$ (mean $\pm$ coefficient of variation; $n = 8$); androstenedione (9.5 ng), $84.4 \pm 17.9\%$ ($n = 6$); testosterone (8.5 ng), $90.9 \pm 3.6\%$ ($n = 6$); oestrone (range 194–776 pg), $98.7 \pm 9.8\%$ ($n = 18$); and oestradiol (274–832 pg), $99.1 \pm 8\%$ ($n = 18$).

Interassay precisions varied considerably; the coefficient of variation was 24.3% for 200 pg progesterone, 38.4% for 95 pg androstenedione, 20.2% for 85 pg testosterone, 4.7% for 24 pg oestrone and 7.9% for 24 pg oestradiol. The recovery of tritiated steroids (Radiochemical Centre, Amersham), which were added routinely to homogenates, ranged from 42 to 67% for granulosa fractions and from 28 to 70% for thecal homogenates. The smallest amount which could be measured with precision was approximately 1 ng for progesterone, androstenedione and testosterone, and 100 pg for oestrone and oestradiol.

Statistics

To normalize the variances, the primary data obtained from the POF analyses were transformed to a logarithmic scale (base 10). Because two POF samples were taken from each bird, a split-plot analysis of variance (Armitage & Remington, 1970) was applied. Means were compared by Student's *t* test before transformation to the arithmetic scale.

Results

Sex steroids in the POF

The data in Table 1 are presented in the arithmetic scale and represent geometric means.

Progesterone was the major hormone in the granulosa and theca cells of the POF and there was no significant difference in their contents. The largest decrease in progesterone occurred in both cell types during the first 24 h after ovulation. The thecal portion had a higher oestrogen content than the granulosa immediately after ovulation but this fell during the first day. The apparent peaks of both oestrogens in the theca at 27 h are due to two high values, 81.4 and 90.4 ng, for oestrone and one of 15.2 ng for oestradiol. Although measurable quantities of androstenedione and testosterone were found in the granulosa and theca 1–2 h after ovulation, considerable fluctuations occurred thereafter. In the granulosa, 38 samples contained less than 1 ng androstenedione, 6 had 1–10 ng, 2 samples had 10–20 ng and 2 samples 20–40 ng. A similar pattern was obtained for testosterone in the granulosa and theca.

Table 1. Steroid hormones (geometric means) in the granulosa and theca of the hen POF at various times after ovulation

Time (h)	No. of birds	Granulosa content (ng)			Theca content (ng)		
		Progesterone	Oestrone	Oestradiol	Progesterone	Oestrone	Oestradiol
1.5	6	55.7 ^a	0.48 ^a	0.78 ^a	32.6 ^a	3.98 ^{ab}	8.65 ^a
9	6	33.5 ^{ab}	0.14 ^{abc}	0.55 ^{ab}	22.2 ^{ab}	0.89 ^{bc}	1.99 ^{ab}
15	6	22.3 ^{bc}	0.11 ^{bc}	0.04 ^c	13.4 ^{ab}	0.08 ^c	0.03 ^c
20	7	17.1 ^c	0.10 ^c	0.18 ^b	10.2 ^b	0.44 ^{cde}	0.53 ^{cd}
27	6	20.6 ^{bc}	0.28 ^{abc}	0.44 ^{ab}	12.6 ^{ab}	5.38 ^a	1.88 ^{bc}
30.5	4	21.9 ^{bc}	0.37 ^{abc}	0.23 ^{ab}	6.4 ^b	0.32 ^{cde}	1.36 ^{cd}
36	6	14.3 ^c	0.42 ^{ab}	0.76 ^a	17.3 ^{ab}	0.59 ^{cd}	0.50 ^{cd}
40	6	5.7 ^e	0.08 ^c	0.03 ^c	11.2 ^{ab}	0.08 ^c	0.04 ^c
45	7	9.0 ^{de}	0.13 ^{bc}	0.16 ^b	19.5 ^{ab}	0.33 ^{cde}	0.25 ^d
52	4	12.8 ^{cd}	0.26 ^{abc}	0.33 ^{ab}	18.3 ^{ab}	0.47 ^{cde}	1.14 ^{cd}
Variance (log ₁₀) within birds		0.035	0.198	0.195	0.121	0.136	0.128
Variance (log ₁₀) between birds		0.045	0.212	0.223	0.148	0.288	0.294

Within columns, means with different superscripts are significantly different ($P < 0.05$).

Dye studies showed that granulosa cells are still viable in 5-day-old POFs, and the changes were not therefore associated with cell death.

Sex steroids in the AOF

Table 2 shows a comparison of the concentrations of various steroid hormones in the thecal layers of POFs collected 1–2 h after ovulation with those in the theca of AOFs 1 h before ovulation. Analyses of the hormones in the kidney, shell gland and blood plasma are also presented to demonstrate their concentrations in non-endocrine tissue and in the circulation at the time of sampling. The concentration of progesterone in the AOF theca was greater than in the POF theca ($P < 0.01$). Although oestradiol concentration did not change and testosterone concentrations fell after ovulation ($P < 0.01$), androstenedione and oestrone in the POF theca were significantly higher ($P < 0.01$) than in that of the AOF.

Table 2. Comparisons of the steroid hormones (mean $\pm$ s.e.m. for 5 birds) in various tissues of the hen before and after ovulation

Tissue	Time before (–) or after (+) ovulation	Progesterone	Andro- stenedione	Testosterone	Oestrone	Oestradiol
AOF theca (ng/g)	–1	313 $\pm$ 67	<1	7.7 $\pm$ 1.3	0.71 $\pm$ 0.08	4.76 $\pm$ 0.8
POF theca (ng/g)	+1.5	64.9 $\pm$ 11.2	5.0 $\pm$ 2.9	1.6 $\pm$ 0.3	4.3 $\pm$ 0.85	11.2 $\pm$ 3.8
Shell gland (ng/g)	–1	19.6 $\pm$ 2.7	<1	<1	0.38 $\pm$ 0.11	0.34 $\pm$ 0.05
Kidney (ng/g)	–1	23.1 $\pm$ 4.0	<1	<1	0.75 $\pm$ 0.39	0.32 $\pm$ 0.09
Plasma (ng/ml)	–1	1.93 $\pm$ 0.10	<1	<1	0.09 $\pm$ 0.02	0.27 $\pm$ 0.05
AOF granulosa (ng)*	–1	2670 $\pm$ 1364	3.0 $\pm$ 1.3	<1	0.42 $\pm$ 0.05	4.5 $\pm$ 1.9
POF granulosa (ng)*	+1.5	59.7 $\pm$ 6.7	5.6 $\pm$ 2.2	1.48 $\pm$ 0.33	0.76 $\pm$ 0.29	1.5 $\pm$ 0.79

* These values are for the total amounts in the tissue.

The granulosa cells of the AOF contained amounts of progesterone ranging from 340 to 6810 ng in different birds. Statistical comparisons of the geometric means of 1291 ng for AOF granulosa and 24.8 ng of the POF granulosa confirmed their difference ($P < 0.002$). There was therefore a 50-fold decrease in the progesterone content of the granulosa cells during and after ovulation.

Discussion

Our findings demonstrate that, in the domestic fowl, a large decrease in the progesterone content of the follicle occurred within 2 h of ovulation. The result provides chemical confirmation that the POF is not homologous with the mammalian corpus luteum which, by contrast, contains large amounts of progesterone until the late luteal phase of the cycle (Swanston, McNatty & Baird, 1977).

The concentrations of steroids in the theca of the AOF are comparable to those reported by Shahabi, Norton & Nalbandov (1975) for the whole follicle. This is because Shahabi *et al.* (1975) split the AOF to remove the yolk before analysis and thus probably removed much of the granulosa layer (Gilbert *et al.*, 1977). Our results show that the granulosa layer is a rich source of progesterone (up to 6 μ g) and the progesterone content of the follicle is therefore about 20 times more than previously reported.

The decline in progesterone content of the granulosa and thecal fractions of the POF during the first 15 h after ovulation is similar to that observed for 3 β -HSD activity in this tissue (Armstrong *et al.*, 1977). The tissue content of other steroid hormones showed erratic patterns but there were transient increases in oestrogens in the POF between 20 and 27 h after ovulation.

Prostaglandins from the POF may be involved in the control of oviposition. Hertelendy, Yeh & Biellier (1974) demonstrated that prostaglandins can induce premature oviposition in the hen, and Day & Nalbandov (1977) found a 100-fold increase in the prostaglandin level of the POF 1–2 h before oviposition. It is tempting to speculate that the fall in progesterone and the rise in oestrogens somehow leads to an increased production of prostaglandins by the POF. Such a mechanism has been demonstrated in the uterus of the ewe (Barcikowski, Carlson, Wilson & McCracken, 1974). Tanaka (1976), however, has obtained a factor from hen follicles which causes premature oviposition but is neither a prostaglandin nor a steroid hormone. There is, as yet, no apparent link between any of these substances.

We thank Mrs Linda M. Currie for steroid measurements; Mrs Caroline C. McCorquodale for statistical analyses; and Dr W. M. Hunter and Dr A. E. Bolton for supplies of anti-progesterone and anti-oestrogen sera.

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Received 10 October 1977