BIOLOGY OF REPRODUCTION 12, 590-598 (1975)

Concentrations of Estrogens, Progesterone and LH During the Ovulatory Cycle of the Laying Chicken [*Gallus domesticus*]

P. C. LAGUË[1], A. VAN TIENHOVEN AND F. J. CUNNINGHAM

Department of Poultry Science, Cornell University, Ithaca, N.Y. 14853 and Department of Physiology and Biochemistry, The University, Whiteknights, Reading RG62AJ, England

Accepted February 11, 1975

The concentrations of estradiol, estrone, progesterone and LH are reported for blood samples taken at different intervals during the ovulatory cycle of the domestic fowl. In general there appears to exist a consistent relationship between peaks of estradiol and estrone concentrations, on the one hand, and the progesterone peaks, on the other hand.

These peaks of estrogen and progesterone precede ovulation by 4-7 hours. Ovulation does, however, occur occasionally in the absence of estrogen peaks and some estrogen peaks appear not to be related to ovulation.

In samples taken at 20-min intervals at about the time of the expected progesterone peak it was found that the progesterone concentrations varied in a pulsatile fashion whereas estrogen and LH concentrations did not, but showed a smooth peak.

Experiments with different doses of estradiol 17β and progesterone failed to show either an inhibitory or facilitory effect of estradiol on spontaneous or progesterone-induced ovulation.

In recent years a number of reports concerning the hormonal control of ovulation of the domestic hen have been published.

Bioassays for avian LH have indicated that there might be three peaks of LH release prior to ovulation (Bullock and Nalbandov, 1967; Imai and Nalbandov, 1971; Nelson *et al.*, 1965). However, when LH concentrations were determined by radioimmunoassay there was only one distinct peak of LH at about 4-7 h prior to ovulation (Cunningham and Furr, 1972; Furr *et al.*, 1973). In 5 out of 24 hens Cunningham and Furr (1972) found a small LH peak at about 23-20 h prior to ovulation.

A distinct peak of progesterone concentration occurs at 4-6 h prior to ovulation according to Kappauf and van Tienhoven (1972) and Peterson and Common (1971), 2-6 h before ovulation according to Haynes *et al.* (1973), and 4-7 h prior to ovulation according to Cunningham and Furr (1972) and Furr *et al.* (1973). Cunningham and Furr (1972) found that the rise in progesterone preceded the peak of LH determined on the same blood samples, whereas Furr *et al.* (1973) stated that the rise in progesterone either preceded or occurred simultaneously with the LH increase, but that the rise in LH never preceded the rise in progesterone.

For estradiol, concentrations in the peripheral plasma of the hen show peaks at 18-22 h and at 2-6 h prior to ovulation (Peterson and Common, 1972). However, Senior and Cunningham (1974) reported an increase in estradiol concentrations 8 h prior to ovulation with a peak concentration at 6 h prior to ovulation, which is about 2 h prior to the LH peak.

Under our experimental conditions the first egg of a sequence is usually ovulated at about 6:00 on the day when no egg is laid. This egg is laid the next day and this oviposition is followed within about 30-75 min by the ovulation of the second egg of the sequence. Each successive egg is ovulated later in the day and each resulting egg is laid later in the day also, till the final egg of the sequence is

[1] Present address: Department of Animal Science, Macdonald College, Ste. Anne de Bellevue, H9X3173, Canada.

laid between 14:00–17:00. This oviposition is followed by an ovulation the next morning at about 6:00 thus starting a new sequence. For a detailed consideration of the ovulation-oviposition cycle and the time relationships between these events in sequences of different numbers of eggs, see the excellent review by Fraps (1955).

It was the purpose of this study to determine the temporal relationships between estrogen, progesterone and LH concentrations during the ovulatory cycle of chickens with sequences of 3–5 eggs. The results are reported here together with a report on experiments designed to test the relationships between exogenous progesterone and estrogen in inducing premature ovulations in chickens.

MATERIALS AND METHODS

Animals

White Leghorn hens of the Cornell University "C" strain were used for these studies. The hens were 6 months or older and in their first year of production. Only hens laying sequences of 2–6 eggs and with intervals of one day between sequences were used (Fraps, 1955).

The hens were housed in individual cages where they received a commercial laying mash and water *ad libitum*. The light regime was 14L:10D with lights on at 5:00. The temperature was 22 ± 1°C.

Time of Oviposition and Ovulation

The time of oviposition was determined every hour on the hour and the time of ovulation was estimated from the laying records and by digital palpation. With this method the time of ovulation can be estimated with an error of not more than 30 min in either direction.

Blood Samples

Blood samples were collected at 4-hour intervals in most experiments, but in one experiment samples were taken at 4-hour intervals till the time of expected release of LH when samples were taken at 20-min intervals for 22 samples. We used the method of Kappauf and van Tienhoven (1972) to collect the blood. In order to minimize the stress of the frequent sampling we took the following precautions: (1) Washed red blood cells, from previous samples from the same bird were resuspended in avian Ringer's solution and reinjected after each blood sampling. (2) Blood samples taken during the dark period were obtained after placing the hen near a dim green light, which allowed the operator to obtain blood, but apparently did not interfere with the hen's sleep. During virtually all samples taken during the dark period the hen seemed to be sleeping or drowsy. The fact that ovulation and oviposition continued normally seems to indicate that any interference may have been slight.

Assay Method for Estrogens

Estradiol and estrone were extracted from duplicate plasma aliquots of 1.0 ml in 60 ml separatory funnels. Internal standards (3000 dpm ^{3}H,6-7 estradiol-17β, Amersham Searle, and 3000 dpm ^{3}H,6-7 estrone, New England Nuclear) were added for the estimation of procedural losses. The plasma was diluted to 10 ml with glass distilled water and extracted three times with 20 ml dichloromethane (Nanograde, Mallinckrodt). The solvent was evaporated to dryness in a 50 ml Erlenmeyer flask under a stream of nitrogen at 39°C. The extract was then dissolved in 5 ml 1 N NaOH by shaking it vigorously for 15 sec and transferred to a separatory funnel containing 15 ml toluene (purified according to Scholler, 1962). This transfer was repeated two more times with the sodium hydroxide and one time with 10 ml toluene. The contents of this funnel were mixed for 1 min and the NaOH phase was drained into a clean separatory funnel. Ten ml 1 N NaOH were added to the toluene phase, mixed for 1 min and the NaOH phase was added to the first NaOH fraction. The NaOH fraction was further purified twice with 15 ml N-hexane (purified according to Scholler, 1962).

The extraction of the estrogens from the NaOH phase was accomplished by adding 1 ml carbonate buffer (190 ml saturated $KHCO_3$ plus 9.5 ml saturated K_2CO_3) to the NaOH phase and brought to pH = 9.0 ± 0.5 with concentrated HCl (AR grade). This mixture was extracted three times with 15 ml dichloromethane and the solvent evaporated. The wall of the vessel was rinsed with 1 ml methanol (purified by refluxing over KOH and zinc and distilled, discarding the first and last 5 per cent fractions). The methanol was evaporated and the extract stored, dissolved in 0.15 to 0.20 ml ethanol (purified according to Peterson, 1957) in a well sealed tube.

Estradiol, estrone and estriol were separated by column chromatography according to the procedure of Mikhail *et al.* (1970).

For the radioimmunoassay the samples were first dried by evaporating the solvent under nitrogen. Then 1 ml purified ethanol was added: 0.1 ml was used for counting of radioactivity to establish procedural losses, and two aliquots of 0.4 ml were transferred in 12 × 75 mm disposable glass tubes. The estrogen determination was carried out similar to the method described by Peterson and Common (1972) except that the counting solution consisted of 3.8 liters toluene containing 18.95 g PPO (Packard Instrument Co.) and 0.379 g POPOP (Packard Instrument Co.) and 95 ml Bio-Solv-BBS-3 (Beckman Instrument Inc.).

Sensitivity of Estrogen Determination

The lowest amount which can be determined consistently on the standard curve is 5 pg. The sensitivity for

each plasma sample is 18 pg for estrone and 21 pg for estradiol; this takes into account procedural losses of 30 percent for estrone and 40 percent for estradiol and the fact that duplicate determinations were carried out on each plasma aliquot with each duplicate amounting to 40 percent of the original sample.

Accuracy of Estrogen Determination

The accuracy of the method is illustrated by Table 1. Water blanks and blood samples from capons yielded zero values in this assay.

Precision of Estrogen Determination

The precision was calculated by the formula of Snedecor (1956). The precision for the values obtained in Table 1 was ±14.8 pg (N = 9) over the range of 33-250 pg for estrone and ±24 pg over the range of 40-246 pg for estradiol. For the samples reported under results the precision was ±5.9 to ±24.5 pg/ml over a range of 15-221 pg/ml for estrone and ±17.4 to ±40.3 pg/ml over a range of 35-278 pg/ml for estradiol.

Progesterone Determination

Progesterone was determined by a protein-binding assay as described by Kappauf and van Tienhoven (1972). The sensitivity of this assay is 0.08 ± 0.04 ng. The accuracy as assessed by recovery of 0.625 and 1.25 ng progesterone added to laying hen plasma is 0.53 ± 0.16 ng and 1.26 ± 0.06 ng, respectively. The precision is S = ±0.18 ng (Kappauf and van Tienhoven, 1972).

LH Radioimmunoassay

This assay was carried out according to the method of Follett *et al.* (1972). The plasma concentration of LH was expressed in terms of a purified preparation of chicken LH (Fraction IRC-2), the purification of which was described by Stockell Hartree and Cunningham (1969). In the assay dilutions of plasma gave inhibition curves parallel with that of IRC-2.

Hormone Injections

Estradiol-17β and progesterone were dissolved in sesame oil so that the desired amount was contained in 0.2 ml. Injections were made intramuscularly between 15:00-17:00 on the day prior to ovulation of the C_1 follicle.

RESULTS

The variations in estrone (E_1) and estradiol (E_2) concentrations which occur during the ovulatory cycle are illustrated in Figs. 1 through 4. In Figs. 1 and 2 the times of oviposition and ovulation were determined but the time of the indicated progesterone peak was estimated from the time of ovulation, using the data of Cunningham and Furr (1972), Furr *et al.* (1973), Kappauf and van Tienhoven (1972), and Peterson and Common (1972). The progesterone peaks indicated in Figs. 3 and 4, and the progesterone concentrations in Fig. 5 were determined on the same blood samples used to determine the estrogen concentrations. The progesterone value in Figs. 3 and 4 have been reported by Kappauf (1971) and Kappauf and van Tienhoven (1972). Fig. 5 illustrates the LH, E_1, E_2 and progesterone concentrations with respect to the time of one ovulation with the samples taken at 1-hour intervals till about 7.5 h before ovulation and at 20-min intervals after this, till 1.5 h after ovulation.

These results show that progesterone peaks with few exceptions are accompanied by E_1 and E_2 peaks. Exceptions are: the lack of an

TABLE 1

ACCURACY OF ESTRONE AND ESTRADIOL DETERMINATION BY RADIOIMMUNOASSAY IN LAYING HEN PLASMA

Plasma volume	Added estrogen	Estrone N	Estrone pg/ml	Estrone % recovery	Estradiol N	Estradiol pg/ml	Estradiol % recovery
0.5 ml		2	31, 41	—	2	36, 43	—
1.0 ml		2	36, 36	—	2	38, 42	—
2.0 ml		2	32, 35	—	2	44, 49	—
1 ml +	50 pg Estradiol	—	—	—	2	97, 93	106
	50 pg Estrone	2	76, 79	85	—	—	—
	100 pg Estradiol	—	—	—	2	138, 141	98
	100 pg Estrone	2	152, 168	115	—	—	—
	200 pg Estradiol	—	—	—	2	241, 250	102
	200 pg Estrone	2	239, 260	107	—	—	—

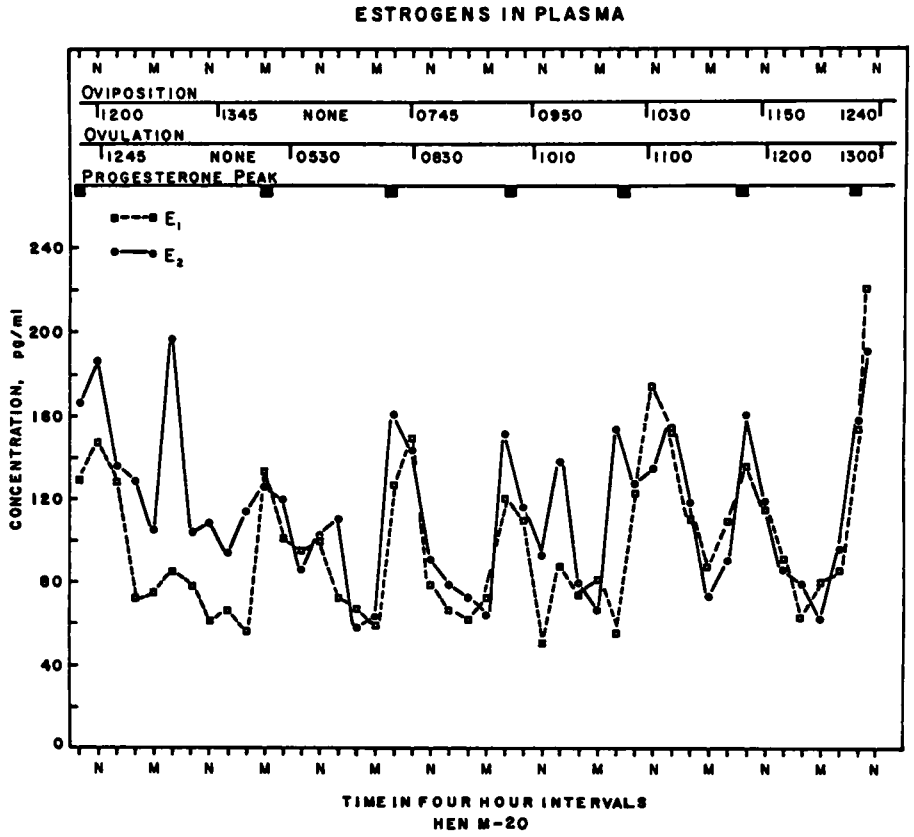

FIG. 1. Concentrations of plasma estrone and estradiol during a seven-egg ovulation cycle in hen M-20. The times of ovulation, oviposition and the calculated time of the progesterone peak are indicated. M = midnight; N = noon.

E_1 peak at the time of the second progesterone peak in Fig. 2, and the low concentrations of E_1 at the time of the progesterone peaks in Fig. 3. In both hens the basal levels of E_1 were markedly lower than in hens M-20 and M-21 which both showed synchronized progesterone and E_1 peaks.

In addition to these estrogen peaks, which appear to be synchronized with progesterone peaks, there are peaks of E_1 and E_2 which appear to be occurring without apparent regularity, e.g. E_2 peaks in hens M-19 (Fig. 3) and M-21 (Fig. 4) which appear between two progesterone-associated peaks. In hen M-20 (Fig. 1) there is a large E_2 peak between progesterone peaks 1 and 2, between 4 and 5, and between 5 and 6 but no such peaks appeared between progesterone peaks 2 and 3, 3 and 4, and 6 and 7. In hen M-8 (Fig. 5) there was a large estrone peak shortly after oviposition, a peak not observed in connection with ovipositions in other hens.

The data presented in Fig. 5 show that between −7:30 and −2:30 before ovulation the progesterone concentration tends to increase but superimposed on this "overall" increase in concentration of progesterone are peaks and valleys of concentrations of this hormone. Presumably if samples had been taken at 4-hour intervals the progesterone concentration would have shown a peak. These variations in progesterone concentrations suggest a pulsatile secretion of these steroids. Fig. 5 also shows that the peak of LH and of progesterone coincide and that both precede the E_1 and E_2 peaks. The rise in LH concentration appears to precede the increase in progesterone concentration.

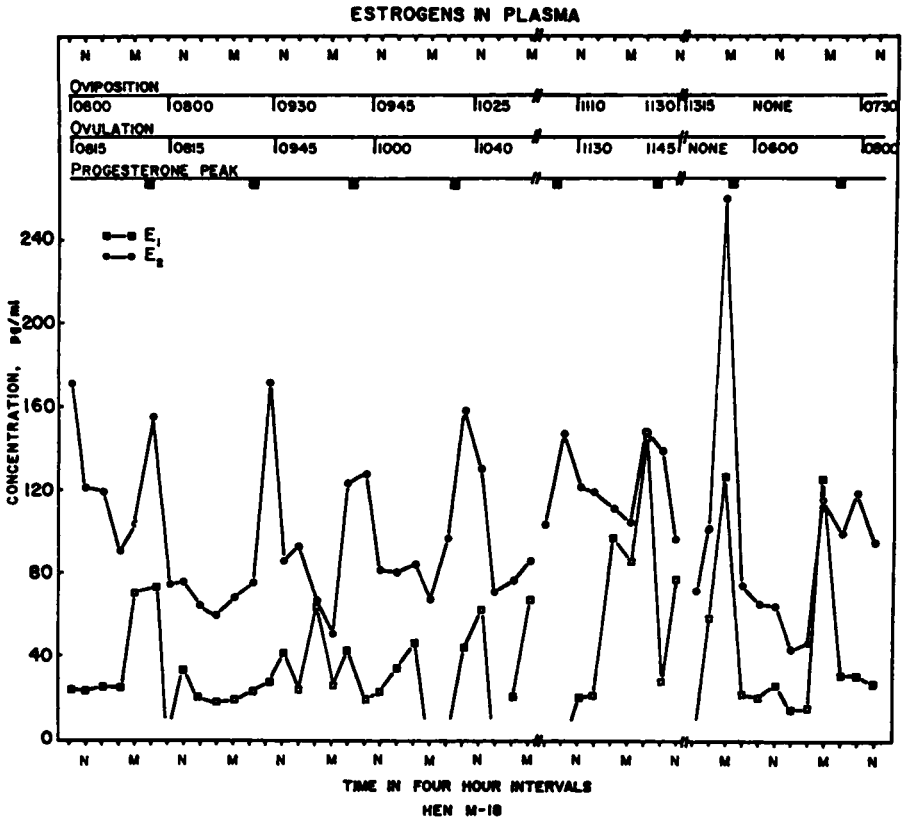

FIG. 2. Concentrations of plasma estrone and estradiol over a period of four successive ovulations, with an interruption of one day and an interruption of seven days during which the hen ovulated at the same hour each day. The times of the progesterone peaks are calculated. M = midnight; N = noon.

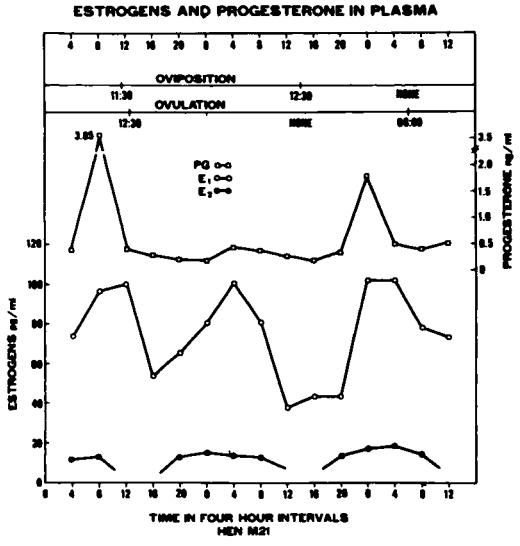

FIG. 3. Concentrations of plasma progesterone, estrone and estradiol in samples taken at the end of one sequence and the start of the next one in the laying hen.

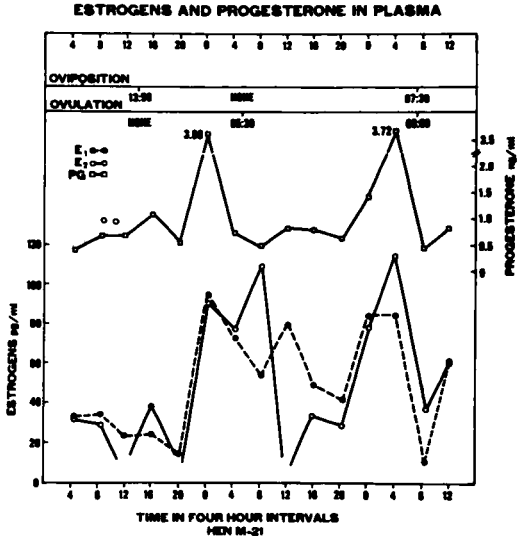

FIG. 4. Plasma progesterone, estrone and estradiol concentrations at the end of one ovulation-oviposition sequence and the start of the subsequent sequence. M = midnight; N = noon.

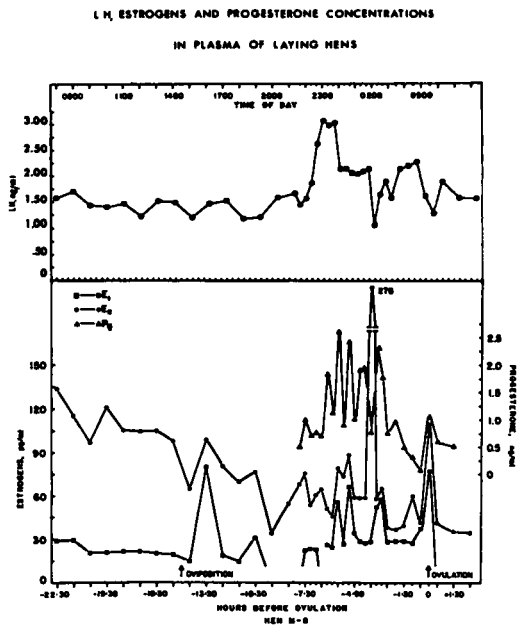

FIG. 5. Plasma estrone, estradiol, progesterone and LH concentrations with respect to the time of ovulation. Progesterone and LH determinations were started at the time of the expected progesterone peak. M = midnight; N = noon.

The apparently close association between estrogen and progesterone peaks suggested that estrogens either alone or in conjunction with progesterone might play a role in regulation of ovulation in the laying hen. We, therefore, designed two experiments to test the hypothesis that exogenous estradiol, alone or in conjunction with progesterone injections might affect ovulation, in view of the fact that exogenous progesterone can cause premature ovulations in chickens (Fraps, 1955).

In the first experiment doses of 0, 125, 250, 500 and 1,000 μg progesterone were injected in combination with 0, 25, 50 and 100 μg estradiol-17β (E_2). The results are presented in Table 2. On the basis of this experiment and after establishing the approximate dose which had been injected per kg body weight, we decided to inject a threshold dose of 125 μg progesterone/kg in combination with 0, 12.5, 25 and 50 μg E_2/kg. The results are reported in Table 2.

The fact that 125 μg progesterone/kg induced premature ovulations in 2 of 15 hens only, suggests that this dose was indeed near

TABLE 2

EFFECTS OF DIFFERENT DOSES OF ESTRADIOL 17β (E_2) AND PROGESTERONE (P) ON OVULATION OF THE C_1 FOLLICLE OF WHITE LEGHORN HENS

Dose P μg/hen	0	0	0	0	125	125	125	125	250	250	250	500	500	500	1000	1000	1000	1000		
Dose E_2 μg/hen	0	25	50	100	0	25	50	100	0	25	50	100	0	25	50	100	0	25	50	100
Ovulation:																				
Normal	4	5	5	4	5	5	3	5	3	2	1	4	0	0	0	0	0	0	0	0
Premature	0	0	0	1	0	0	2	0	2	3	4	1	5	5	5	6	5	5	6	5
No ovulation	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Dose P μg/kg b.w.		0	0	0	0	125	125	125	125											
Dose E_2 μg/kg b.w.		0	12.5	25	50	0	12.5	25	50											
Ovulation:																				
Normal		4	3	4	6	13	13	16	15											
Premature		0	0	0	0	2	4	3	0											
No ovulation		0	0	0	0	2	0	0	0											

threshold. It appears from the results that estradiol, at the doses tested, did not facilitate the ovulation-inducing effect of progesterone.

DISCUSSION

The concentrations of E_1 and E_2 reported here are in good agreement with values reported by Schröcksnadel *et al.* (1973), Peterson and Common (1972), Senior (1974) and Senior and Cunningham (1974), who also used radioimmunoassay methods. The values published by these authors and our values are, however, only about 1 percent of those reported by O'Grady (1968), who used a double isotope derivative method and about 10 percent of those reported by Ozon (1966), who used a fluorimetric method for analysis.

We are inclined to accept the concentrations obtained after the radioimmunoassay, not only because of the agreement with values reported by the investigators cited above, but also because there was a good agreement between results obtained on the same samples after use of the radioimmunoassay and use of a gas liquid chromatography-electron capture detection method (Laguë, 1972).

The laying and ovulatory cycles of the hens used in these studies have the desirable characteristic that the time at which each of these events occur shifts about 2 h each successive day till the end of the sequence is reached with the oviposition of an egg (usually) late in the afternoon and the lack of an ovulation shortly after this oviposition. The "internal clock" of the reproductive cycle has a period of about 26–28 h and is not synchronized by the 24-hour cycle of light and darkness. The clock is apparently reset at the end of the oviposition sequence (van Tienhoven and Planck, 1973). This asynchronous cycle with respect to the 14L:10D cycle permits separation of events which are imposed by the light cycle, events which occur each day at about the same time, from events which are linked to the ovulation-oviposition sequence events, which should shift about 2 h from day to day.

The results indicate clearly that the peaks of estrone, estradiol and progesterone concentrations indeed shift about 2–3 h every day and retain a constant time relationship with respect to the time of ovulation.

Peterson and Common (1972) also found that estrogen concentrations reached a peak about 6–8 h before ovulation. Senior and Cunningham (1974) established that the estradiol peak occurred about 6 h prior to ovulation and that this peak preceded the LH peak by about 2 h.

In our data, without exception, E_2 and progesterone peaks preceded ovulation by about 6–8 h. With respect to E_1 concentrations the situation may be different, as illustrated by the lack of an E_1 peak prior to the third ovulation in hen M-18 (Fig. 2) and the low concentration of E_1 in hen M-19 (Fig. 3). In this hen the concentration of E_1 was so low that no reasonable conclusion could be drawn about peak levels. These observations suggest that E_2 and progesterone might be causally involved in the induction of spontaneous ovulations. However, the experimental results obtained with estradiol-progesterone injections, using either ovulation-inducing doses or threshold doses of progesterone, indicate that estradiol, over the dose range tested, neither facilitated nor inhibited progesterone-induced ovulations.

The data concerning the concentrations of progesterone, E_1, E_2 and LH revealed that the LH is apparently secreted at a relatively steady rate and not in a pulsatile fashion [such as occurs in ovariectomized monkeys (Atkinson *et al.*, 1970; Dierschke *et al.*, 1970) and ewes (Reeves *et al.*, 1972)], whereas progesterone concentrations vary in a manner which suggests pulsatile release of progesterone. For estradiol and estrone the data appear equivocal at this time.

It is also of interest that even when samples are taken at 20-min intervals, it is not possible to determine which occurs first, the rise in LH or the rise in progesterone secretion. On the basis of experimental data it appears a justifiable speculation that there may be a positive feedback relationship between hypophyseal LH and ovarian progesterone in the laying chicken.

We are now engaged in determining these relationships in greater detail under different experimental conditions.

The presence of one LH peak only, in our experiments and in those reported by Cunningham and Furr (1972) and Furr *et al.* (1973) is at variance with the three peaks found by Bullock and Nalbandov (1967), Imai and Nalbandov (1971) and Nelson *et al.* (1965). This difference is probably the result of the techniques used. The three peaks were detected by using an ovarian ascorbic acid depletion (OAAD) bioassay whereas the one peak was detected by using a radioimmunoassya (RIA). The OAAD test is, however, not specific for LH, but also shows a response to arginine vastocin (Jackson and Nalbandov, 1969) whereas the RIA is specific for avian LH (Follett *et al.*, 1972).

We can not explain the reasons for the similarity in E_1 and E_2 concentrations found for hens M-20 (Fig. 1) M-21 (Fig. 3) and the lower concentrations for E_1 than for E_2 in hens M-18 (Fig. 2), M-19 (Fig. 3) and M-8 (Fig. 5).

ACKNOWLEDGMENTS

We wish to express our appreciation to Mrs. B. Kappauf Andreassen for doing the progesterone assay, to Dr. E. Echternkamp for his help with the estrogen assays, to Dr. W. Hansel for sharing the estrogen antibodies donated in turn by Dr. B. V. Caldwell. We thank Dr. R. K. Cole for making the progeny from the "C" strain available.

REFERENCES

ATKINSON, L. E., BHATTACHARYA, A. N., MONROE, S. E., DIERSCHKE, D. J., AND KNOBIL, E. (1970). Effects of gonadectomy on plasma LH concentrations in the rhesus monkey. Endocrinology 87, 847-849.

BULLOCK, D. W., AND NALBANDOV, A. V. (1967). Hormonal control of the hen's ovulation cycle. J. Endocrinol. 38, 407-415.

CUNNINGHAM, F. J., AND FURR, B. J. A. (1972). Plasma levels of luteinizing hormone and progesterone during the ovulatory cycle of the hen. *In:* "Egg Formation and Production" (B. M. Freeman and P. E. Lake, eds.), pp. 51-64. British Poultry Science Ltd., Edinburgh.

DIERSCHKE, D. J., BHATTACHARYA, A. N., ATKINSON, L. E., AND KNOBIL, E. (1970). Circhoral oscillations of plasma LH levels in the ovariectomized rhesus monkey. Endocrinology 87, 850-853.

FOLLETT, B. K., SCANES, C. G., AND CUNNINGHAM, F. J. (1972). A radioimmunoassay for avian luteinizing hormone. J. Endocrinol. 52, 359-378.

FRAPS, R. M. (1955). Egg production and fertility in poultry. *In:* "Progress in the Physiology of Farm Animals" (J. Hammond, ed.), Vol. 3, pp. 661-740. Buttersworth, London.

FURR, B. J. A., BONNEY, R. C., ENGLAND, R. J., AND CUNNINGHAM, F. J. (1973). Luteinizing hormone and progesterone in peripheral blood during the ovulatory cycle of the hen, *Gallus domesticus*. J. Endocrinol. 57, 159-169.

HAYNES, N. B., COOPER, K. J., AND KAY, M. J. (1973). Plasma progesterone concentrations in the hen in relation to the ovulatory cycle. Br. Poultry Sci. 14, 349-357.

IMAI, K., AND NALBANDOV, A. V. (1971). Changes in FSH activity of anterior pituitary glands and of blood plasma during the laying cycle of the hen. Endocrinology 88, 1465-1470.

JACKSON, G. L. AND NALBANDOV, A. V. (1969). A substance resembling arginine vasotocin in the anterior pituitary gland of the cockerel. Endocrinology 84, 1218-1223.

KAPPAUF, B. H. (1971). Plasma progesterone concentrations in the laying hen. M.S. Thesis, Cornell University.

KAPPAUF, B., AND VAN TIENHOVEN, A. (1972). Progesterone concentrations in peripheral plasma of laying hens in relation to the time of ovulation. Endocrinology 90, 1350-1355.

LAGUË, P. C. (1972). Plasma estrone and estradiol concentrations in the laying hen in relation to the ovulatory cycle. Ph.D. Thesis, Cornell University.

MIKHAIL, G., WU, C. H., FERIN, M., AND VANDE WIELE, R. L. (1970). Radioimmunoassay of plasma estrone and estradiol. Steroids 15, 333-352.

NELSON, D. M., NORTON, H. W., AND NALBANDOV, A. V. (1965). Changes in hypophysial and plasma LH levels during the laying cycle of the hen. Endocrinology 77, 889-896.

O'GRADY, J. E. (1968). The determination of oestradiol and oestrone in the plasma of the domestic fowl by a method involving the use of labelled derivatives. Biochem. J. 106, 77-86.

OZON, R. (1966). Mise en évidence d'hormones stéroïdes oestrogènes dans le sang de la poule adulte et chez l'embryon de poulet. C. R. Acad. Sci. 261, 5664-5666.

PETERSON, A. J., AND COMMON, R. H. (1971). Progesterone concentration in peripheral plasma of laying hens as determined by competitive protein-binding assay. Can. J. Zool. 49, 599-604.

PETERSON, A. J., AND COMMON, R. H. (1972). Estrone and estradiol concentrations in peripheral plasma of laying hens as determined by radioimmunoassay. Can. J. Zool. 50, 395-404.

PETERSON, R. E. (1957). The identification of corticosterone in human plasma and its assay by isotope dilution. J. Biol. Chem. 225, 25-37.

REEVES, J. J., O'CONNELL, D. A., AND DENOISCIA, F. (1972). Effects of ovariectomy on serum luteinizing hormone (LH) concentrations in the anestrous ewe. J. Animal Sci. 35, 73–78.

SCHOLLER, R. (1962). Solvants et réactifs purs. *In:* "Analyse des Stéroïdes Hormonaux" (M. F. Jayle, ed.), Vol. II, pp. 447–480. Masson et Cie, Paris.

SCHRÖCKSNADEL, H., BATOR, A., AND FRICK, J. (1973). Plasma estradiol-17β level in the domestic fowl. Steroids 22, 767–773.

SENIOR, B. E. (1974). Radioimmunoassay of oestrone and oestradiol in the peripheral plasma of the domestic fowl in various physiological states of hypophysectomized and ovariectomized fowls. Acta Endocrinol. 75, 133–140.

SENIOR, B. E., AND CUNNINGHAM, F. J. (1974). Oestradiol and luteinizing hormone during the ovulatory cycle of the hen. J. Endocrinol. 60, 201–202.

SNEDECOR, G. W. (1965). "Statistical Methods", 5th Edition, Iowa State University Press, Ames, Iowa.

STOCKELL HARTREE, A., AND CUNNINGHAM, F. J. (1969). Purification of chicken pituitary follicle-stimulating hormone and luteinizing hormone. J. Endocrinol. 43, 609–616.

VAN TIENHOVEN, A., AND PLANCK, R. J. (1973). The effect of light on avian reproductive activity. *In:* "Handbook of Physiology" Section 7, Vol. II, Part 1, pp. 79–107. American Physiological Society, Washington, D.C.

WILSON, SUSAN C., AND SHARP, P. J. (1973). Variations in plasma LH levels during the ovulatory cycle of the hen, *Gallus domesticus*. J. Reprod. Fert. 35, 561–564.

RECOMMENDED REVIEWS

FRAPS, R. M. (1955). Egg production and fertility in poultry. *In:* "Progress in the Physiology of Farm Animals" (J. Hammond, ed.), Vol. 3, pp. 661–740, Buttersworth, London.

VAN TIENHOVEN, A., AND PLANCK, R. J. (1973). The effect of light on avian reproductive activity. *In:* "Handbook of Physiology." Section 7, Vol. II, Part 1, pp. 79–107, American Physiological Society, Washington, D.C.