

### Sex Hormones During the Laying Cycle of Hens

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ONE HUNDRED female chicks at 2 months of age were used in this study. Date and time of sexual maturity and egg position in the clutch were recorded for individual hens. Blood samples were collected by direct veinepuncture of the wing. Radioimmunoassay was used to determine the level of progesterone and estradiol in plasma.

The levels of the two hormones were the lowest 4 weeks prior to sexual maturity. The more the approach to sexual maturity, the higher the levels of progesterone and 17 $\beta$  estradiol. This suggests that the ovaries begin to induce increasing amounts of sex hormones 2 to 3 weeks prior to sexual maturity. The highest levels of progesterone and estradiol were at the second day prior to the first oviposition of the first egg.

During the ovulatory cycle the progesterone and estradiol reached their highest levels 3 to 2 and 4 to 3hr before oviposition respectively, and dropped to their lowest level at the time of oviposition. The progesterone ranged from 0.09 to 4.5 ng/ml, while the estradiol ranged from 34.8 to 275.0 pg/ml during the ovulatory cycle.

There is a critical ratio between estrogen and progesterone required to prime the LH positive feed back mechanism (Wilson and Sharp, 1975 a,b). The release of LH is facilitated by the positive feedback of combination of estrogen and progesterone in 2 phases. The first, is the priming phase which dependent on the presence of estrogen and progesterone in the blood. The second, is the inductive phase which depends only on incremental change in plasma progesterone concentration.

Many workers determined progesterone levels at short intervals. They found that the maximum concentration was about 2 to 6hr prior to ovulation and fell there after to the basal levels at ovulation (Peterson and Common, 1971 ; Kapauf and Van Tienhoven, 1972 ; Haynes *et al.*, 1973 ; Wilson and Sharp, 1975 a,b ; and Etches, 1979). These results have been substantiated in studies by Cunningham and Furr (1972) , using a radioimmuno assay (RIA) technique.

Furr *et al.* (1973) observed a consistent pattern in the plasma level of LH and progesterone. Significantly more LH and progesterone was present in the plasma 4 to 7 hr before ovulation than at other times during the cycle. An increase in the level of progesterone either preceded that LH or the 2 hormones increased simultaneously.

Williams and Sharp (1977) found that just before the first egg was laid, the level of progesterone in the plasma rose, established within the range 0.4 to 2.2 ng/ml and remained in the range while birds were in lay.

Recently Hammond *et al.* (1978 a,b) showed that the level of progesterone rose to a broad preovulatory peak 2 to 6 hours before oviposition, followed by a precipitous fall before oviposition, reaching a minimum of 0.4 ng/ml shortly after oviposition.

The changes in the level of circulating estrogen control the timing of ovulation in the domestic fowl (Heald *et al.*, 1967). Senior and Cunningham (1974) found that the lowest concentration of both estradiol and LH occurred 10 to 14 hr before ovulation. They added that while the concentration of estradiol increased (significantly eight hours before ovulation to reach a peak 6 hr before it, LH did not increase significantly until 6 hours before ovulation. Hammond *et al.* (1978 a) reported that estrogen level began to rise at 7hr prior to oviposition, reaching a maximum of over 200 pg/ml 1 hour before oviposition.

### Material and Methods

One hundred female chicks 2 months of age were used in this study. They were brooded on floor pens for 6 weeks, then were grown in pens with open yards. They were fed an ad libitum ration containing 16.82% protein and 2780 Cal KE/kg (Table 1). As each pullet reached sexual maturity, it was transferred to an individual cage receiving ad libitum food and water. At 18 weeks of age, all the birds received continuous lighting with intensity of 0.75 ft c. from sunset to sunrise. Date of sexual maturity was recorded for individual hens. Data and time of egg position for every hen were recorded. The approximate time of ovulation for each hen was obtained from the time of oviposition. The time lapse between oviposition and ovulation was considered to be 30 min (Nalbandov, 1958 ; and Kappanf and Van Tienheven, 1972). Monitoring oviposition was continued for a period of 12 weeks from the age of sexual maturity of the bird.

The time needed for the egg formation was also calculated from the approximate ovulation time and the next oviposition.

Blood samples of 3 to 5ml were collected by direct veinepuncture of the wing into a heparinized syringe, then transferred to a centrifuge tube. The samples were centrifuged at 720gr. and at 4°. The plasma was then separated and stored at -20°C. until the time of hormonal determination. Date and time of collection were recorded for each sample the date and time of collection was then retabulated according to the oviposition for each hen. Radioimmunoassay was used in this study for determination the level of progesterone and estradiol in plasma as reported by Hunter (1972). All re-agents used in progesterone and estradiol assay were produced and supplied by New England Nuclear Biochemical assay Laboratories and were supplied concentrated in a lyophilized form.

TABLE 1. Composition and calculated analysis of the layer Ration used.

Feed Stuff (Kg)	%	Crude Protein	M.E./ Kg	Calcium :	Phosphorus	Meth.	Lys.	Cys.
Yellow corn	45	3.96	1527.3	0.013	0.126	0.054	0.099	0.067
Decorticated seed meal	7	3.14	132.2	0.010	0.068	0.037	0.120	0.045
Rice bran	14	2.23	291.6	0.010	0.055	0.021	0.083	0.014
Rice germ meal	15	2.62	408.0	0.030	0.180	0.054	0.121	—
fish meal	6	1.82	208.2	0.137	0.107	0.125	0.321	0.093
Molasses yeast	7	2.49	108.6	0.077	0.008	—	—	—
Limestone	2.5	—	92.5	0.906	—	—	—	—
Bone meal	2	0.56	12.0	0.388	0.232	0.004	0.018	—
NaCl	0.5	—	—	—	—	—	—	—
Mineral mix.	1.0	—	—	—	—	—	—	—
Total	100	16.82	2780.0	1.57	0.77	0.295	0.782	0.169

### Results and Discussion

#### *Hormone levels in immature hens*

Table 2 shows that the more the approach to sexual maturity (the point of lay) the higher the levels of 17 $\beta$  estradiol and progesterone in plasma.

The estradiol level increased from 9.0 pg/ml at 27th day prior to sexual maturity to 34.0 pg/ml at 6 days prior to it. The level of estradiol ranged from 37.0 to 39.0 pg/ml during the fifth and fourth days before the first egg was laid. It was ranged from 35.0 to 47.0 pg/ml during the three days prior the onset of lay. On the second days prior to the onset of lay the level was the highest.

The levels of progesterone were the lowest during the period from 42 *nd* to the 11th day before sexual maturity. The level increased from this period to reach 0.048 ng/ml 10 days before sexual maturity, then it reached 0.100 ng/ml on the 6th day before sexual maturity. On the days prior to the first egg being laid, the progesterone levels ranged from 0.15 to 0.40 ng/ml during the fifth and fourth days and from 0.25 to 0.28 ng/ml on the third day. The highest levels of progesterone were on the second day prior to the first oviposition of the first egg with an average of 0.487 ng/ml (Table 2).

TABLE 2. Levels of 17B estradiol and Progesterone of the Fayoumi pullets prior to the Day of Sexual Maturity.

Days before sexual maturity	17 B Estradiol pg/ml	Pogestcone ng/ml
42	9.0	0.012
27	9.0	0.020
26	11.0	0.020
26	14.0	0.024
19	19.0	0.026
19	23.0	0.024
13	24.0	0.030
12	25.0	0.028
11	23.0	0.028
10	26.0	0.048
9	31.0	0.050
8	37.0	0.050
7	35.0	0.100
6	34.0	0.100
5	37.0	0.150
5	39.0	0.150
4	37.0	0.400
4	38.0	0.150
3	35.0	0.280
3	41.0	0.270
3	47.0	0.280
2	47.0	0.380
2	67.0	0.500
2	77.0	0.460
2	85	0.600

The levels of the two hormones were the lowest about 4 weeks prior to sexual maturity, and increased gradually when the birds approached sexual maturity. These results suggested that the ovaries of Fayoumi hens began to induced increasing amounts of sex hormones 2 to 4 weeks prior to sexual maturity. Hafez and Kamar (1955) reported that the ovary undergoes a steady increase in weight until the age of sexual maturity. The development changes in the reproductive organs were more marked just prior to sexual maturity than after its onset. Williams and Sharp (1977) reported that the plasma progesterone level remained at a low level in the immature hens until 1 week prior to the onset of lay.

#### *Basal levels of 17 $\beta$ Estradiol and progesterone in mature hens*

On the day of missed ovulation (during the time interval between clutches, with hens that did not lay the next day) the levels of the 2 hormones averaged 37.62 pg/ml and 0.22 ng/ml for the estradiol and progesterone respectively. These findings agree with those of Bullock and Nalbandov (1967), Peterson and Common (1971), Arcoss and Opel (1971); Kappanf and Van Teinhoven (1972); and Furr *et al.* (1973) who reported that no hormonal peaks were observed on the day of missed ovulation.

#### *Hormone levels during the ovulatory cycle*

It was noticed that the lowest concentrations of both hormones were at 28 to 26 hr before oviposition and started to a peak at 21hr prior to oviposition (Table 3). Levels of both hormones dropped to their basal levels from 12 to 7hr before oviposition. It was noticed that the estradiol and progesterone reached their highest levels 4 to 3 and 3 to 2 hr before oviposition respectively. Both hormones dropped to their lowest level at the time of oviposition.

Progesterone level increased significantly at 6.5 hours before ovulation to reach a maximum level at 4.5 hours before ovulation (Furr *et al.*, 1973; and Etches and Cunningham, 1975). Etches (1979) reported that it reached a maximum level of 6.9 ng/ml 3 to 2 hr before ovulation, then returned to baseline concentrations at the time of ovulation. In this study the results of progesterone peaks were similar to previously reported data (Cunningham and Furr, 1972; Furr *et al.*, 1973 and Etches, 1979). While in previously published data the progesterone level rose gradually to its peak, its rise was abrupt in this study. This difference may be due to the breed and the light treatment effect. Williams and Sharp (1977) reported that the progesterone level in immature hens ranged from 0.1 to 0.5 ng/ml, while it ranged from 0.012 to 0.6 ng/ml in this study.

During ovulatory cycle the progesterone ranged from 0.4 (Williams and Sharp, 1977) to 17.1 ng/ml (Furr and Pope, 1970), while it ranged from 0.09 to 4.5 in this study.

Estradiol concentration came to the lowest at 14 to 10 hr before ovulation, and increased significantly to reach a peak 6 hr before ovulation (Senior and Cunningham, 1974). This previous data differ slightly from the present when

the estradiol peak was at 4 to 3 hr before oviposition. The breed difference and light treatment may also have affected these results.

While the estradiol level ranged from 32.0 to 160 pg/ml during the ovulatory cycle as reported by Senior and Cunningham (1974), it ranged from 34.8 to 275.0 pg/ml in this study. The higher values obtained in this study may be due to continuous illumination and breed difference.

TABLE 3. 17-B Estradiol and Progesterone levels During the Laying Cycle of the Fayoumi Hens.

Hours before oviposition	17-B Estradiol Mean $\pm$ SE pg/ml	Progesterone Mean $\pm$ SE ng/ml
0	34.83 $\pm$ 5.8 <sup>b</sup>	0.09 $\pm$ 0.02 <sup>b</sup>
1	36.00 $\pm$ 14.2 <sup>b</sup>	0.49 $\pm$ 0.17 <sup>b</sup>
2	57.00 $\pm$ 9.1 <sup>b</sup>	3.46 $\pm$ 0.61 <sup>a</sup>
3	260.00 $\pm$ 77.6 <sup>a</sup>	4.51 $\pm$ 1.33 <sup>a</sup>
4	253.00 $\pm$ 42.3 <sup>a</sup>	1.51 $\pm$ 0.49 <sup>b</sup>
5	177.50 $\pm$ 25.5 <sup>b</sup>	0.60 $\pm$ 0.31 <sup>b</sup>
6	89.00 $\pm$ 62.9 <sup>b</sup>	0.32 $\pm$ 0.07 <sup>b</sup>
7	55.67 $\pm$ 11.2 <sup>b</sup>	0.35 $\pm$ 0.13 <sup>b</sup>
10	37.00 $\pm$ 8.0 <sup>b</sup>	0.75 $\pm$ 0.13 <sup>b</sup>
12	53.50 $\pm$ 41.5 <sup>b</sup>	0.75 $\pm$ 0.35 <sup>b</sup>
21	275.00 $\pm$ 50.0 <sup>a</sup>	1.45 $\pm$ 0.62 <sup>b</sup>
22	252.50 $\pm$ 27.5 <sup>a</sup>	0.90 $\pm$ 0.05 <sup>b</sup>
23	240.00 $\pm$ 44.69	0.53 $\pm$ 0.27 <sup>b</sup>
24	93.33 $\pm$ 17.6 <sup>b</sup>	0.17 $\pm$ 0.05 <sup>b</sup>
25	86.33 $\pm$ 42.1 <sup>b</sup>	0.17 $\pm$ 0.03 <sup>b</sup>
26	51.00 $\pm$ 27.0 <sup>b</sup>	0.17 $\pm$ 0.02 <sup>b</sup>
27	44.50 $\pm$ 33.5 <sup>b</sup>	0.10 $\pm$ 0.00 <sup>b</sup>
28	41.33 $\pm$ 6.1 <sup>b</sup>	0.08 $\pm$ 0.02 <sup>b</sup>
29	6.67 $\pm$ 23.4 <sup>b</sup>	0.73 $\pm$ 0.32 <sup>a</sup>
30	150.00 $\pm$ 56.7	2.76 $\pm$ 0.68 <sup>a</sup>

\* Values within hormones with different superscripts differ significantly ( $P \leq 0.01$ ) from each other (Duncan, 1955).

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## References

- Arcos, M. and Opel, H. (1971) Plasma progesterone in hen during the ovulatory cycle of the domestic hen (*Gallus domesticus*), *Poultry Sci.* 34, 776.
- Bullock, D.W. and Nalbandov, A.V. (1967) Hormonal control of hen's ovulatory cycle, *J. Endocr.* 38, 407.
- 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. Eds B.M. Freeman and P.E. Lake, *British Poultry Sci. Ltd. Edinburgh*.
- Duncan, D.P. (1955) Multiple range and multiple t tests. *Biometrics*, 11:1-42.
- Etches, R.J. (1979) Plasma concentrations of progesterone and corticosterone during the ovulatory cycle of the hen (*Gallus domesticus*) *Poultry Sci.* 58, 211,
- Etches, R.J. and Cunningham, F.J. (1975) The role of preovulatory release of progesterone in chicken. *J. Endocr.* 64, 74p.
- Furr, B.J.A. and Pope, G.S. (1970) Identification of cholesterol, progesterone, 20 hydroxy-pregn-4-one zone epimers and 5 $\beta$  and rosterone 3, 17 dione in plasma and ovarian tissue of the domestic fowl. *Steroids* 16, 471,
- 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. *J. Endocr.* 57, 159.
- Hafez, E.S.E. and Kamar, G.A.R. (1955) Development changes in the reproductive organs of the domestic fowl, *Poultry Sci.* 34, 1002.
- Hammond, R.W., Olson, D.M. and Hertelendy, F. (1978a) striking changes in prostaglandin E (PGE) and F (PGE) and progesterone levels in pre and post ovulatory follicles during the laying cycle, *Poultry Sci.* 57, 1141,
- Hammond, R.W., Olson, D.M. and Hertelendy, F. (1978b) Plasma levels of prostaglandin and progesterone during the laying cycle of the domestic hen. *Poultry Sci.* 57, 1141,
- Haynes, N.B., Cooper, K.J. and Kay, M.J. (1973) Plasma progesterone concentration in the hen in relation to the ovulatory cycle. *Br. Poultry Sci.* 14 : 349-357.
- Heald, P.J., Eurnival, B.E. and Rookledge, K.A. (1967) Changes in the levels of luteinizing hormone in pituitary of the domestic fowl during ovulatory cycle. *J. Endocr.* 37, 73.
- Hunter, W.M. (1972) The radioimmunoassay of hormones In Brown. P.S. Proc. Soc. Endocr. *J. Endocr.* 54, 1.
- Kappanf, B. and Van Teinhoven, A.V. (1972) Progesterone concentration in peripheral plasma of laying hens in relation to the time of ovulation, *Endocrinology* 90, 1350.
- Nalbandov, A.V. (1958) Reproductive physiology, Freeman and Co., San Francisco.
- Peterson, A.J. and Common R.H. (1971) Progesterone concentration in peripheral plasma of laying hens as determined by radioimmunoassay. *Can J. of Zool.* 50, 395,
- Senior, B.E. and Cunningham, F.J. (1974) Oestradiol and Luteinizing hormone during the ovulatory cycle of the hen. *J. Endocr.* 60, 201.
- Williams, J.B. and Sharp, P.J. (1977a) comparison of plasma progesterone in growing hens from eight weeks of age to sexual maturity. *J. Endocr.* 75, 447,
- Wilson, S.C., and Sharp, P.J. (1975a) Changes in plasma concentrations of luteinizing hormone at various times during the ovulatory cycle of the domestic hen. *J. Endocr.* 67, 59.
- Wilson, S.C., and Sharp, P.J. (1975b) changes in plasma concentrations of luteinizing hormone after injection of progesterone at various times during the ovulatory cycle of the domestic hen. *J. Endocr.* 67, 59.

### الهرمونات الجنسية أثناء سلسلة وضع البيض

جمال قمر - حمدي حسان - مختار عيد الفتح - فريد استينو و محمد النادى

استخدم في هذا البحث ١٠٠ كتكوت أنثى عمر شهرين \* وقد تم تسجيل تاريخ ووقت النضج الجنسي وميعاد وضع البيض في السلسلة \* كذلك اخذت عينات الدم من وريد الجناح على فترات متتالية - وقت وضع البيضة \* واستخدمت النظائر المشعة لاجل تقدير مستوى البروجستيرون والاستراديول في البلازما \*

مستوى الهرموني كان منخفض قبل النضج الجنسي بمدة ٤ أسابيع \* وكلما قربنا من ميعاد النضج الجنسي زاد مستوى الهرموني \* هذا يبين أن المبيض بدأ في زيادة انتاجه من الهرمونات الجنسية وذلك قبل النضج الجنسي بمدة ٤ - ٣ أسابيع . أعلى مستوى من البروجستيرون والاستراديول حدث قبل وضع اول بيضة بمدة يومين \*

خلال سلسلة وضع البيض نجد ان مستوى البروجستيرون زاد قبل وضع البيض في داخل السلسلة بمدة ٣ - ٢ ساعة أما الاستراديول فقد زاد قبل وضع البيض بمدة ٤ - ٣ ساعة ، ثم بعد ذلك ينخفض مستوى هذه الهرمونات أثناء وضع البيضة \*