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PLASMA CONCENTRATIONS OF ESTRADIOL, PROGESTERONE, TRIIODOTHYRONINE AND SOME BLOOD CONSTITUENTS DURING OVULATORY CYCLE OF LAYING HENS

Nagwa A. Ahmed, A.A. El-Far, M.A. Kicka and G.K. Mehaisen

Department of Animal Production, Faculty of Agriculture, Cairo University

SUMMARY

Fayoumi and Brown Lohman Selected Leghorn (LSL) at the peak of egg production were used in this study. Birds were individually housed and fed laying diet. Egg production was recorded daily and blood samples were collected from eight hens of each strain at 0, 2, 6, 12, 18, 22, 25 hr post oviposition to determine erythrocyte count (RBC's), packed cell volume% (PCV) and hemoglobin concentration (Hb). Plasma estradiol (E2), progesterone (P4) and Triiodothyronine (T3) were also determined.

Plasma E2 and T3 were higher in Fayoumi than LSL all over the studied sampling time. However, plasma P4 of LSL was significantly higher at 18 and 22 hr than that of Fayoumi. The highest plasma concentrations of E2 and P4 occurred at 0 and 22 hr post oviposition, while T3 maintained almost constant at high level throughout the six hr post oviposition. Total RBC's count, Hb concentrations and PCV% values were lower in LSL than Fayoumi hens and the highest values were recorded at 12 hr post oviposition for RBC's and PCV% and at six hr for Hb.

Keywords: Laying hens, E_2, P_4, T_3 , RBC's, PCV, Hb, ovulatory cycle

INTRODUCTION

Significant changes occur in hormonal balance of the birds during the ovulatory cycle. The ovary of the mature hen contains a hierarchy of yolk-filled follicles. This hierarchy is organized so that a new follicle is recruited for next ovulation as soon as the largest follicle ovulates. The ovarian steroides include progesterone, estrogens and androgens. Few studies discussed the relationship between these steroids during the ovulatory cycle, especially, in high and low producing laying hens. Sturkie (1986) reported that the metabolic rate of progesterone is lower in laying than in non-laying birds. Nirmalan and Robinson (1972) stated that estrogen tends to decrease erythrocytes number mainly by depressing erythropoiesis initially. Some investigators have reported

levels of hemoglobin lower in laying than in non-laying chickens, but others have reported no difference (Sturkie, 1965).

The objective of this study was to determine the changes in estradiol, progesterone and triiodothyronine level during the ovulatory cycle in the plasma of low producer (Fayoumi) and high producer (LSL) laying hens. Also, to study the relationship between these changes and some hematological parameters.

MATERIALS AND METHODS

The present study was carried out in the Poultry Research Farm and Laboratory of Poultry Physiology, Animal Production Department, Faculty of Agriculture, Cairo University.

Birds

One hundred and eighty Egyptian Fayoumi and Brown Lohman selected Leghorn (LSL) were individually housed in wire cages. Starting from 18 weeks of age, birds were exposed to 14 hr light daily.Artificial light was applied being increased half an hour daily every two weeks up to 17hr. Feed (17% crude protein, ME, 2800kcal/kg diet, 3.7% calcium and 0.66% phosphorus according to NRC (1984) requirements) and water were provided for ad libitum consumption. The age of sexual maturity was 150 day and 187 day for LSL and Fayoumi hens, respectively. Egg production was recorded (from the first egg to the seventh month of production) for each female throughout the experiment.

Blood analysis

At the peak of egg production blood samples were collected from 16 hens (8 of each strain) at 0, 2, 6, 8, 12, 18, 22 and 25 hr post-oviposition to determine the hormonal changes and related hematological properties. Blood samples were withdrawn from the wing vein into a hyparinized syringe. Total RBC's count (10^6/mm^3 blood), PCV value (%) and hemoglobin concentration (g/100m/blood) were immediately determined for each individual hen. Blood samples were centrifuged at 3000 rpm for 5 minutes to collect plasma which was stored at -20°C for hormonal assays.

Hormonal assay

Direct radio immunoassay (R.I.A) technique was performed for plasma progesterone, estradiol (Etches *et al.*, 1981) and triiodothyronine (May, 1978) assessments ready antibody coated tube kits (Diagnostic Product Corporation, Los Angeles) were used according to the procedure outlined by the manufacturer. Crossreaction of progesterone, while it was 2.2%, 3.4%, 9.0% and 3.2% with 11-Deoxycorticosterone, 17∝-Hydroxyprogesterone, 5∝-Pregnan- 3,20 dione and 5β-pregnan-3, 20 dione, respectively. It also was

less than 1.0% with corticosterone, 20β-dihydroprogesterone, pregnenolone, midroxyprogesterone and tesosterone, and less than 0.1% with any of other steroids. Estradiol antibody (at 50% binding) showed 100% cross reaction with estradiol while it was 4.4%, 10.0% and 1.8% with d-equilenin, estrone and estrone -β-D-glucuronide and ethinyl estradiol, respectively. It was less than 1.0% with any of the other steroids. Total triiodothyronine antiserum is highly specific for triiodothyronine. The crossreactivity of total T3 antiserum was 100% with T3, while it was 19.8% and 1.1% with triiodothyroacetic acid and D-thyroxine, respectively. It was less than 1% with any of the other natural compounds that might be present in the samples.

Statistical analysis

Data collected were subjected into two factor-factorial analysis of variance (MSTAT, 1986). Differences among means were tested using Duncan Multiple Range Test (Duncan, 1955).

RESULTS

The levels of estradiol hormone (E2) were significantly higher in Fayoumi than LSL hens throughout the ovulatory cycle (Table 1and Fig.1). The average E2 level in LSL was 113.3 pg/ml plasma versus 200.5 pg/ml plasma in Fayoumi hens. The highest levels of hormone in LSL hens were recorded during 2 hr after and 22 to 25 hr post oviposition (Fig.1). Plasma estradiol levels in Fayoumi hens fluctuated in a predictable pattern in relation to the ovulatory cycle with a major and minor peak occurring at zero and 22 hour post-oviposition, respectively.

Table 1. Plasma estrogen (E2), progesterone (P4), E2 / P4 ratio and triiodothyronine (T3) during ovulatory cycle in LSL and Fayoumi laying hens

Item	Time (hr)							SE
	0	2	6	12	18	22	25	
E_2 (pg/ml)								
LSL	121.7^{cd}	124.7^{bcd}	108.6^{cd}	104.6^{d}	100.1^{d}	116.0^{cd}	117.0^{cd}	30.3
Fayoumi	221.9^{ab}	166.4^{bcd}	197.3^{abcd}	207.8^{abc}	166.7^{bcd}	265.1^{a}	178.4^{abcd}	
P_4(ng/ml)								
LSL	0.649^{bc}	0.236^{e}	0.216^{e}	0.267^{de}	0.529^{cd}	1.334^{a}	0.446^{cde}	0.084
Fayoumi	0.871^{b}	0.314^{de}	0.343^{de}	0.331^{de}	0.441^{cde}	0.521^{cd}	0.365^{de}	
E_2/P_4 ratio								
LSL	0.185	0.528	0.503	0.392	0.189	0.087	0.262	
Fayoumi	0.255	0.530	0.575	0.628	0.378	0.509	0.489	
T_3 (ng/dl)								
LSL	89.13^{bc}	96.77^{b}	86.69^{bc}	72.77^{cd}	51.32^{e}	48.05^{e}	68.22^{d}	5.37
Fayoumi	90.28^{bc}	88.74^{bc}	116.7^{a}	98.12^{b}	92.46^{b}	88.06^{bc}	83.63^{bcd}	

^{a,b,c,d,e} Means within each traits having different superscripts significantly differ (P<.05)

Plasma P4 concentration of LSL and Fayoumi hens had the same trend during the ovulatory cycle. That it decreased significantly from zero to 2 hr post-oviposition, then gradually increased significantly to reach the maximum of hormone level at 22 hr post-oviposition and decreased significantly at 25 hr post-oviposition (Table 1 and Fig.1). The values of P4 levels in Fayoumi plasma were higher than in plasma of LSL hens from zero to 12 hr post-oviposition then, the opposite trend was observed at 22 and 25 hr post-oviposition (Fig.1). At 22 hr stage the P4 concentration was augmented in LSL hens to reach more than double the corresponding concentration in Fayoumi hens.

Figure 1 illustrates that the levels of T3 hormone had an opposite trend in LSL and Fayoumi hens during the first six hours of ovulatory cycle, then similar decreasing trend was detected in both strains. The levels of T3 hormone tended to decrease, except at 25 hr post-oviposition in plasma of LSL hens, it was increased significantly (Table 1). Generally, values of T3 hormone of Fayoumi were higher than LSL through the oviposition cycle (Fig.1).

Approximately similar trend was found in the count of RBC's in the two strains through ovulatory cycle (Fig.2). However, the values of total RBC's in blood of Fayoumi were significantly higher than that in LSL (Table 2). The maximum count of RBC's was observed at 12 hr post-oviposition.

Table 2. Red blood cells (RBC's), packed cell volume (PCV %) and hemoglobin (Hb) during ovulatory cycle in LSL and Fayoumi laying hens

Item	Time (hr.)							SE
	0	2	6	12	18	22	25	
RBC's (10^6/ml)								
LSL	2.64^{def}	2.53^{def}	2.70^{def}	2.79^{cde}	2.41^{ef}	1.81^{g}	1.74^{g}	0.12
Fayoumi	3.11^{abc}	3.18^{ab}	3.26^{a}	3.38^{a}	2.86^{bcd}	2.77^{cde}	2.35^{f}	
PCV%								
LSL	25.9^{ab}	23.7^{bcdefg}	24.0^{bcdef}	25.3^{abc}	24.6^{bcde}	22.0^{fg}	21.3^{g}	0.8
Fayoumi	25.7^{ab}	25.0^{abcd}	25.1^{abcd}	27.2^{a}	22.6^{defg}	22.9^{cdefg}	22.3^{efg}	
Hb (g/ 100 ml)								
LSL	9.11^{abc}	9.57^{ab}	9.89^{a}	9.14^{abc}	8.54^{c}	9.00^{bc}	8.39^{c}	0.27
Fayoumi	9.68^{ab}	9.57^{ab}	9.96^{a}	8.29^{c}	8.96^{bc}	8.82^{bc}	8.29^{c}	

^{a,b,c,d,e,f,g} Means within each traits having different superscripts significantly differ ($P<0.05$)

Almost similar trend was observed in backed cell volume (PCV%) in blood of both strains. It decreased until 6 hr post-oviposition, then it increased at 12 hr and decreased again at 18 hr (Fig.2). However, the decrease in PCV% was sharp in blood of Fayoumi hens. After that PCV% decreased significantly in LSL hens, while an increase occurred at 22 hr in Fayoumi (Table 2).

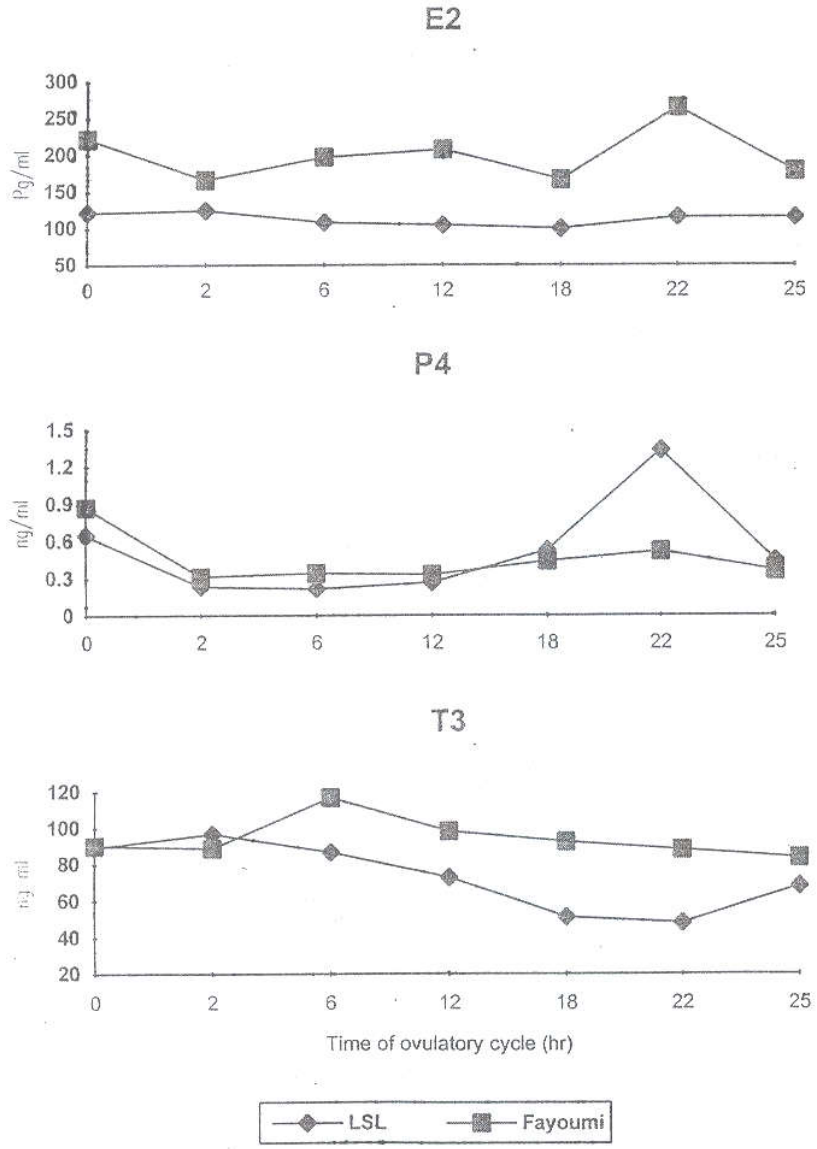

Figure 1. Plasma estrogen (E2), progesterone (P4) and triiodothyronine (T3) during ovulatory cycle in LSL and Fayoumi laying hens

The highest value of hemoglobin concentration (g/100ml) was noticed in blood of LSL and Fayoumi at 6 hr post-oviposition. However, the minimum value was determined at 25 hr post-oviposition in LSL and at 12 and 25 hr post-oviposition in Fayoumi hens (Table 2).

DISCUSSION

In the present study there was a tendency for higher E2 concentration in Fayoumi hens (the low producing strain) than in LSL hens (the high producing strain). This result was in harmony with Mehaisen (1997) who found that during the ovulatory cycle the values of E2 was higher in Fayoumi (237.5 pg/ml) than in LSL hens (116.3pg/ml). Ahmed Nagwa *et al.* (1997) found that E2 concentration of Fayoumi plasma at the peak of egg production was higher (218.8 pg/ml) than LSL (113.1 pg/ml). They reported that higher production rate of LSL hens (20-30 eggs per sequence) than Fayoumi (3-5 eggs per sequence) indicated that the ovary of Fayoumi hens has a large proportion of small follicles which mature at a slower rate than that of LSL hens. This conclusion agreed with the results of Senior and Furr (1975) that estradiol is not an important secretion of the large follicles and the largest quantity of estradiol was present in the numerous small (<5mm) follicles and ovarian stroma. Also, Sharp (1980) reported that as the follicle enlarges, it produces increasing quantities of Estrogen, but after it becomes the second largest follicle in the hierarchy, progesterone and testosterone synthesis increases. The main steroid synthesized in the mature preovulatory follicle is progesterone. Furthermore Yu *et al.* (1992) suggested that small follicles rather than large ones, are the main source of estrogen in domestic fowl.

There is a physiological basis for the classification of follicles as small yolk follicle (SYF, 5-10mm) and large white follicle (LWF, 2-3mm). The SYF under basal conditions produce more estradiol, androstenedione and dehydroepiandrosterone than the LWF (Robinson and Etches, 1986 and Etches, 1990). These basis explain the higher level of E2 in Fayoumi than LSL. Another reason for rising E2 level in Fayoumi allover the stages during the ovulatory cycle is the better eggshell quality of Fayoumi than LSL. It is well known that E2 regulates yolk formation and calcium deposition in hens (Palmer and Bahr, 1992). Moreover, Taylor (1965) suggested that E2 might help in building up the reserves of calcium in preparation for eggshell formation.

In the present study two peaks of E2 hormone were observed in plasma of hens through the ovulatory cycle (Fig. 3). The first peak occurred at oviposition and the second one was obtained at 22 hr post-oviposition. These results are in agreement with several authors (Senior, 1974; Lague *et al.*, 1975; Graber and Nalbandov, 1976; Tsang *et al.*, 1981 and Etches, 1990). They reported that the major peak of estradiol occurred at about 4 to 2 hr before ovulation. The present results confirm the findings of Curl *et al.* (1985)

who found that E2 concentrations were greater at 21 and 22 hr than at 18 or 24 hr post-oviposition. Also, Kawashima *et al.* (1993) stated that plasma concentration of E2 in laying hens increased significantly at 3 hr before ovulation (22 hr post-oviposition). The peak of hormonal level was observed 4 -5 hr before oviposition by Etches and Cheng (1981) and at 6 hr prior to ovulation by Tixier-Biochard *et al.* (1990).

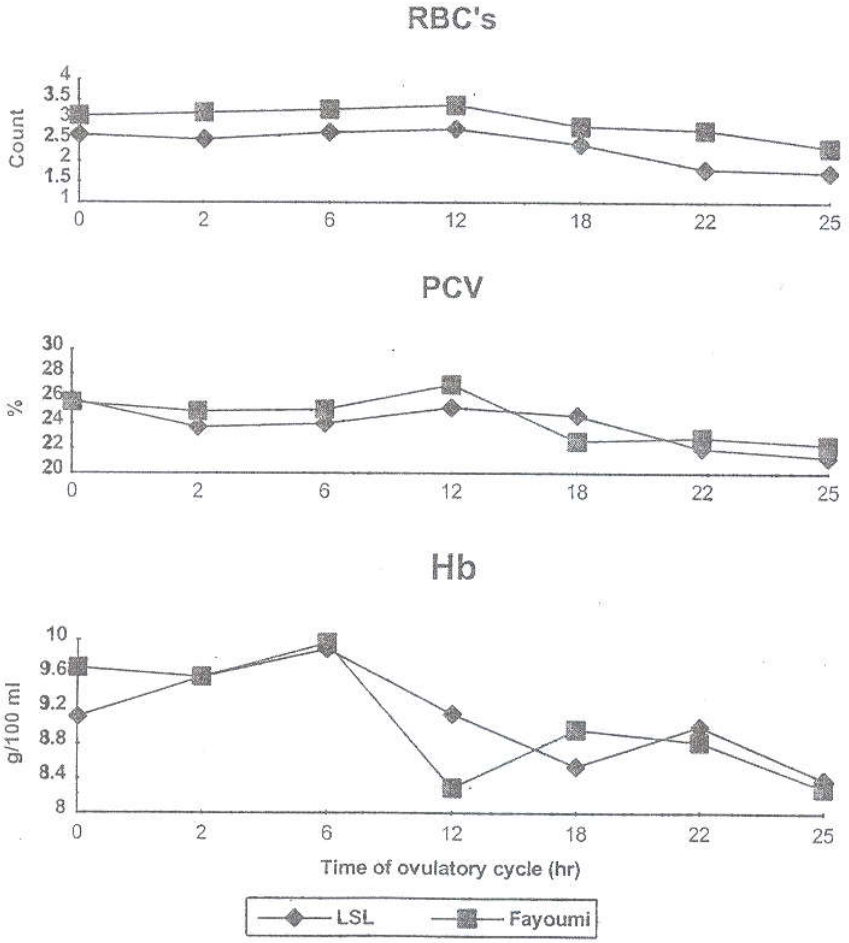

Figure 2. Red blood cells count (RBC's), packed cell volume (PCV%) and hemoglobin (Hb) during ovulatory cycle in LSL and Fayoumi laying hens

The average of P4 hormone in Fayoumi had a tendency to be lower (0.455ng/ml plasma) than that in LSL hens (0.525 ng/ml plasma). The reduced rate of follicular growth in Fayoumi hens (the lower egg production rate) may be the result of the low plasma P4 level in Fayoumi than LSL hens. There is a tendency for the most high producing layers to have high basal plasma progesterone levels (Leszczynski *et al.*, 1985). Also, they stated that, generally, egg production appears to be associated more strongly with circulating P4 than with estrogen. Moreover Yoshimura and Bahr (1991) suggested that progesterone, acting via its receptor, might have an important role in regulating follicular maturation, ovulation and oviposition in the domestic hens.

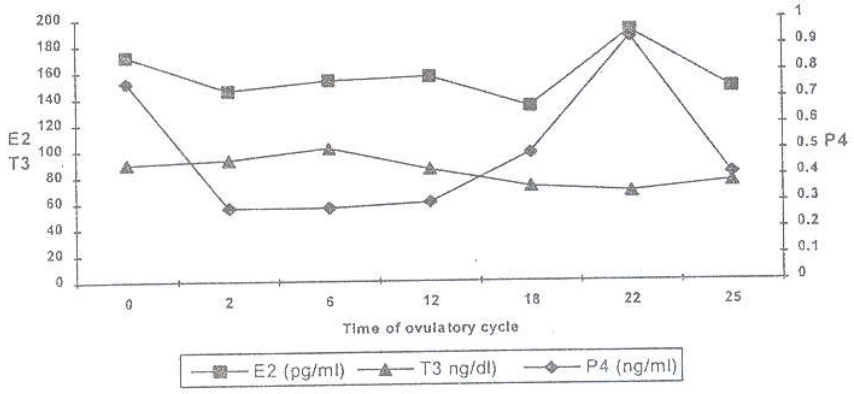

Figure 3. Plasma estradiol (E2), progesterone (P4) and triiodothyronine (T3) during ovulatory cycle in hens

The present study demonstrated that the increase in plasma P4 during the ovulatory cycle started at 12 hr post-oviposition and peaked at 22 hr post-oviposition (Fig. 3). The formation of the preovulatory peak of plasma progesterone in the ovulating hens indicates the important role of the hormone in ovulation. Progesterone is the principal steroids produced by the largest mature follicle, exerting a positive feedback effect on LH release in the hen and ovulation. The results of the present study support the aforementioned conclusion. Etches (1996) reported that at ovulation, the granulosa cells of the F1 follicle have attained their maximum capacity to produce progesterone. He added that, within 12 hr after F2 follicles are recruited into the F1 position, theca loses the ability to produce androgens. It is not clear if the absence of androgens releases an enhancement of P4 synthesis or if the failure to metabolize P4 to androgens causes a massive increase in the progesterone output from the follicle, but the net result of either interaction is the secretion of P4 into the follicular veins. Similar results were found by Gulati *et al.* (1981),

Etches (1990) and Ahmed Nagwa *et al.* (1997) and in agreement with Kappauf and Van Tienhoven (1972) and Etches and Cheng (1981). Etches *et al.* (1981) reported that the increase of plasma progesterone at 4 -6 hr before ovulation was almost directly related to progesterone secretion by the largest preovulatory follicle. On the other hand, Curl *et al.* (1985) found that P4 concentrations in laying hens were greater at 21 hr than that at 18 and 24 hr post-oviposition. The significant greater plasma progesterone level at oviposition (zero time) in the present study was observed by Roland *et al.* (1983). Wilson Susan and Sharp (1976) stated that "it appears that once an egg has been in the oviduct for more than 15 to 18 hr, an increased concentration of P4 in the circulation will cause it to be oviposited. Since a naturally occurring ovulation is always preceded by increase in the concentration of plasma P4, this mechanism ensures that an oviductal egg is expelled at about the time of ovulation, so that the oviduct rarely contains two eggs".

Results in the present study show that the major peak of progesterone was accompanied with the major peak of estradiol in plasma of fayoumi hens (Fig. 1 and Table 1). This incidence of two peaks at the same time during ovulatory cycle (22 hr post-oviposition) was observed by Lague *et al.* (1975). They stated that the apparently close association between estrogen and progesterone peaks indicated that estrogen either alone or in conjunction with progesterone might play a role in regulating ovulation in the laying hens. On the other hand Kawashima *et al.* (1992) suggested a possible role of estrogen through the stimulation of the progesterone receptor pinding to progesterone for the gonadotropin secretion through the ovulatory cycle in the hen.

The higher ratio of E2/P4, mainly a result of reduced plasma progesterone, in Fayoumi hens (Table 1) seems to be consistent with their lower number of fast-growing follicles, because small follicles secret more E2 than the largest follicles which mainly secret P4 (Robinson and Etches, 1986 and Tixier-Boichard *et al.*, 1990). The higher E2/P4 ratio found in Fayoumi hens is associated with a reduced laying rate (90 egg no. in LSL versus 70 egg no. in Fayoumi through 100 days)

This study proved that the concentration of T3 hormone in plasma of LSL was lower (73.3 ng/dl) than that in Fayoumi hens (94.0 ng/dl). This result is consistent with the findings of Shoukry (1987) with LSL and Fayoumi hens. The changes in plasma T3 hormone of the two strains were significant from 6 to 25 hr post-oviposition (Table 1). The difference between the two strains may be due to the difference in the rate of egg production. The peak of hormone in LSL plasma was observed at 2 hr post-oviposition, while the peak in Fayoumi plasma was detected at 6 hr post-oviposition. Awad (1979) and Soliman *et al.* (1980) revealed that thyroid activity, as measured biologically, showed cyclic variations being most active after ovulation by 15 to 16 hr in Dokki-4 and HNL hens. The changes in the concentration of T3 throughout the

ovulatory cycle may be due to the inverse relationship between gonadal and thyroidal function as pronounced at 22 hr post-oviposition. Similar results were obtained by Sharp and Klandorf (1981) and Mehaisen (1997).

The average RBC's count in Fayoumi was significantly higher ($2.99x10^6mm^3$) than in LSL hens ($2.37 x10^6mm^3$) throughout the ovulatory cycle. Freeman (1971) stated that RBC's count increased with the low rate of egg production. Similar results were found by Mehaisen (1997). The present study proved an opposite trend between erythrocytes number and the level of plasma sex hormones (E2 and P4) from 12 to 22 hr post-oviposition (Fig. 3 and 4). Nirmalan and Robinson (1972) stated that estrogen administration to quail depressed erythrocyte numbers. The depression of RBC's was pronounced at 22 hr post-oviposition, in LSL and Fayoumi hens coinciding with, the maximum levels of plasma E2 and P4. Also, a parallel relationship was realized between T3 hormone concentration and RBC's count from 6 to 22 hr post-oviposition (Fig. 3 and 4). This may be due to the erythropiotic effect of T3 and T4 hormones that compensate to the negative effect of estrogen (Sturkie, 1951 and Gilbert, 1963).

Averages PCV% were 23.8% and 24.4% for LSL and Fayoumi hens, respectively through the ovulatory cycle. Similar average was obtained by Wood *et al.* (1971) and Mehaisen (1997). Results showed that the decrease in PCV% from 12 to 25 hr post-oviposition was associated with a drop in RBC's at the same period reflecting the negative effect of E2 and P4 on these blood parameters (Fig. 3 and 4).

The value of Hb was $9.1g/100mm^3$ in both LSL and Fayoumi hens throughout the ovulatory cycle. Similar result was obtained by Pilaski (1972). Fluctuating trend of Hb concentration was observed during ovulatory cycle. The levels of Hb were higher in the period from zero to 6 hr post-oviposition than that in the period from 6 to 25 hr (Fig. 4). The low values of Hb concentration from 6 to 25 hr post-oviposition were consistent with the decrease in the number of RBC's in the same period. Jaffe (1960) attributed the Hb changes during egg production to the influence of gonadal hormones. Sturkie (1965) reported that factors affect erythropoiesis and red cell number also affect hemoglobin level.

CONCLUSION

This study proves that the major hormonal factor affecting the capacity of egg production is the P4. A high concentration of P4 and lower E2/P4 ratio results in more egg yield. Thus it can be concluded that E2/P4 ratio may be a better parameter for estimating egg production than the P4 alone. There is inverse relationship between sex hormones and both of T3 concentration and blood parameters during ovulatory cycle.

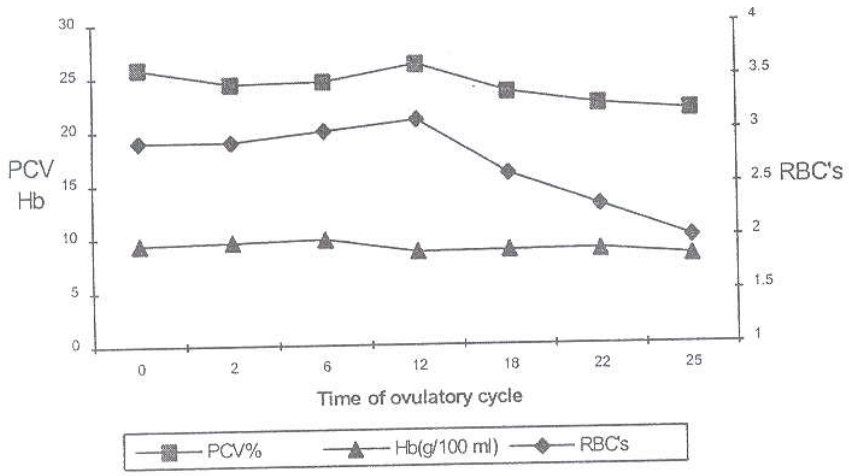

Figure 4. RBC's count (10^6/ml), packed cell volume (%) and hemoglobin concentration (g/100 ml) during ovulatory cycle in hens.

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تركيز هرمونات الاستراديول و البروجستيرون و التراى ايودوثيرونين في البلازما و بعض مكونات الدم أثناء دورة التبويض في الدجاج

نجوى عبد الهادى أحمد – أحمد عبد اللطيف الفار – مختار قيقة – جمال محيسن

قسم الإنتاج الحيواني – كلية الزراعة – جامعة القاهرة – الجيزة – مصر

استخدم في هذه الدراسة عدد ١٨٠ دجاجة بياضة LSL و فيومي في قمة الإنتاج. وضعت الدجاجات بصورة فردية في بطاريات و تغذت على عليقة بياض. تم تسجيل إنتاج البيض يومياً. تم جمع عينات الدم من ٨ دجاجات في كل نوع على فترات صفر – ٢ – ٦ – ١٢ – ١٨ – ٢٢ و٢٥ ساعة بعد وضع البيض لتقدير عدد كرات الدم الحمراء و نسبة المكونات الخلوية و تركيز الهيموجلوبين. كما تم تقدير تركيز كل من هرمون الاستراديول و البروجستيرون و التراى ايودوثيرونين في البلازما.

وجد أن تركيز الاستراديول و التراى ايودوثيرونين في الفيومي أعلى من LSL خلال دورة التبويض. بينما وجد أن البروجستيرون أعلى في دجاج LSL من ١٨ – ٢٥ ساعة بعد وضع البيضة. و أعلى تركيز للاستراديول و البروجستيرون وجد عند ٢٢ ساعة بعد التبويض. تركيز هرمون التراى ايودوثيرونين استمر عالياً في البلازما من صفر إلى ١٢ ساعة بعد التبويض. العدد الكلى لكرات الدم الحمراء و نسبة المكونات الخلوية و تركيز الهيموجلوبين كان أعلى في الفيومي عن LSL و سجلت أعلى القيم لمكونات الدم من صفر حتى ١٢ ساعة بعد وضع البيضة.