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EFFECTS OF PREGNANT MARE SERUM GONADOTROPIN ON EGG PRODUCTION AND SOME BLOOD HORMONES OF FAYOUMI CHICKENS

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ABSTRACT

One hundred and twenty Fayoumi pullets at 20 weeks of age were used to study the effect of pregnant mare serum gonadotropin (PMSG) on egg production and some related blood serum hormones. Pullets were randomly divided into four equal groups Pullets of all groups were housed in floor pens; and food and water were offered *ad libtum.* Birds were fed a diet contained 14% crude protein, 2880 Kcal ME/Kg diet, 3.7% calcium and 0.66% total phosphorous. The experiment strarted from 20 weeks and extended up to 35 weeks of age.

Birds of group 1 served as control (which were injected with 2ml saline solution/bird) at weeks 20 and 30. Those of groups 2 and 3 were subcuteonuosly injected with 75 IU of PMSG /bird/day for four consecutive days at weeks 20 and 30, respectively. While those of group 4 were twice injected at weeks 20 and 30.

Obtained results indicate that PMSG injection improved ($P < 0.05$) egg production (number and weight) and feed conversion. Moreover, the injection reduced ($P < 0.05$) the level of triiodothyronine (T_3) in blood serum. While it increased ($P < 0.05$) serum progesterone (P_4), estradiol (E_2), and T_4/T_3 ratio. However, serum thyroxin (T_4) level was not affected by treatment. According to the obtained results it could be concluded that PMSG injection might have a useful effect on egg production of low producer birds when injected slightly before sexual maturity .

Keywords: *Pregnant mare serum* gonadotropin, egg production, thyroid function, ovarian hormones.

INTRODUCTION

Ovarian follicles are the most important steroid producing structures of the avian ovary. The ovary of a mature chicken generally contains 7-9 large preovulatory follicles arranged in a follicular hierarchy, several postovulatory follicles, and numerous small follicles, which have not entered the follicular hierarchy. It has been reported that in avian preovulatory follicles biosynthesis of sex steroids changes during maturation (Gomez *et al.*, 1998; Lee *et al.*, 1998). The cell theory for steroid production suggested that granular layer of preovulatory follicles primarily produce progesterone that are required as substrate for the production of androgen and estradiol by theca layer (Huang et al., 1979). The recently multiple-cell theory of steroidogenesis suggest that theca layer can also synthesize progesterone, androgen and estradiol independent of granulosa layer (Nitta *et al.*, 1991).

In birds, ovulation and oviposition are processes controlled by LH and sex steroids, including progesterone (P4). Surges of LH and P4 have been observed between 4 and 7 h before ovulation in laying chicken (Johnson, 1993). Ovulation of the largest and most mature ovarian follicles occurred 15–30 min after oviposition in turkey hens. (Liu *et al.*, 2001).

Egg production is associated with intensive metabolic activities under the action of gonadotropins (Sturkie, 1986). These hormones affect egg production within physiological limits, to afford behoof metabolic activities for the action of gonadotropins and female steroid hormones. They collectively regulate egg formation from ovulation to oviposition (Armstrong, 1984). Pregnant mare serum gonadotropin (PMSG) is glycoprotein, with two α and β subunits, structurally similar to FSH and LH with higher carbohydrates content, especially sialic acid (Sherwood and McShan, 1977). The higher sialic acid content in PMSG appears to account for its long half-life for several days. Thus, a single injection of PMSG can be of a biological effect, at the target organ for more than a week (Hafez, 1985).

The purpose of the present study was to determine the possibility of accelerating the process of egg formation and thus increasing production rate of Fayoumi chickens by PMSG injection, in accordance with metabolic and steroid hormones.

MATERIALS AND METHODS

Experimental design

This experiment was carried out at private poultry farm near Cairo , Egypt A total number of 120 female Fayoumi pullets aging 20 weeks with body weight averaged (928± 38 gm) was randomly chosen from a large commercial flock. Pullets were randomly assigned to four equal groups. Birds of each group were subdivided into three replicates,with 10 birds each. Birds were housed in a floor pen of 1×1.5m per replicate and were fed a diet containing 14% crude protein, 2880 Kcal ME/kg diet, 3.7% calcium and 0.66% total phosphorous according to NRC (1994), recommended requirements of low producer strains . The experiment was started from 20 weeks to 35 weeks of age . feed and water were offered *ad lib.*, while the natural day light was only used . All birds have the same hygeinic and mangerial conditions .

Birds' in-group 1 (G1) were untreated and served as control, which were injected at weeks 20 and 30 of age with 2ml saline solution/bird for 4 consecutive days. While those of group 2, 3 and 4 were subcuteonusly injected (2ml/bird) of an aquatic solution containing 75 IU of PMSG (Folligon, Intervet international B.V. Boxmeer. Holland) which was immediately dissolved prior to administration. Time of injection differed among groups for 4 consecutive days. Injection of pullets for the second, third and forth group was applied at 20; 30 and at both 20 and 30 weeks of age, respectively.

Egg production (number and weight) was recorded from the first laid egg up to 35 weeks of age. Age at sexual maturity (SM) was estimated as the day at which the bird laid its first egg.

Blood Sampling

Blood samples were collected between 7:00 to 8:00 O'clock at 20 weeks (before PMSG injection) and at 21 , 25 , 30 , 31 and 35 weeks of age from five randomly chosen birds within each group. About 3ml of blood

were withdrawn from the brachial vein into collecting tubes ,immediately centrifuged at 3000 rpm for 15 minutes, serum was then decenated and stored at -20°C till analyses.

Hormonal assays

Direct radioimmunoassay (RIA) technique was performed to determine the serum hormones. Ready antibody coated tube kits of Avian (Diagnostic Product Corporation, Los Angelos) were used according to the procedure outlined by the manufacturer. Serum thyroxine (T_4) and triiodothyronine (T_3) were determined according to May (1978). The T_4/T_3 ratio was then calculated. Serum progesterone (P_4) and estradiol (E_2) were determined according to Etches *et al.* (1981).

Statistical Analysis

Data collected were statistically analyzed by the analysis of variance using the General Linear Model (GLM) procedure of the SAS institute (SAS, 1992). All statements of significance are based on the 0.05 level of probability. Significance of differences among means was tested using Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

1 - Productive performance

Data of productive performance of birds as affected by PMSG injection are listed in Table 1. Obtained data reveal that injected pullets at week 20 of age maturated ($P < 0.05$) earlier by about 12 days than controls. Moreover, injected birds at week 20, recorded ($P < 0.05$) higher egg number and ($P < 0.05$) heavier egg weight throughout the experimental period than those of control and those which were injected only at week 30,while the highest egg number and egg weight were recorded for the birds which injected twice at week 20 and 30. The laying intensity could be mainly related to metabolic activity. This observation may also indicate a more complex relationship between thyroid hormones and gonodal function of laying chickens. Since thyroidal hormones are essential for energy metabolism, as reported by Queen *et al.*, (1997); Gomez *et al.*, (1998); Lee *et al.*, (1998) and Liu *et al.*, (2001).

Data of feed intake and feed conversion are listed in Table 1. It seems that injected birds in (G2 and G4) ate ($P < 0.05$) more and converted food to eggs ($P < 0.05$) better than controls. It is possible that nutrient intake increased, in part to meet the additional metabolic requirements of estrogen-sensitive target tissues. This relationship provides an indirect evidence to support the proposal that the increase in nutrient intake following PMSG treatment was required for oviductal tissues development and function for the formation of yolk precursors (Zadworny and Etches, 1988).

2 - Hormonal changes

Thyroid hormones: Data of thyroid activity T_3, T_4 and T_4/T_3 are presented in Table 2. Obtained data indicate that serum T_3 decreased ($P < 0.05$) due to PMSG injection, while serum T_4 had ($P > 0.05$) unchangeable values. However, T_4/T_3 ratio increased ($P < 0.05$) by PMSG injection.

Table 1: Least squares means ±SE for productive parameters of Fayoumi chickens as affected by PMSG injection.

Item	Age in weeks	G1	G2	G3	G4
Age at sexual maturity (Days)		159 ±2.3^a	147 ±1.6^b	156 ±2.1^a	147 ±1.7^b
Egg number/ Bird / day	20 – 25	0.63 ±0.1^b	0.78 ±0.2^a	0.66 ±0.1^b	0.79 ±0.2^a
	25 – 30	0.60 ±0.1^c	0.75 ±0.2^a	0.65 ±0.2^b	0.76 ±0.3^a
	30 – 35	0.64 ±0.1^c	0.72 ±0.3^b	0.77 ±0.3^a	0.80 ±0.3^a
Egg weight (gm)	20 – 25	34.2 ±1.2^b	37.3 ±1.2^a	34.3 ±1.1^b	38.2 ±1.4^a
	25 – 30	37.8 ±1.4^b	43.6 ±1.6^a	36.8 ±1.0^b	43.3 ±1.4^a
	30 – 35	38.2 ±1.5^b	43.8 ±1.8^a	43.3 ±1.2^b	44.4 ±1.7^a
Feed intake gm/bird/day	20 – 25	88.2 ±6.4^b	114.1 ±7.3^a	90.8 ±7.1^b	118.4 ±8.2^a
	25 – 30	93.7 ±8.5^b	127.6±10.2^a	102.4 ±8.3^b	124.5 ±9.9^a
	30 – 35	105.8±10.0^b	132.8±12.5^a	129.8±10.2^b	133.8±13.8^a
Feed conversion kg feed· kg eggs	20 – 25	4.1 ±0.07^a	3.9 ±0.02^b	4.0 ±0.06^a	3.9 ±0.02^b
	25 – 30	4.1 ±0.06^a	3.9 ±0.04^b	4.2 ±0.07^a	3.8 ±0.04^b
	30 – 35	4.3 ±0.09^a	4.2 ±0.07^a	3.9 ±0.09^b	3.8 ±0.08^b

Least squares means in the same row with no common superscripts differ significantly (P<0.05).
G1: Control. G2: Birds injected by 75 IU of PMSG at week 20 of age.
G3: Birds injected by 75 IU of PMSG at week 30 of age.
G4: Birds injected by 75 IU of PMSG at week 20 and 30 of age.

This is scientifically logic since the ratio is a relation between the concentrations of the two hormones. The results of egg production and thyroid hormones demonstrated that thyroid activity was of essential importance for both initiation and maintenance of egg production. Since it is correlated with the energy metabolism needed for biological endothermic reactions. May (1989) and Queen *et al.*, (1997) speculated that a higher metabolism may caused a rapid conversion from T_4 to T_3 which is considered the more potent thyroidal hormone. Such mechanism could have been responsible for the egg production levels observed in the current study of PMSG injected chickens.

The decline observed in serum T_3 level could also represent other mechanisms being related to feed intake. Sturkie, (1986) and Queen *et al.*, (1997) pointed out that plasma T_3 decreased ($P < 0.05$) by increasing feed intake and speculated that the decrease of T_3 may be also due to the advance of productive status.

Progesterone (P_4) and estradiol (E_2): Obtained data of P_4 and E_2 are shown in Table 3. It was found that both of P_4 and E_2 concentrations in blood serum increased ($P < 0.05$) due to PMSG injection by about 3 times and 5 times for P_4 and E_2, respectively when compared with controls. The strong positive relationship between gonadotropin and ovarian hormones as reported by Etches (1993) may be strictly indicates the greater increase of P_4 and E_2 concentrations in the blood serum due to PMSG. Tixier-Biochard *et al.*, (1990); Johnson (1993); Lee *et al.* (1998) and Liu *et al.*, (2001) speculated that release of gonadotropins stimulate the ovarian follicles

progesterone secretion. In addition, the variation in serum E_2 level may be a function of existed rate of yolk protein biosynthesis and eggshell calcium deposition (Gruber, 1972). It is well known that laying hens require more calcium during eggshell formation (Goldenberg and Fernandez, 1966). The later authors speculated that an estrogen-induced hypocalcaemia occurs several days before ovulation. Estrogen increases the production of blood-calcium binding proteins. Yolk proteins complexes to calcium are transported to the ovary under the influence of estrogen, (Scheideler and Robeson, 1997).

Table(2): Least squares means ± SE for the serum thyroid hormones of Fayoumi chickens as affected by PMSG injection.

Item	Age in weeks	G1	G2	G3	G4
T_3 (ng/ml)	20	3.2±0.3^a	2.9±0.3^a	2.8±0.4^a	2.9±0.2^a
	21	3.1±0.2^a	1.7±0.2^b	2.9±0.4^a	1.6±0.2^b
	25	3.3±0.2^a	1.5±0.5^b	3.4±0.1^a	1.6±0.3^b
	30	4.0±0.2^a	1.6±0.3^b	3.3±0.2^a	1.7±0.2^b
	31	3.6±0.4^a	1.8±0.2^b	2.1±0.3^b	2.0±0.4^b
	35	3.4±0.3^a	2.1±0.3^b	1.9±0.3^b	1.9±0.2^b
T_4 (ng/ml)	20	14.4±0.7^a	14.7±0.8^a	14.6±0.6^a	14.3±0.8^a
	21	15.2±0.8^a	14.7±0.9^a	15.3±0.7^a	14.7±0.8^a
	25	12.8±0.8^a	12.6±0.8^a	12.5±0.9^a	13.1±0.8^a
	30	12.5±0.8^a	12.4±0.9^a	12.2±0.9^a	12.3±0.9^a
	31	11.1±0.9^a	11.3±0.8^a	11.0±0.7^a	10.8±0.9^a
	35	11.3±0.7^a	11.2±0.9^a	11.4±0.9^a	11.2±0.8^a
T_4 / T_3	20	4.5±0.9^a	5.0±1.0^a	5.2±0.8^a	5.0±1.1^a
	21	4.9±0.8^b	8.6±1.6^a	5.3±0.9^b	9.2±1.7^a
	25	3.9±0.9^b	8.4±1.2^a	3.7±0.8^b	8.2±1.1^a
	30	3.1±0.7^b	7.7±1.4^a	3.7±0.7^b	7.2±1.3^a
	31	3.1±0.8^b	6.3±1.1^a	5.2±0.9^a	5.4±0.8^a
	35	3.3±0.7^b	5.3±1.0^a	6.0±0.9^a	5.9±0.9^a

Least squares means in the same row with no common superscripts differ significantly (P<0.05).
G1: Control. G2: Birds injected by 75 IU of PMSG at week 20 of age.
G3: Birds injected by 75 IU of PMSG at week 30 of age.
G4: Birds injected by 75 IU of PMSG at week 20 and 30 of age

However, PMSG may create a state of coordination along hypothalamo-hypophyseal-ovarian axis in favor of an efficient egg formation process that resulted in higher egg production in the injected birds. This observation is also supported by the findings of Bedecarrats *et al.* (1997) and Karatzas *et al.* (1997). They speculated that Prolactin (PRL) might act at the neural or pituitary level to inhibit gonadotropin secretion or acts directly on the ovary because gonadotropin-stimulated ovulation and steroid production are inhibited by exogenous PRL. In the present study, PMSG injection showed to activate ovarian function. Obviously, Luteinzing hormone (LH) level is affected by the environmental conditions, especially, photostimulation period.

Alternately, changes in the activity of the hypothalamo-hypophyseal-ovarian axis, due to PMSG injection, luteinzing hormone-releasing factor (LHRF) stimulates the release of LH, which in turn stimulates the secretion of steroids in the ovary (Bedecarrats *et al.*, 1997). These hormonal activities are concomitant with the maturation of the reproductive tract, which leads to augment laying of eggs.

Table(3):Least squares means ± SE serum progesterone (P_4) and estradiol (E_2), of Fayoumi chickens as affected by PMSG injection.

Item	Age in weeks	G1	G2	G3	G4
P_4 (ng/ml)	20	0.108±0.01^a	0.108±0.01^a	0.107±0.01^a	0.107±0.01^a
	21	0.104±0.01^b	0.356±0.06^a	0.108±0.01^b	0.341±0.07^a
	25	0.117±0.01^b	0.371±0.04^a	0.123±0.02^b	0.372±0.04^a
	30	0.162±0.02^b	0.389±0.05^a	0.168±0.02^b	0.396±0.06^a
	31	0.179±0.02^c	0.380±0.07^b	0.414±0.06^a	0.549±0.08^a
	35	0.191±0.02^c	0.398±0.07^b	0.491±0.07^a	0.538±0.08^a
E_2 (pg/ml)	20	34.7±2.6^a	34.2±2.3^a	34.3±2.2^a	36.5±2.3^a
	21	36.3±3.4^b	208.7±6.6^a	41.6±2.3^b	214.8±5.7^a
	25	41.4±2.9^b	232.5±4.8^a	42.7±2.8^b	233.4±4.5^a
	30	43.8 ±3.2^b	213.7±4.3^a	53.3±2.2^b	218.5±4.3^a
	31	56.6±3.1^c	228.6±4.1^b	290.9±5.5^a	295.6±5.4^a
	35	72.5±2.7^c	176.9±3.9^b	289.7±4.6^a	294.8±5.8^a

Least squares means in the same row with no common superscripts differ significantly (P<0.05).
G1: Control. G2: Birds injected by 75 IU of PMSG at week 20 of age.
G3: Birds injected by 75 IU of PMSG at week 30 of age.
G4: Birds injected by 75 IU of PMSG at week 20 and 30 of age.

In conclusion, subcutaneous PMSG injection for laying Fayoumi chickens at level of 75 IU/bird/daily for four subsequent days, resulted in significant ($P < 0.05$) increase of egg number, egg weight, feed intake and improved the conversion of food to egg. Moreover, the injection reduced ($P < 0.05$) the blood serum level of T_3, while it increased ($P < 0.05$) the blood serum concentrations of P_4, E_2 and T_4/T_3 ratio. However, serum thyroxin (T_4) level was not affected by treatment.

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تأثيرا لحقن بالهرمونات المنشطة للغدد الجنسية (سيرم الفرس الحامل) علـى إنتاج البيض و بعض هرمونات الدم في الدجاج الفيومي

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استخدم في هذه الدراسة عدد ١٢٠ دجاجة فيومي عمر ٢٠ أسبوع متماثلة في الـوزن بمتوسط وزن ٩٢٨ ±٣٨ جم. قسمت الطيور إلى أربعة مجا ميع، تحتوي كل مجموعة علـى ٣ مكررات. (بكل منها ١٠ طيور) وضعت الطيور في مساكن مفتوحـة بمسـاحة ١,٥×١م / ١٠ دجاجات استمرت التجربة من الأسبوع الـ ٢٠ إلى الأسبوع الـ ٣٥ من العمر . غذيت الطيـور على عليقه تحتوى على ١٤% بروتين خام، ٢٨٨٠ كيلو كالورى طاقة ممثلة/كجم عليقـه، ٣,٧% كالسيوم و ٠,٦٦% فوسفور كلى.

عوملت المجموعات كالآتي: المجموعة الأولى استخدمت كمجموعة مقارنة حيـث تـم حقنها بالمحلول الفسيولوجي (٢مل محلول فسيولوجي/طائر يوميا لمدة ٤ أيـا م متتاليـة عنـد الأسبوع ال ٢٠ و ال ٣٠) المجموعة الثانية حقنت الطيور بـ PMSG بمستوى ٧٥ وحـدة دولية لكل طائر تحت الجلد ولمدة ٤ أيا م متتالية في الأسبوع الـ ٢٠ من العمر. طيور المجموعة الثالثة عوملت بالحقن كالمجموعة الثانية ولكن تم الحقن في الأسبوع الـ ٣٠ من العمر. بينما فـي المجموعة الرابعة حقنت الطيور بنفس المعدل (٧٥ وحدة لمدة ٤ أيام متتالية) في الأسبوع الـ ٢٠ والـ ٣٠ .

أوضحت النتائج أن الحقن زاد معنويا من عدد ووزن البيض وكذلك من كمية الغـذاء المستهلك وحسن من معدل تحويل الغذاء إلى بيض. بينما قلـل الحقـن معنويـا مـن مسـتوى هرمون T_3 في سيرم الدم وزاد معنويا من مستوى تركيز البروجسترون والاستراديول E_2، P_4 في سيرم الدم. وبصفة عامة فإن الحقن بـ PMSG كان له تأثير ايجابي على زيادة إنتاج البيـض في الدجاج الفيومي سواء من ناحية العدد أو الوزن خاصة إذا تم الحقن مرتـان عنـد الأسـبوع العشرين ثم الثلاثين .