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EFFECT OF PROSTAGLANDINS ON OVIPOSITION AND SOME RELATED PLASMA CONSTITUENTS IN JAPANESE QUAIL

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SUMMARY

The effects of prostaglandin-E_1 (PGE_1) on the induction time of oviposition, and plasma mineral levels during egg shell calcification were studied in sexually mature female Japanese quail (*Coturnix coturnix japonica*). The birds were maintained under photoperiod (LD 14:10) till the end of the experiment. Five levels of PGE_1 (5, 4, 3, 2 and 1 µg/bird) were i.m. injected into 75 (8-wk old) females, while 45 birds served as controls. Each female was given a single injection of PGE_1/4 hrs before the predicted oviposition.

Results indicated that:-

1) the induction time of oviposition increased with the decrease in PGE_1 doses.
2) Plasma Na, K, Ca and total protein concentrations increased significantly with the increase of PGE_1 doses. There were no significant differences in the concentrations of plasma inorganic phosphorus.
3) Significant differences were noticed in egg shell colour and calcification between the groups injected with different doses of PGE_1.

It is concluded that PGE_1 level plays an important role in the timing of oviposition, increasing plasma mineral levels and total protein concentrations in the female Japanese quail.

Keywords: Japanese quail, egg shell color and calcification, oviposition, PGE_1, plasma mineral levels, total protein

INTRODUCTION

The oviposition in birds and the parturition in mammals appear to be similar phenomena, although the underlying fundamental mechanisms are not fully understood (Goto *et al.,* 1985). The oviposition in birds is the result of a

coordinated series of precisely timed physiological events culminating in shell gland muscles contraction and vaginal relaxation to expel the egg (Saito *et al.*, 1987).

The neurohypophyseal hormones which are implicated in regulating oviposition in birds and initiating labor in mammals have received prominent attention (Gilbert and Lake, 1963; Coch *et al.*, 1965). Several additional lines of evidence indicated that a highly potent endogenous substances termed prostaglandins (PGs), present in most tissues, play a functional role in the regulation of oviposition (Wechsung and Houvenaghel, 1976 and Hertelendy and Biellier, 1978). Several PGs particularly the E-series, when injected into the shell gland can induce premature oviposition within a few minutes (Hertelendy *et al.*, 1974b). Interestingly, PGE_1 and PGE_2 may also play a physiological role in the Ca^{2+} mobilizing process during the time of active shell calcification when the demand for Ca^{2+} is maximal (Hertelendy *et al.*, 1984).

Since the high producing female Japanese quail lays an egg almost daily, it is a convenient experimental model for studying the roles of PGs in oviposition. The objectives of this investigation were as follows:
1- To determine the effect of PGE_1 on the time of oviposition in Japanese quail. 2- To determine the effect of PGE_1 on the level of some minerals such as Na^+, K^+, Ca^{2+} and P, which are related to egg shell calcification and muscle activity. 3- To assess the effect of PGE_1 on the total plasma protein and its fractions.

MATERIALS AND METHODS

Two hundred and sixty eight (268) one-day old Japanese quail chicks *Coturnix coturnix japonica* were individually weighed and their weights were recorded to the nearest tenth gram. Chicks were brooded in starting five deck batteries. The brooding temperature was 35°C, then reduced gradually until it reached room temperature at 7 days of age. Chicks were fed on turkey starter ration contained 24% protein, 3.3% crude fiber and 2954 Kcal/kg ME (Table 1). Birds were supplied with feed and water *ad. libitum*. At sexual maturity, birds were reared in 18x18 cm cages (pair per cage) with a sloping floor for collecting the eggs. The cages were in an air conditioned room with the temperture maintained at approximately 23°C. A light period of 14 hrs/day was provided.

Age at sexual maturity was measured in days as indicated by the lay of the first egg. A record of egg laying for each female was kept for several weeks before starting the experiment. The mean length of the clutches was recorded for all individuals. The time of oviposition was determined manually every 15 min and the experimental females which laid relatively at regular sequence of 7-16 eggs on consecutive days were selected for the experiment.

Table 1. Composition of ration

Ingredient	%
Ground yellow corn	62.0
Soybean meal (44% protein)	26.5
Fish meal	6.00
Meat meal (60% protein)	4.00
Bone meal (12.6%protein)	1.00
Sodium chloride	0.25
Vitamins (a) & Minerals (b)	0.25
	100
Calculated analysis	%
Crude protein	24.0
Crude fat	3.48
Crude fibers	3.33
Lysine	1.39
Methionine and cystine	0.80
Calcium	0.80
Available phosphorus	0.49
Kcal ME/kg	2954.0

a) Vitamin mixture: Each kilogram of diet contains = A, 12.000 I.U. ; D3, 2000 I.U.; E, 10mg.; K, 2mg.; B1, 1mg.; B2, 4mg.; B6, 1.5mg.; B12, 10µg.; Niacin, 20 mg.; Pantothenic acid, 10mg.; Biotine, 50µg.; Folic acid, 1000µg. and Coline chloride, 005 mg.

b) Mineral mixture: Each kilogram of diet contains = Copper, 10mg.; Iodine, 1.0 mg.; Iron, 30mg.; Manganese, 55mg. ; Zinc, 55mg, and Selenium, 1 mg. The carrier was wheat bran.

Prostaglandin treatments

A total of one hundred and twenty sexually mature females were randomly assigned to eight treatments with three replications. Since it was impossible to collect blood samples for plasma analysis from each female, so it became necessary to classify the laying females within each treatment into groups according to the time of oviposition before being treated with PGE_1. Prostaglandin-E_1 (Sigma Chemical Co., St. Louis, Missouri, U.S.A. No. P-5515) was dissolved in 95% ethanol (1 mg/ml) and diluted with saline solution (0.9% Nacl) immediately before injection. Each female was given a single i.m. injection, 4 hrs before the predicted oviposition. The period between treatment and induced oviposition "induction time" was recorded in Tables 2 and 3. The amounts of PGE_1 injected were 5, 4, 3, 2, 1 µg/ml/bird for T_1, T_2, T_3, T_4 and T_5, respectively. As controls; females were given ethanol and saline solution (C^+).

Table 2. The percentage incidence of oviposition in female Japanese quail injected with PGE1 four hours before expected oviposition

Treatments	No. of bird	Mean Induction %	µg/ bird Induction
		Time	
Control	15	199.5	2.9
T5	15	206.0	0.0
T4	15	155.8	25.0
T3	15	106.1	33.3
T2	15	67.30	58.3
T1	15	160.8	25.0

Table 3. Induction of oviposition in female Japanese quail injected with PGE1

Treatment[a]	Dose No. of µg/ bird	No. of Test	No. of premature oviposition	Average induction time (min.)[b]
PGE	15	12	3	12
	4	12	7	21
	3	12	4	13
	2	11	3	20
	1	12	0	--
C+	1ml	12	0	--

a PGE1 was injected four hours before expected oviposition.

b The response of the hen was regarded as positive when oviposition was induced within 3 – 22 min. after injection.

c+ The females were given ethanol and saline solution 0.9% (1 ml)

Blood analysis

Blood samples were collected at 10 min. before the expected oviposition, zero "induced oviposition", 5, 15, and 30 min. after induced oviposition. Selected females were decapitated and blood was collected directly into heparinized centrifuge tubes, and were centrifuged immediately at the speed of 6500 r.p.m for 10 min. After centrifugation, plasma were decanted into plastic tubes, stoppered tightly and stored in deep freezer at-20°C until mineral and hormonal assays were conducted. The plasma samples were analysed for Ca^{++}, P^{-}, K^{+} and Na^{+} using 400 Flame Photometer except for Phosphorus element, which was determined by use of phosphorus Kit (Inorganic phosphorus 12601, 3 Palm City FL 34990 U.S.A.).

Egg shell

At the time of oviposition, the egg shell color was scored as the following: Score 3 for prematare eggs, their shells had no color, score 2 for inadequate color or little color eggs and score 1 for normal shell color eggs (control). The frequency distribution of eggs was 4% for score 3, 6% for score 2 and 90% for score 1. Egg shell calcification was scored according to the following: hen

laying soft shelled egg (Partial calcification) took rank 2, hen laying hard shelled egg (normal calcification) took rank 1 and control took rank zero.

Plasma proteins

Total plasma proteins were determined by the (Hitach Apparatus No. 705 manufactured by Japan). Separation of plasma protein by electrophoresis was accomplished by use of protein electrophresis Kit, P/N 655900 manufactured by Beckman, U.S.A. according to Beckman Instructions 015-556458-G (Paragon Electrophoresis System) CA 92621-6209.

Statistical Analysis

The data were statistically analysed by the use of General Linear Models procedure as described by Statistical Analysis System (SAS), SAS/STAT User's Guide (1988). Duncan's New Multiple Range test was used to estimate significant differences among means.

RESULTS AND DISCUSSION

Effect of PGE_1 on the induction time

The results clearly showed that the PGE_1 only at relatively high doses (4 µg per bird) induced premature oviposition in a few minutes when injected 4 hrs before the expected oviposition, while low dose, (1 µg PGE_1 per bird) had no effect on inducing oviposition. The induction time was significantly lower in T_2 (4 µg/bird) than all other groups except T_3 (3 µg/bird), while no significant differnce was found between low (1 µg/bird) and high doses (5 µg/bird). Mean while, all treated groups had significantly lower induction time than the control groups as shown in Tables 2, 3 and 4 and Fig. 1. These results are in agreement with the findings of Hertelendy (1972), (1973) and (Hertelendy *et al.*, 1974a and b); Hertelendy *et al.* (1975); Hammond *et al.* (1981); Goto *et al.* (1985); Saito *et al.* (1987) and with those reported by Shimada and Saito (1989).

Table 4. Analysis of variance for the effect of PGE1 on induction time in female Japanese quail

S.O.V.	d.f.	M.S.
Total	5	43607.87**
Error	75	6979.28**

** P< 0.01

High and low doses of PGE_1 (5 and 1 µg/bird), produced less response in inducing oviposition than 4 µg/bird. This may be attributed to the position of the egg in the clutch to variations in the response of the same tissues to different doses of the same PG (Hertelendy *et al.*, 1975), or to a temporary inhibition of the shell gland (Hertelendy, 1972).

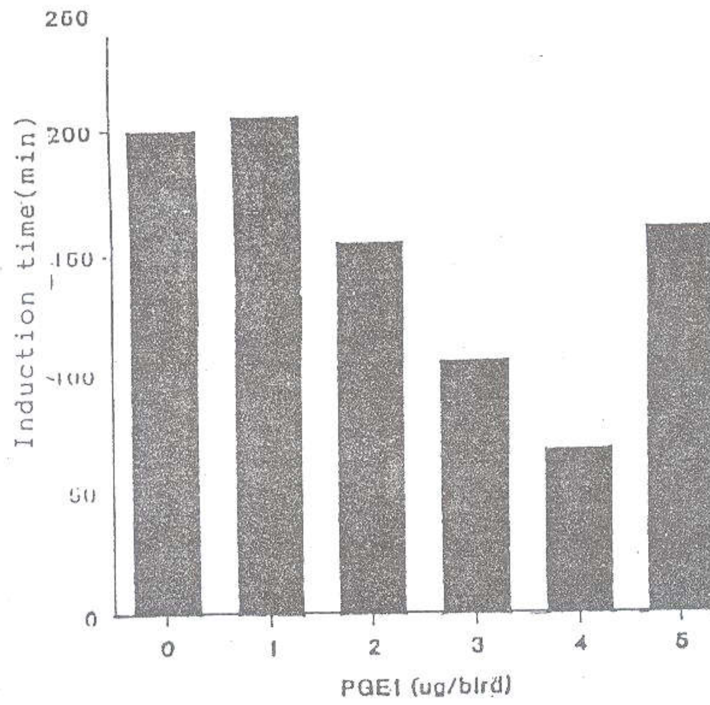

Fig. 1. Effect of different doses of PGE1 on the induction time of female Japanese quail.

Table 5. Analysis of variance for the effect of PGE1 on plasma potassium concentration in female Japanese quail

S.O.V.	d.f.	M.S.
Total	5	51533.53
Error	92	7821.36**

** $P< 0.01$

The increased plasma calcium concentration observed in this study was similar to the findings of Hertelendy *et al.*, (1984). In addition, they reported an increase in cytosolic free Ca^{2+} concentration above a threshold level, which was an essential prerequisit in the activation of the contractile elements of shell gland smooth muscle. Such increase in Ca^{2+} was derived either from intracellular stores (release of bound and/or decreased uptake by intracellular organelles). PGs had been implicated in both of these events. They also added that, a specific receptors for PG appear to exist in the cell membrane of shell gland muscle. Occupancy of such receptors by certain PGs should alter some of the properties of the cell membrane in such a way as to cause *inter alia* "change in ion fluxes, particularly in transport of Ca".

It is apparent that birds injected with 5 µg PGE_1 had highly significant higher calcium concentration as compared to birds injected with 1 µg PGE_1. This might indicate that PG had an enhancing effect on calcium metabolism. Gilbert *et al.* (1982) postulated that PGs were involved in calcium metabolism in both mammals and birds. They noted that PGs synthetase inhibitors (PGI) caused hypocalcemia. In addition, elevated plasma levels of PGE_1 in laying hens, that were injected with high dose of PGE_1 during the time of active shell calcification (4 hrs before oviposition), might had stimulated the mobilization of skeletal calcium (Hertelendy, 1980). Molnar *et al.* (1987) found that both Ca^{2+} and cAMP were key components of the mechanism regulating shell gland smooth muscle contractility. Uterotonic PGs and other myometrial agonists have been proposed to initiate their physiological response (contraction of the muscles) by increasing intracellutar Ca^{2+} levels, wherease substances that increase cAMP accumlation caused relaxation.

There appear to be an interrelation between PGs and calcium metabolism, but the available literature contains no studies on the mechanism of action of PGE_1 on bone resorbtion.

Phosphorus concentration increased only slightly using 3 and 2 µg of PGE_1. However, the mean differences between treatments were not significantly different. These results indicated that PGs may have an indirect effect on the phosphorus, through its effect on the cAMP.

Data of the present study showed that when PGE_1 was injected into female laying quail 4 hrs before expected oviposition, egg shell appeared with no pigments. These effects were due to PGE_1 which enhanced the uterine

Table 6. Average values for different parameters in laying female Japanese quail injectad with prostaglandin - E1.

Parameters	Control	T 1	T2	T3	T4	T5	Level of sign.
Induction Time	199.54^{a}	160.75^{ab}	67.33^{c}	106.08^{bc}	155.82^{ab}	206.00^{a}	**
Na^{+}meq/L	4332.51^{bc}	4559.20^{a}	4423.65^{ab}	4489.42^{ab}	4412.01^{ab}	4225.89^{c}	**
K^{+}meq/L	143.54^{bc}	268.03^{a}	204.43^{ab}	245.14^{a}	172.37^{bc}	133.37^{c}	**
Ca^{2+} meq/L	276.02^{b}	311.94^{a}	293.19^{ab}	303.51^{ab}	291.96^{ab}	275.07^{b}	*
Pi mg/L	90.99^{a}	99.02^{a}	80.49^{a}	102.88^{a}	107.75^{a}	77.61^{a}	Ns
progesterone ng/ml	1.34^{a}	1.57^{a}	1.80^{a}	1.34^{a}	1.29^{a}	1.61^{a}	Ns
UIU/ml LH	0.36^{a}	0.35^{a}	0.27^{a}	0.38^{a}	0.34^{a}	0.27^{d}	Ns
Egg shell color	0.80^{b}	1.14^{ab}	1.13^{ab}	1.33^{a}	1.07^{ab}	0.80^{b}	*
Egg shell calcific.	0.80^{b}	1.00^{ab}	1.00^{ab}	1.07^{a}	0.92^{ab}	0.80^{b}	*

Means not having the same superscripts within a row are signifcantly different at ** 0.01 and at * 0.05 levels of probability by Duncan's multiple range Test.

mechanism by which PGs interact with the feedback and neurotransmitter systems known to regulate the hypothalamo-pituitary unit.

Table 7. Effect of prostaglandin-E I on the total plasma protein and plasma protein electrophoretic fractions

T ug/ml/bird	Total plasma Concentrations gm/dl	Plasma protein electrophoresis fractions				A/G Ratio
		Albumin	alpha-globulin	beta-globulin	gamma-globulin	
1	3.71	51.38	29.6	13.63	7.85	1.39
2	2.61	47.48	22.0	19.13	9.78	1.20
3	3.70	44.03	26.05	19.28	8.88	1.12
4	4.21	49.88	16.5	22.4	11.23	1.34
5	4.77	44.4	18.4	24.2	12.93	1.06
C+	2.22	39.35	16.63	6.43	8.0	0.91

All values presented as means.

REFERENCES

Asem, K., H. Todd and F. Hertelendy, 1987. In vitro effect of prostaglandins on the accumulation of cyclic AMP in the avian oviduct, Gen. Comp. Endo., 66: 244-247.

Coch, J.A., J. Brovetto, H. Cabot, C.A. Fielitz and R. Caldeyro-Barcia, 1965. Oxytocin equivalent activity in the plasma of women in labor and during the *puerperium*. Amer. J. Obstet. Gynecol. (9):10-17. Cited from Gen. Comp. endocrinol., 22: 529-531.

Duncan, D.B., 1955. Multiple range and Multiple f-tests. Biometrics, 11: 1-42.

Etches, R.J., J.D. kelly, L. Anderson and D.M. Olson, 1991. Prostaglandin production by the largest preovulatory follicles in the domestic hen (*Gallus domesticus*). Biol. Reprod., 43: 378-384.

Gilbert, A.B. and P.E. Lake, 1963. The effect of oxytocin and vasopressin in the domestic hen. J. Physiol., London, (169): 52-53, Cited from Jap. Poult. Sci., 1985 22: 55-63.

Gilbert, A.B., G. Mitchell, M. Davidson, k. Laughlin and B. Hughes, 1982. Effect of administring two prostaglandin synthetase inhibitors (indomethacin and aspirin) on egg production in the domestic fowl. Res. Vet. Sci., 33: 216-220.

Goto, K., Y. Nakanishi and K. Ogawa, 1985. The effect of arginine vasotocin and prostaglandins on oviposition in the domestic fowl. Jap. Poult. Sci., 22: 55-63.

Hammond, R., K. Koelkebeck, C., Scanes, H. Biellier and F. Hertelendy, 1981. Plasma prostaglandin, LH and progesterone levels during the ovulatory cycle of the turkey (*Meleagris gallopavo*). Gen. Comp. Endo., (44): 400-403.

Hertelendy, F., 1972. Prostaglandin-induced premature oviposition in the coturnix quail. Prostaglandins, 2: 269-279.

Hertelendy, F., 1973. Block of oxytocin-induced parturition and oviposition by prostaglandin inhibitors. Life Sci., 13: 1581-1589.

Hertelendy, F., 1980. Prostaglandins in avian Endocrinology. In "AVIAN ENDOCRINOLOGY" (A. Epple and M.H. Stetson Eds.) Academic Press. Inc. III Fifth Avenue, New York, U.S.A. pp. 455-479.

Hertelendy, F. and H. Biellier, 1978. Evidence for a physiological role of prostaglandins in oviposition by the hen. J. Reprod. Fert., 53: 71-74.

Hertelendy, F., H. Biellier and H. Todd, 1975. Effects of the egg cycle and route of administration on prostaglandin-induced oviposition of hens and Japanese quail. J. Reprod. Fert., 44: 579-582.

Hertelendy, F., Y. Mary and H. Biellier, 1974a. Induction of oviposition in the domestic hen by prostaglandins. Gen. Comp. Endo., 22: 529-531.

Hertelendy, F., D. Olson and G. Asboth, 1974b. Roles of prostaglendin-E_2 in inducing oviposition in domestic fowl. Biol. Reprod., 20: 390-398.

Hertelendy, F., D. Olson, H. Todd, R. Hammond, M. Toth and G. Asboth, 1984. Role of prostaglandins in oviposition and ovulation. In "*Reproductive Biology of Poultry*" (F.J. Cunningham, P.E. Lake and D. Hewitt Eds.) British Poultry Science Ltd. Longman group, Harlow. pp. 89-102.

Molnar, M., E. Asem and F. Hertelendy, 1987. Differential effects of prostaglandin-$F_2\alpha$ and of prostaglandin-E_1 and E_2 on cyclic 3, 5-monophosphate production and intracellular calcium mobilization in avian uterine smooth muscle cells. Biol. Reprod., 36: 384-391.

Olson, D. and F. Hertelendy, 1983. Avian shell gland contractility: interaction of PGF_2 and arginine vasotocin with Ca^{2+}. Amer. Physiol. Soc., 83: 150-157.

Saito, N., K. Shimada and T. Koike, 1987. Interrelationship between arginine vasotocin, prostaglandin, and uterine contractility in the control of oviposition in the hen (*Gallus domesticus*). Gen. Comp. Endo., 67: 342-347.

SAS. 1988. Statistical Analysis system SAS user's guide: Statistics. SAS Inst., Inc., Cary, NC.

Shimada, K. and N. Saito, 1989. Control of oviposition in poultry. Poult. Biol., 2: 235-253.

Wechsung, E. and A. Houvenaghel, 1976. A possible role of prostaglandins in the regulation of ovum transport and oviposition in the domestic hen. Prostaglandins, 12: 599-608. Cited from Biol. Reprod., 1986, 35: 1147-1153.

تأثير البروستاجلانديندات على بعض مكونات البلازما فى السمان اليابانى

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كان الغرض من هذه التجربة هو تأثير البروستاجلاندين (PGE_1) على إستحداث ميعاد وضع البيض ومستوى العناصر المعدنية أثناء عملية تكوين القشرة فى أناث السمان اليابانى.

تم تربية الطيور تحت نظام إضاءة ١٤ ساعة ضوء: ١٠ ساعات اظلام حتى نهاية التجربة. تم حقنها بخمس مستويات من البروستاجلاندين (٥، ٤، ٣٠، ٢، ١ ميكروجرام/طائر) فى العضلات وكان عدد الطيور التى تم حقنها ٧٥ أنثى عمر ٨ أسابيع بينما كان عدد طيور المقارنة ٤٥ وحقنت كل أنثى مرة واحدة قبل الميعاد المتوقع لوضع البيض باربعة ساعات.

وكانت نتائج التجربة كالاتى:

١– كانت هناك علاقة عكسية بين ميعاد إستحداث وضع البيض والجرعة المحقونة من PGE_1.

٢– زادت مستويات كل من الصوديوم والبوتاسيوم والكالسيوم وتركيز البروتين الكلى زيادة معنوية مع زيادة الجرعة من الصوديوم والبوتاسيوم والكالسيوم وتركيز البروتين الكلى زيادة معنوية مع زيادة الجرعة من PGE_1 بينما لم يزد مستوى الفوسفور زيادة معنوية

٣– كانت هناك فروق معنوية فى لون القشرة وتكلسها بين المجاميع المختلفة

تشير النتائج السابقة إلى أن مستويات PGE_1 تلعب دوراً هاماً فى ميعاد وضع البيض – زيادة مستويات العناصر المعدنية فى البلازما وتركيز البروتين الكلى فى إناث السمان اليابانى.