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EFFECTS OF EXOGENOUS CALCIUM OR HUMAN CHORIONIC GONADOTROPIN (hCG) ON SOME REPRODUCTIVE HORMONES, TESTICULAR AND THYROID ACTIVITIES IN MALE CHICKENS.

(With 4 Tables and 7 Figures)

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تأثيرالكالسيوم وهرمون المشيمة الأدمى على بعض هرمونات التناسل فى الديوك البالغة بالإشارة إلى نشاط الخصية والغدة الدرقية

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أجريت هذه الدراسة لبحث علاقة الكالسيوم المتأيين فى البلازما مع وظائف هرمونات الغدة النخامية والمناسل. وقد استخدم لهذا الغرض عدد ٤٥ ديك بالغ قسمت إلى ٣ مجاميع متساوية كما يلى: *المجموعة الأولى:* استخدمت كمجموعة ضابطة وحقنت بمحلول الملح الفسيولوجى فى الوريد الجناحى. *المجموعة الثانية:* حقنت فى الوريد الجناحى بجرعة من جلوكونات الكالسيوم مقدارها ٥٥٠ مجم / كجم من وزن الجسم الحى. *المجموعة الثالثة:* حقنت فى الوريد الجناحى بجرعة من هرمون المشيمة الأدمى مقدارها ٢٠٠ وحدة دولية / كجم من وزن الجسم الحى. وقد اخذت عينات الدم بعد الحقن بـ ٢٠ دقيقة باستخدام طريقة الوخز الوريدى من الوريد الساقى لطيور المجموعات الثلاثة واستخدمت فى قياس هرمونات الغدة النخامية والمناسل على النحو التالى: *أولاً:* تم تقدير الهرمون الحاث للخلايا البينية والهرمون الحاث للحويصلات وهرمون البرولاكتين وكذا الهرمون الحاث للغدة الدرقية بطريقة التقدير المناعى الانزيمي. *ثانياً:* تقدير الهرمزن الذكرى وكذا هرمون الاستراديول باستخدام طريقة التقدير المناعى الإشعاعى. ثالثاً: تقدير الكالسيوم المتأيين فى البلازما. هذا وقد عضدت النتائج بالدراسة النسيجية لكل من الغدة الدرقية والخصية فى الديوك البالغة . وقد أوضحت النتائج ما يلى: (١) ارتفاع ملحوظ فى مستويات الهرمون الذكرى والهرمون الحاث للحويصلات والهرمون الحاث للخلايا البينية وهرمون البرولاكتين وكذا الهرمون الحاث للغدة الدرقية وذلك بعد حقن ذكور الطيور بجلوكونات الكالسيوم فى حين سجل مستوى أيونات الكالسيوم انخفاضاً ملحوظاً. (٢) ارتفاع ملحوظ فى مستويات الهرمون الحاث للحويصلات والهرمون الحاث للخلايا البينية مع انخفاض ملحوظ فى مستويات هرمون البرولاكتين والهرمون الحاث للغدة الدرقية وكذا فى مستوى الكالسيوم المتأين استجابة للحقن بهرمون المشيمة الآدمى. (٣) فى المجموعة الضابطة. سجلت علاقات طردية بين مستويات كل من الهرمون الذكرى والهرمون الحاث للغدة الدرقية وأيونات الكالسيوم. وكذلك بين هرمون الإستراديول وأيونات الكالسيوم وبين الهرمون الحاث للخلايا البينية والبرولاكتين.

كما سجلت علاقات عكسية بين الهرمون الحاث للحويصلات وكلا من هرمون الإستراديول وأيونات الكالسيوم. (٤) وفى الطيور المحقونة بجلوكونات الكالسيوم سجلت علاقات طردية جديدة بين مستويات كلا من البرولاكتين والهرمون الذكرى والاستراديول والهرمون الحاث للحوصلات وبين الاستراديول ولاهرمون الذكرى والهرمون الحاث للخلايا البينية. أيضاً سجلت علاقات عكسية جديدة بين الهرمون الحاث للغدة الدرقية والاستراديول أو أيونات الكالسيوم. (٥) أدى حقن ذكور الطيور بهرمون المشيمة الآدمى لظهور علاقات طردية جديدة بين مستويات كل من الهرمون الذكرى والاستراديول وبين البرولاكتين والهرمون الحاث للغدة الدرقية. (٦) أوضحت الدراسة النسيجية ان لجلوكونات الكالسيوم تأثير محفز على حويصلات الغدة الدرقية التى ظهرت بقدرة عالية على التكوين والافراز. فى حين كان للحقن بهرمون المشيمة الآدمى تأثيراً مثبطاً جداً على تكوين هرمونات الدرقية. كما أدى حقن جلوكونات الكالسيوم إلى تغيير مراحل تكوين الحيوانات المنوية واختفاءها فى بعض الأنابيب المنوية بالاضافة إلى تكوين خلايا كبيرة متعددة الانوية يظهر بعضها انحلالا. ولم يظهر حقن هرمون المشيمة الآدمى أى تأثير على مراحل تكوين الحيوانات المنوية بينما زادت خلايا لايدج فى العدد والحجم وظهرت بمظهر يدل على زيادة قدرتها على تكوين الهرمون الذكرى. ومما سبق يتضح ان حقن كل من جلوكونات الكالسيوم او هرمون المشيمة الآدمى قد أدى إلى تغيرات فى مستويات وكذلك العلاقات البينية لهرمونات الغدة النخامية وكذلك هرمونات الخصية فى ذكور الدجاج البالغ. فقد كان لحقن جلوكونات الكالسيوم تأثيراً محفزاً للغدة الدرقية وتأثير مثبطاً على وظيفة الخصية. فى حين كان لحقن هرمون المشيمة الآدمى تأثيراً مثبطاً على الغدة الدرقية مصحوبا بتأثير محفز على وظيفة الخصية. ولذلك يجب توخى الحذر الشديد عند استعمال مثل هذه المركبات فى مزارع الدواجن لتجنب التاثيرات الضارة لهما على خصوبة ذكور هذه الطيور. كما تلفت نتائج هذه الدراسة النظر الى ضرورة استكمال البحث وامكانية استخدام مثل هذه المركبات سوياً لما لها من تأثير متكامل على خصوبة ذكور الطيور البالغة.

SUMMARY

In the present investigation exogenous administration of calcium or human chorionic gonadotropin (hCG) to mature male chickens modulated the secretions and correlations between different reproductive hormones, either pituitary or gonadal hormones. A stimulatory effect on thyroid gland with an adverse effect on testicular activity were observed with calcium administration, while an inhibitory effect on thyroid gland with a stimulatory effect on testicular activity were observed with hCG administration. So, it is advisable to use calcium or hCG in poultry farm, especially in mature male birds, with caution. Further studies on the use of calcium in combination with hCG should be taken in considerations.

Key Words: *Calcium, hCG, Reproductive hormones, Thyroid activity in male chicken.*

INTRODUCTION

It is well known that hormones regulating reproductive function in male chickens are interstitial cell stimulating hormone (ICSH) that responsible for proliferation activity of Leydig cells which produce steroid hormones, follicle stimulating hormones (gametogenic hormones-FSH) that stimulates seminiferous tubules, testicular growth and spermatogenesis (Patricia and Scanes, 1977), prolactin (PRL) that believed to influence behaviour and mineral metabolism (Harold et al., 1985), thyroid stimulating hormone (TSH) that stimulates thyroid hormones synthesis and release which are involved in the regulation of growth and sexual development (Sturkie, 1986), testosterone (Test) that stimulates growth of the testis and spermiogenesis (Sharp et al., 1977 and Tag El-Din, 1995) and estradiol-17β (E_2) that stimulates the release of neurotransmitter, dopamine, which is able to increase the release of LH-RH from the hypothalamus (Hutchinson et al., 1989) and inhibits the secretion of PRL by inhibiting the activation of messenger system, Ca^{2+} and cAMP (Delbeke and Dannis, 1985 and Johnson and Tilly, 1991).

Noor et al. (1986) and Jamaluddin et al. (1992) reported that calcium is essential for hormonal regulation of steroidogenesis and is important for hormone synthesis and secretion such as ICSH, FSH, PRL, TSH, GH, oxytocin, insulin, glucagon and adrenal hormones.

The variation in plasma concentrations of pituitary hormones and sex steriods in male and female chicken have been documented (Sharp, 1975, Saleh et al., 1977 and Al-Suhaimi, 1994). Scarce data has been found on endocrine profile changes in mature chickens in relation to ionic calcium, therefore, the present study was planned to clarify the effects of exogenous calcium or hCG on some reproductive hormones and ionic calcium levels in mature male chickens. Correlations between reproductive hormones, ionic calcium and exogenously administrated calcium or hCG were also studied. Thyroid and testicular activities were also included.

MATERIALS and METHODS

Forty five mature male chicken of lohman selective line (LSL) breed were obtained from the General Poultry Company. They were highly active, free from any abnormalities and apparently healthy. Their average body weight was 1000 ± 100 gms and of 13 months old (Sharp et al., 1987). Birds were housed in caged metal batteries. Food was

provided in a trough feeder and drinking water from nipples. The experimental birds were fed on standared layer ration obtained from the General Poultry Company. Food additives (Premix-Roche), free from any hormonal supplementation, were added to the ration. Chickens were raised under standard system of management and lightening regimen of 14 hours light and 10 hours dark. Food and water were available ad libitum. Birds were classified into 3 main groups of 15 birds each, as follow:

1-Group I: birds were injected I.V by 0.3 ml/bird physiological saline (0.85% Nacl) in the wing vein.

2-Group II: birds were injected I.V by 0.3 ml/bird calcium gluconate solution (Sandoz LTD, Basle, Switzerland) in a dose equal to 550 mg/kg body weight (Morimoto et al., 1984).

3-Group III: birds were injected I/V by 200 i.u. /kg body weight of human chorionic gonadotropin (Pregnyl, each ampule contains 500 i.u. of human chorionic gonadotropin, Organon, Holland) dissolved in 0.4 ml of physiological saline (Kuwayama et al., 1991).

Heparinized blood samples were collected from birds of control and treated groups at 20 minutes post-injection. Samples collected by tibial vein puncture. Plasma were sepsreted by centrifugation and stored at -18°C until hormonal and free calcium assays. Plasma levels of FSH, ICSH, PRL and TSH were measured by using Enzyme-immuno assay kits according to Engvall (1980). Plasma levels of testosterone and estradiol-17β were measured by using radioimmuno-assay kits according to the method of Abraham (1981). Plasma free calcium level was measured according to the method of Gindler and King (1972).

After blood samples collection, 5 birds from each group were slaughtered. Testes and thyroid tissues were dissected out and fixed in Bouin's solution and 10% neutral formaline, respectively. Routine histopathological examination for testicular or thyroid tissues was done according to Erlich's Haematoxyline (1886).

Statistical analysis of data inculding analysis of variance, protected least significant difference (PSD) and correlation matrix between different hormones and free calcium of control and treated groups was done according to procedures reported by Snedecor and Cochrane (1980) at 5% probability.

RESULTS

1- Effects of calcium gluconate or human chorionic gonadotropin (hCG) on plasma levels of some reproductive hormones and ionic calcium:

Table (1) shows that the levels of testosterone, FSH, ICSH, PRL and TSH were significantly increased in response to exogenous calcium administration. The plasma level of ionic calcium was significantly decreased. No significant alterations was detected in the level of estradiol-17β when compared with control levels. Also, data presented in table (1) illustrates that at 20 minutes post-hCG administration, the plasma levels of FSH and ICSýH were significantly increased in comparison to their control levels, while the levels of PRL, TSH and ionic calcium were significantly decreased. No significant alterations were detected concerning testosterone and estradiol-17β plasma levels.

2- Correlation matrix between different reproductive hormones and ionic calcium levels in control and calcium or hCG-treated mature male chickens:

Table (2) illustrates that, in control birds, a significant positive correlations existed between testosterone and TSH or ionic calcium; estradiol and ionic calcium; and ICSH and PRL. Also, a significant negative correlations existed between FSH and estradiol or ionic calcium levels. In birds injected with calcium gluconate, a new positive correlations between PRL and testosterone or estradiol or FSH; and between estradiol and testosterone or ICSH were recorded. In addition, a new significant negative correlation were also existed between TSH and estradiol or ionic calcium levels (Table 3). In response to hCG-administration, a significant positive correlation between testosterone and estradiol levels and between PRL and TSH levels were also recorded (Table 4).

3- Histopathological findings:

(A) thyroid gland:- in control birds, thyroid acini were lined by a single layer of cuboidal cells with pale basophilic dye and rounded nuclei. The lumen of the thyroid acini were filled with thyroid secretion (Fig. 1). In birds treated with calcium gluconate, the thyroid acini were lined by a simple columner cells layer. Clear spaces separating thyroid colloid and the lining cell layer were observed that indicate a higher ability for

synthesis and secretion of thyroid hormones (Fig. 2). The thyroid tissues of hCG-treated birds revealed an inhibitory effect of hCG on thyroid function that was manifested by flattened cell lining with scant cytoplasm and spindle nuclei (Fig. 3).

(B) Testis: Testicular tissues of control birds revealed seminiferous tubules with different spermatogenic cells and Sertoli cells. Sperms similar to the well developed chock's sperms were seen in the lumen of the tubules (Fig. 4).

Few interstitial tissue between the tubules were also observed. Few large polygonal Leydig cells were also observed (Fig. 5). In birds treated with calcium gluconate, disappearance of different cellular stages of spermatogenesis was recorded. Moreover, giant cells with multiple nuclei which showed heterolysis were also observed (Fig. 6). However, in hCG-treated birds, no changes in the cellular stages of spermatogenesis were seen. Mature sperms were also seen in the centers of the seminiferous tubules. Leydig cells appeared numerous (Fig. 7).

DISCUSSION

① Effects of calcium gluconate on some reproductive hormones, ionic calcium level, testicular and thyroid functions:

In the present study, the obtained results revealed that exogenous calcium gluconate injection to adult male chickens induced a significant increase in the plasma levels of testosterone, FSH, ICSH, PRL and TSH with a significant decrease in the ionic calcium level. No significant change was recorded in the plasma estradiol level at 20 minutes post-administration. The increased testosterone level may be attributed to the calcium-induced Gn-RH velease that stimulates ICSH release which inturn led to testosterone release as reported by Lin (1985). Another explanation depends on the direct stimulatory action of clacium on Leydig cell. Lin (1985) showed that addition of calcium and calmodulin on testicular tissues stimulated androgen formation. Carr et al. (1986) observed the same findings on adrenal steroids. The significant positive correlations existed between testosterone and estradiol or between ICSH and estradiol may confirm the possibility of two pathways.

Regarding FSH and ICSH incressed plasma levels, the obtained results could be attributed also to Gn-RH release that stimulates their secretion from pituitary glands via calcium influx. This suggestion was

supported by the work of many authors; Kneight (1983) found that depolarization of mediobasal hypothalamus leads to depolarization-induced release of LH-RH. This stimulatory effect was diminished by removal of calcium ions. McArdle et al. (1987) reported that stimulation of Gn-RH for LH release is inhibited by chelation of extracellular calcium ions and initiated by increased Ca^{2+} uptake and increased intracellular Ca^{2+} concentration. In addition, Scammell and Dannies (1983) and Judd et al. (1985) demonstrated the essential role of calcium in PRL release from the pituitary gland. The decreased ionic calcium level that was reported in the present study in association with the increased level of PRL may confirmed by the findings of Tan and Tashjan (1984) who attributed that decrease to the increased Ca^{2+} uptake by the pituitary cells. Moreover, Gershengorn and Thaw (1985) reported that the increased PRL release might be through TRH that also depends on the presence of extracellular calcium. Meanwhile, the positive correlation between PRL and FSH and the negative correlation between TSH and Ca^{2+} that were induced by exogenous calcium administration may suggest that the nature of relationships between Ca^{2+} and PRL or FSH or TSH are nearly the same and give an idea about the mechanisms involved in the release of such hormones that appear to be calcium-dependent.

Concerning TSH level, Greerspan (1991) reported that the increased TSH level in response to calcium administration that was also recorded in ourpresent study may be mediated through TRH pathway. This suggestion may also confirms the already recorded negative correlation existed between TSH and Ca^{2+}. Moreover, Singh and Bharaduaj (1982) demonstrated that the increased TSH level was responsible for the increased thyroid activity that was also shown in the histopathological studies of thyroid gland during the present study. The same results was augmented by those of Scammel and Danies (1983) who reported that substances that stimulate PRL release and depends on Ca^{2+} stimulate also other pituitary hormones such as TSH and Habener et al. (1977) who mentioned that calcium is important for the secretion of different hormones including TSH. Sharp et al. (1990) attributed the unchanged estradiol level, that was also recorded in the present study, in response to calcium treatment to that the injected calcium might stimulated release of cLH-RH-II (ICSH-inducer) more than cLH-RH-I (FSH-inducer). However, the negative correlation recorded between TSH and estradiol may augment the suggestion of Sharp et al. (1990)

considering that the mechanism controlling cLH-RH-II release is the same for TRH (Greenspan, 1991).

Inspite of the elevated levels of ICSH and FSH, the injected calcium adversely affected the seminiferous tubules. Also, disturbance of spermatogenic process was recorded. Molcho et al. (1984) attributed such adverse effects to the stimulatory role of calcium to the inhibitory testicular enzymes or due to stimulation of certain proteins that may have an adverse effects on the testis (Kimball and Jefferson, 1992). Calcium receptors were detected on Sertoli cells by Akerstrom and Walters (1992).

② Effects of exogenous human chorionic gonadotropin (hCG) on some reproductive hormones, ionic calcium level, testicular and thyroid function

In the present study, administration of hCG to adult male chickens was associated with significantly elevated level of ICSH. Similar result was obtained by Soliman et al. (1980b) who found that injection of hCG in adult male rats induced significant increase in the level of LH at 5 minutes post-injection. Nelson et al. (1965) reported that the elevated LH level that was observed in chicken at 36 minutes post-hCG injection was attributed to the increased endogenous LH secretion as the half-life of the injected hCG was recorded to be only few minutes. In addition, the plasma FSH was also significantly increased at 20 minutes post-hCG injection. This obtained result coincides with that of Gabriel et al. (1986) who attributed such finding to the effect of LH-induced increased testosterone level that suppress FSH utilization leading to its increase in the plasma. In the present study, no significant alteration was recorded in the level of plasma testosterone in response to hCG injection, so the increased FSH level could not explained in relation to increased testosterone level. Other explanation could be based on the initiation of feed-back mechanism by the decreased testosterone level through hypothalamic-pituitary axis resulting in increased FSH level. The increased LH level that also recorded in the present study parallel with the FSH level may augmentes this suggestion.

The level of PRL was significantly decreased at 20 minutes post-hCG injection. The same result was also obtained by Kutsky (1973) who attributed such decrease to the known antagonistic relationship between LH and PRL. However, Dobozy et al. (1981) mentioned that the suppressing effect of injected LH on PRL level may be mediated through thyroid hormones. In the present study, the plasma TSH level was

recorded to be significantly decrease accompanied by decreased thyroid activity as shown in the histopathological finding. In the present study, the decreased TSH level could not explain to be mediated through testosterone levels as mentioned by Chiasson and Carr (1985) but it may be mediated through another unclear mechanism. However, the decreased thyroid activity could be attributed to the binding of injected hCG to the less sensitive thyroid receptors. The positive correlation existed between PRL and TSH may indicates existance of another mechanism rather than hypothalamic-pituitary pathway regulating such hormonal changes.

Regarding gonadal steroids, the testosterone and estradiol levels were recorded to be unchanged in response to hCG-injection. On the other hand, histopathological examination revealed increased testicular activity. This contraversal findings could be explained on the basis that the increased activities of Leydig and Sertoli cells for production of testosterone and estradiol may be so fast or delayed beyond the time of blood collection (20 minutes post-hCG injection). Hertelendy et al. (1989) found that testosterone level was significantly increased at 5 minutes post-hCG administration and they attributed such fast action to the rapid mobilization of calcium that takes place one minute after LH administration. The significant decrease in plasma level of ionic calcium may confirm such suggestion. Moreover, Verhoeven et al. (1986) attributed the delayed response of Leydig cells to hCG administration to the higher hormonal level that may lead to delayed response phenomenon with subsequent delayed testosterone secretion. In addition, Williams (1988) found that the level of estradiol-17β did not change in response to ovine LH-administration. The presence of positive correlation between testosterone and estradiol may augments the possibilities of 2 mechanisms. Moreover, the decreased level of plasma ionic calcium could be attributed to the rapid influx of calcium ions through the cell membrane to initiate cellular responses for hormonal regulation and secretion (Habener et al., 1977).

So, it could be concluded that calcium administration was associated with increased secretion of gonadotropins, testosterone and TSH with a stimulatory effect on thyroid gland and an adverse effects on testicular tissues. However, hCG-administration was associated with a stimulating effects on gonadotropins secretion and testicular functions with an inhibitory effects on PRL, TSH and thyroid gland activity. So, the use of either calcium gluconate or human chorionic gonadotropin in poultry farms should be taken with cautiousness.

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Table 1. Effects of exogenous calcium and hCG (LH) on plasma levels of some reproductiv hormones and ionic calcium in mature male chickens.

Parameters Groups	Testosterone (ng / ml)	FSH (miu / ml)	ICSH (miu / ml)	PRL (ng / ml)	Estradiol-17β (pg / ml)	TSH (miu / ml)	Ca^{2+} (mg / dl)
Control (n=16) (Saline)	0.66 ± 0.04	2.16 ± 0.44	3.88 ± 0.45	6.80 ± 0.65	15.60 ± 0.74	2.60 ± 0.20	9.27 ± 0.66
Calcium-treated (n=15)	2.87 ± 0.52	4.68 ± 0.54	51.22 ± 7.34	9.87 ± 1.10	14.64 ± 0.87	6.73 ± 1.17	7.04 ± 0.21
HCG-treated (n=15)	0.35 ± 0.02	14.30 ± 1.34	68.80 ± 7.85	0.34 ± 0.07	17.00 ± 2.26	0.74 ± 0.05	5.51 ± 0.57
F-value	16.82	44.80	24.57	35.87	0.54	16.56	10.90
PSD	0.33	0.93	6.65	0.79	1.57	0.73	0.56

Values indicate means ± SE, n = number of birds, $P<0.05$

Table 2. Correlation matrix between reproductive hormones and ionized calcium levels of control mature male chickens

	Testosterone	FSH	ICSH	PRL	Estradiol-17β	TSH	Ca^{2+}
Testosterone	1.00						
FSH	-0.23	1.00					
ICSH	-0.51	0.15	1.00				
PRL	-0.23	0.20	0.90*	1.00			
Estradiol-17β	0.08	-0.91*	-0.22	-0.21	1.00		
TSH	0.83*	-0.43	-0.27	-0.20	0.08	1.00	
Ca^{2+}	0.64*	-0.75*	-0.61	-0.42	0.75*	0.46	1.00

Table 3. Correlation matrix between reproductive hormones and ionized calcium levels of calcium-treated birds.

	Testosterone	FSH	ICSH	PRL	Estradiol-17β	TSH	Ca^{2+}
Testosterone	1.00						
FSH	0.47	1.00					
ICSH	0.55	0.55	1.00				
PRL	0.80*	0.79*	0.93*	1.00			
Estradiol-17β	0.86*	0.17	0.80*	0.65*	1.00		
TSH	-0.51	0.15	-0.23	-0.47	-0.80*	1.00	
Ca^{2+}	-0.08	-0.76*	0.06	-0.31	0.39	-0.68*	1.00

Table 4. Correlation matrix between reproductive hormones and ionized calcium levels of hCG-treated birds.

	Testosterone	FSH	ICSH	PRL	Estradiol-17β	TSH	Ca^{2+}
Testosterone	1.00						
FSH	0.49	1.00					
ICSH	0.43	0.47	1.00				
PRL	0.47	-0.44	0.02	1.00			
Estradiol-17β	0.94*	0.50	0.23	0.30	1.00		
TSH	0.52	-0.32	-0.18	0.94*	0.4	1.00	
Ca^{2+}	-0.38	0.02	0.01	0.01	-0.58	0.04	1.00

- The critical value at 5% = ± 0.64

- The critical value at 1% = ± 0.83

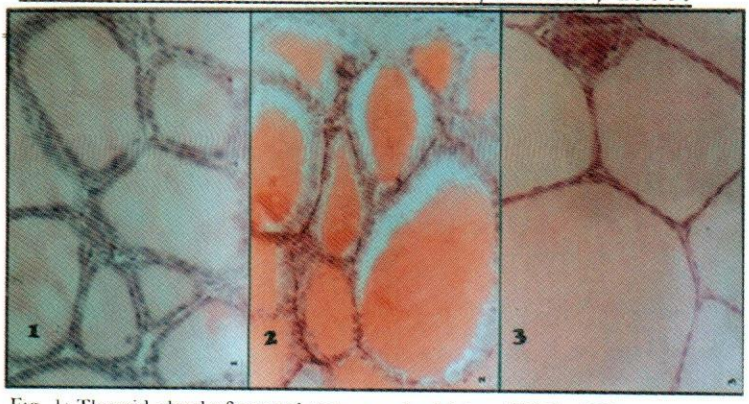

Fig. 1: Thyroid gland of control mature male chicken (H&E x400).
Fig. 2: Thyroid gland of mature male chicken after 20 minutes of calcium gluconate injection (H&E x400)
Fig. 3: Thyroid gland of mature male chicken after 20 minutes of hCG injection (H&E x400).

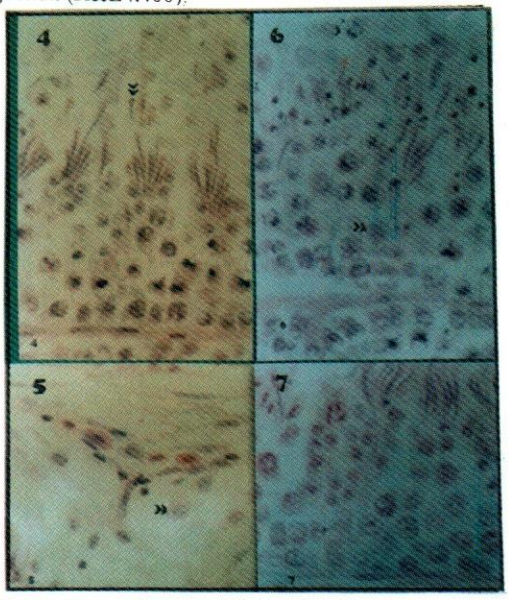

Fig. 4: Testis of control mature chicken showing mature sperms (arrow) (H&E, x800).
Fig. 5: Testis of control mature chicken showing few large polygonal Leydig cells (>>) and few interstitial tissues (H&E, x800).
Fig. 6: Testis of mature chickens after 20 minutes of calcium injection showing disturbed spermatogenic steps, giant cells with multiple nuclei (>) and degenerated spermatocytes (>>) (H&E, x800).
Fig. 7: Testis of mature chickens after 20 minutes of hCG injection showing no changes in cellular stages of spermatogensis (H&E, x800).