

EFFECT OF PROLONGED ADMINISTRATION OF HALOFUGINONE AND MADURAMICIN ON SEMEN QUALITY AND SERUM TESTOSTERONE LEVELS IN BOVANS COCKERELS

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Abstract

In the present work, the effects of Halofuginone hydrobromide and Maduramicin ammonium mixed with ration for 120 days in Bovans cockerels were studied on serum levels of testosterone , semen quality and histopathological changes of testes. It could be concluded from the present study that Halofuginone hydrobromide at a dose of 1.5 p.p.m can be used successfully for Bovans cockerels. However, Halofuginone hydrobromide at a dose of 3 p.p.m cannot be used in Bovans cockerels as it caused significant decrease in serum testosterone levels, semen volume, sperm count, and increased sperm abnormalities as well as marked histopathological changes in testes. On the other hand, the recommended dose (5 p.p.m.) of Maduramicin ammonium can be used successfully in Bovans cocks.

INTRODUCTION

Coccidiosis is undoubtedly the most important parasitic disease of poultry caused by specific protozoa (genus *Eimeria*) which damages the lining of the intestinal tract resulting in enteritis and diarrhoea. Coccidiosis outbreaks vary from mild to severe infection that depends upon the number of the sporulated oocysts causing the infection (Hofstad *et al.*, 1984).

Halofuginone hydrobromide and Maduramicin ammonium were widely used as effective coccidiostats for broiler chicken. Apart from rapid development of oocysts resistance with continual use, problems also arose with adverse effects on laying performance.

On the other hand, semen quality was found to have significant correlation with fertility percentage (Kamar *et al.*, 1984 and Radwan, 1991). Highly significant correlation was observed between motility and live sperm percentage and between

motility and fertility. So, the quality of semen determines cock's fertilizing ability to a high extent.

Thus, the present work was carried out to evaluate the effects of Halofuginone hydrobromide and Maduramicin ammonium on reproductive performance of cockerels (serum levels of testosterone and semen quality). Moreover their effects on the histopathological changes of the testes were also studied.

MATERIALS AND METHODS

A- Materials

A.1. Drugs

A.1.1. Halofuginone hydrobromide (Stenorol)

It was obtained from HOECHST-ROUSSEL-Vet. in a package of 25 kg each. Each kilogram of (Stenorol) contained six grams of Halofuginone hydrobromide. It is dispensed in the form of white premix powder to be mixed with starter ration.

A.1.2. Maduramicin ammonium (Cygro)

It was obtained from HOFFMAN-LA ROCHE France, in a package of 20 kg each. Each kilogram of (Cygro) contained ten grams of Maduramicin ammonium. It is dispensed in the form of tan premix crystalline powder to be mixed with starter ration.

A.2. Birds

Thirty five birds, one day old Bovans cockerels were brought from Tiba company. The birds were kept on a commercial starter ration for four months (rearing period) to which the tested anticoccidial drugs were mixed. The birds were then kept on unmedicated commercial growing ration from 120 days to 148 days (production period). Feed and water were offered *ad-libitum*, temperature was adjusted by using an electric heater during rearing period and using an electric fan with adequate ventilation during production period. Light and vaccination programs were adjusted as described by Bovans chicken strain. Birds were kept in strictly hygienic conditions with regular removal of litter to avoid parasitic and bacterial infection.

Experimental design

Thirtyfive birds, one day old Bovans cockerels were divided into five equal groups (7 males each). The first group was fed on unmedicated starter ration for 120 days. The second group was given Halofuginone hydrobromide mixed with starter

ration for 120 days (1.5 p.p.m ration ,coccidiostatic dose). The third group was given Halofuginone hydrobromide (3 p.p.m. ,coccidiocidal dose) mixed with starter ration for 120 days. The fourth group was given Maduramicin ammonium at a dose of 5 p.p.m. (the recommended dose) mixed with starter ration for 120 days. The fifth group was given Maduramicin ammonium (10 p.p.m.) mixed with starter ration for 120 days. Birds in all groups were fed on unmedicated growing ration from 120-148 days.

B-Methods

B-1- Hormone assay

Blood samples were collected from the brachial vein of each bird at age 120, 127, 134, 141 and 148 days-old without anticoagulant, left to clot then, centrifuged at 3000 r. p. m for 15 minutes to separate serum. Serum samples were kept at -20°C for assay of testosterone hormone at age 120, 127, 134, 141 and 148 days-old (Amin and Gilbert, 1970). Testosterone was measured according to Yalow and Berson (1971) who followed the basic principle of radioimmunoassay using DSL- 4000 Active TM testosterone coated tube radioimmunoassay Kit obtained from Diagnostic Systems Laboratories Inc, Webster, Texas U.S.A.

B-2- Semen analysis

Semen samples were collected from cockerels by abdominal massage (Burrows and Quinn, 1937) weekly from 120 to 148 days-old cocks for evaluating semen quality. Semen samples were collected in clean and dry labeled test tubes from individual cocks in each group previously isolated from females in corresponding groups 5-days prior to sampling. Semen samples were subjected to the following analysis:

- Semen volume

Semen volume was measured by using micropipette of 1000 microlitre capacity.

- Sperm concentration

Sperm concentration was estimated by using Neubauer haemocytometer.

- Motility, livability and abnormalities of spermatozoa:

The motility was determined by subjective evaluation using high power microscope, live spermatozoa and abnormalities were determined by using eosin and negrosin staining as described by El Jack and Lake (1966).

B-3- Tissue samples

Tissue samples from testes were taken and fixed in formalin 10 % for 24 hours, then, trimmed and washed by water for four hours then, they were dehydrated by transferring them into a series of graded concentrated ethyl alcohol (70 %, 95 % & 100 %). The tissues were cleared in xylene for 2 hours, then, embedded in paraffin wax at 60-70 °C for four hours. The sections were cut by microtome, and dewaxed by xylene for 10 minutes, then, in ethyl alcohol 3 minutes then, methyl alcohol for 3 minutes, washed by distilled water for 5 minutes. The sections were routinely stained with haematoxylin and eosin [H & E]. The stained sections were washed by tap water, dehydrated in alcohol, cleared through xylene, mounted in Canada balsum.

Statistical analysis

The results obtained were statistically analyzed using student "t" test according to Snedecor (1969).

RESULTS AND DISCUSSION

Coccidiosis is considered as a very dangerous parasitic disease that affects poultry industry and causes economic losses, so, anticoccidial drugs are widely used to prevent the disease and minimize such losses. Today, cockerels are products of interest due to their importance in semen with high quality.

Halofuginone hydrobromide and Maduramicin ammonium representing two different groups of anticoccidial drugs (Quinazolinone & Polyether ionophores) that are widely used for prophylactic control of coccidiosis by mixing them with ration (Brander *et al.*, 1991). The adverse effects of these drugs may affect productive and reproductive performance. In the present work, the effect of two commonly used anticoccidial drugs (Halofuginone hydrobromide and Maduramicin ammonium) administered for four months in Bovans cockerels were studied on serum testosterone levels and semen quality. Histopathological changes of testes were also recorded. Table 1 showed that Halofuginone hydrobromide decreased the levels of testosterone significantly ($P < 0.05$) at a dose of 3 p.p.m at 127 days-old and with both doses ($P < 0.01$) at 134 days-old. Maduramicin ammonium given at a dose of 5 p.p.m. significantly increased testosterone levels at 120 days-old, while, significantly decreased it at a dose of 10 p.p.m at 127 & 134 days-old (Table 1). Levorse *et al.* (1991) recorded that extended periods of increased cytoplasmic calcium induced by carboxylic ionophores may serve as a negative feed back mechanism to reduce steroid

production. This results in a dose dependent inhibition of androstenedione production by LH stimulated theca cells. Johnson *et al.* (1991) also reported that carboxylic ionophore stimulates the release of endogenous arachidonic acid from theca cells resulting in inhibition of androstenedione production, and the carboxylic ionophore appears to mobilize Ca^{2+} from inositol 1, 4, 5-triphosphate sensitive and insensitive pools. Therefore, cytosolic Ca^{2+} can approach millimolar concentration in response to the carboxylic ionophore. Halofuginone hydrobromide (3 p.p.m.) administration in Bovans cockerels caused a significant decrease in serum testosterone levels which may be supported by degenerative changes of spermatogenic and Leyding cells and absence of sperms as found in histopathological finding. The data of the present work pointed that Maduramicin ammonium administration decreased serum testosterone levels when given at a high dose (10 p.p.m.). Similar findings had been reported by Zobell *et al.* (1987) and Tag El-Din (1995). The increased cytosolic calcium concentration (as a result of ionophores) may be involved in the suppression of androstenedione production as a result of an interaction with protein kinase C (Levorse *et al.* 1991).

It is clear that Halofuginone hydrobromide given to Bovans cockerels at a dose of 3 p.p.m. for 4 months, significantly decreased semen volume and sperm count, (Table 2) and increased sperm abnormalities (Table 3). No changes were observed in sperm motility with both doses (Table 2). Semen volume and sperm count showed non-significant changes in groups given 1.5 p.p.m. as compared with non-medicated control group.

Maduramicin ammonium at a dose of 10 p.p.m. given for 4 months decreased semen volume ,sperm count and sperm motility (Table 2) and increased sperm abnormalities (head, mid-piece and tail) as compared with non-medicated control group (Table 3). It was noticed that, Maduramicin ammonium at a dose of 5 p.p.m. showed a significant increase in semen volume and sperm motility at 120 days-old. Regarding the effect of the two tested drugs on semen quality, it was observed that Halofuginone hydrobromide (3 p.p.m.) and Maduramicin ammonium (10 p.p.m.) decreased semen volume and sperm count and increased sperm abnormalities. These results are supported by the decrease in serum testosterone levels and the observed histopathological changes in the testes. The effect of prolonged administration of the tested anticoccidial drugs on histopathological findings of testes were studied. Cockerels medicated with Halofuginone hydrobromide at a dose of 3 p.p.m. showed

insufficient spermatogenesis with empty sperm lumina in most of seminiferous tubules (Fig. 1). Cockerels medicated with 10 p.p.m. of Maduramicin ammonium, showed insufficient spermatogenic process in seminiferous tubules which had no sperm or spermatid (Fig. 2). This finding was confirmed by Akcrstorm and Walters (1992) and Kimball and Jefferson (1992). They attributed this pathological changes to increase intracellular Ca^{2+} as a result of Maduramicin which also caused some degenerative changes in both spermatogenic and Leyding cells. The absence of sperms may be a result of the decrease in serum testosterone levels. It could be concluded from the present study that, Halofuginone hydrobromide at a dose of 1.5 p.p.m can be used successfully for Bovans cockerels. However, Halofuginone hydrobromide at a dose of 3 p.p.m. cannot be used in Bovans cockerels as it caused significant decrease in serum testosterone level, semen volume, sperm count, and increased sperm abnormalities as well as marked histopathological changes in testes. On the other hand, the recommended dose of Maduramicin ammonium (5 p.p.m.) can be used successfully in Bovans cockerels.

Table 1. Effect of Halofuginone hydrobromide (1.5 & 3.0 p.p.m.) and Maduramicin ammonium (5.0 & 10.0 p.p.m) administered for 120 successive days on the level of serum testosterone levels (ng/ml) of Bovans cockerels (Mean \pm S.E.) n = 7.

Time of sampling (day-old)	Unmedicated Control	Groups			
		Halofuginone hydrobromide		Maduramicin ammonium	
		1.5	3.0	5.0	10
120	1.48 \pm 0.16	1.49 \pm 0.37	1.41 \pm 0.1	2.11 \pm 0.1**	1.2 \pm 0.1
127	1.15 \pm 0.1	0.87 \pm 0.1	0.77 \pm 0.1*	1.02 \pm 0.03	0.83 \pm 0.03**
134	2.16 \pm 0.24	1.29 \pm 0.15**	1.12 \pm 0.16**	1.95 \pm 0.21	1.64 \pm 0.07**
141	1.66 \pm 0.23	1.31 \pm 0.29	1.15 \pm 0.17	1.54 \pm 0.15	1.51 \pm 0.02
148	1.16 \pm 0.27	1.16 \pm 0.16	1.12 \pm 0.19	1.16 \pm 0.1	1.16 \pm 0.29

* P < 0.05

** P < 0.01

Table 2. Effect of Halofuginone hydrobromide (1.5 & 3.0 p.p.m.) and Maduramicin ammonium (5.0 & 10.0 p.p.m.) administered for 120 successive days on semen picture of Bovans cockerels (Mean \pm S.E.) n = 7.

Time of sampling (day-old)	Semen picture	Groups				
		Unmedicated Control	Halofuginone hydrobromide		Maduramicin ammonium	
			1.5	3.0	5.0	10
120	Semen volume (ml)	0.59 \pm 0.04	0.72 \pm 0.04	0.47 \pm 0.03*	0.77 \pm 0.05*	0.48 \pm 0.03*
	Sperm count (10^9 /ml)	4.01 \pm 0.38	3.76 \pm 0.54	3.23 \pm 0.67	5.09 \pm 0.5	2.49 \pm 0.24**
	Motility %	84.57 \pm 2.86	84.86 \pm 2.36	79.86 \pm 1.24	93.86 \pm 2.19*	30.29 \pm 4.26***
	Livability %	96.29 \pm 1.75	94.86 \pm 1.83	95.0 \pm 1.57	94.29 \pm 0.87	94.57 \pm 1.17
127	Semen volume (ml)	0.74 \pm 0.01	0.71 \pm 0.02	0.61 \pm 0.04**	0.76 \pm 0.04	0.64 \pm 0.03**
	Sperm count (10^9 /ml)	4.04 \pm 0.31	3.25 \pm 0.27	2.71 \pm 0.2**	4.59 \pm 0.38	2.62 \pm 0.24**
	Motility %	91.0 \pm 3.35	83.29 \pm 2.3	88.5 \pm 2.36	90.29 \pm 1.49	30.29 \pm 5.68***
	Livability %	98.86 \pm 0.46	97.43 \pm 1.21	94.96 \pm 2.68	97.71 \pm 1.08	91.14 \pm 3.03***
134	Semen volume (ml)	0.79 \pm 0.05	0.75 \pm 0.06	0.54 \pm 0.05**	0.76 \pm 0.06	0.53 \pm 0.03***
	Sperm count (10^9 /ml)	3.69 \pm 0.2	3.31 \pm 0.18	3.1 \pm 0.17*	3.78 \pm 0.37	3.06 \pm 0.19*
	Motility %	85.14 \pm 3.39	80.57 \pm 1.41	76.14 \pm 2.06*	85.29 \pm 1.86	41.43 \pm 5.34**
	Livability %	96.86 \pm 1.45	95.43 \pm 1.61	96.86 \pm 1.2	98.57 \pm 0.95	95.75 \pm 1.43
141	Semen volume (ml)	0.75 \pm 0.03	0.86 \pm 0.04	0.52 \pm 0.02***	0.85 \pm 0.04	0.46 \pm 0.01***
	Sperm count (10^9 /ml)	3.92 \pm 0.16	3.9 \pm 0.18	3.31 \pm 0.16*	4.23 \pm 0.16	3.3 \pm 0.13*
	Motility %	86.71 \pm 1.84	80.57 \pm 0.89	80.57 \pm 2.85	88.14 \pm 1.62	4.43 \pm 3.22***
	Livability %	99.29 \pm 0.42	98.57 \pm 0.81	88.14 \pm 1.62	97.71 \pm 1.49	96.57 \pm 1.63
148	Semen volume (ml)	0.76 \pm 0.04	0.75 \pm 0.03	0.56 \pm 0.03**	0.83 \pm 0.03	0.53 \pm 0.03**
	Sperm count (10^9 /ml)	4.29 \pm 0.16	4.11 \pm 0.23	3.35 \pm 0.2**	4.61 \pm 0.15	3.1 \pm 0.13***
	Motility %	89.57 \pm 0.75	83.86 \pm 1.65**	9.57 \pm 3.0**	91.43 \pm 0.89	54.71 \pm 3.39***
	Livability %	98.71 \pm 1.28	99.14 \pm 0.86	96.57 \pm 1.44	99.43 \pm 0.43	97.71 \pm 1.2

* P < 0.05

** P < 0.01

*** P < 0.001

Table 3. Effect of Halofuginone hydrobromide (1.5 & 3.0 p.p.m.) and Maduramicin ammonium (5.0 & 10.0 p.p.m.) administered for 120 successive days on sperm abnormalities (%) of Bovans cocks (Mean \pm S.E.) $n = 7$.

Time of sampling (day-old)	Sperm Abnormalities	Groups				
		Unmedicated Control	Halofuginone hydrobromide		Maduramicin ammonium	
			1.5	3.0	5.0	10
120	Head	-	-	-	-	-
	Mid. Piece	4.0 \pm 1.09	4.86 \pm 0.77	4.29 \pm 0.18	4.0 \pm 0.62	7.29 \pm 0.99*
	Tail	5.57 \pm 0.99	6.43 \pm 0.81	8.43 \pm 0.22*	6.86 \pm 0.94	26.0 \pm 1.04***
127	Head	2.57 \pm 0.48	2.43 \pm 0.53	3.57 \pm 0.73	2.71 \pm 0.61	6.15 \pm 1.16*
	Mid. Piece	1.0 \pm 0.31	1.57 \pm 0.2	2.42 \pm 0.37*	1.43 \pm 0.2	4.43 \pm 0.92**
	Tail	5.15 \pm 0.18	4.43 \pm 0.53	7.14 \pm 0.88*	5.29 \pm 0.61	22.14 \pm 1.99***
134	Head	-	-	-	-	-
	Mid. Piece	3.83 \pm 0.56	3.43 \pm 0.48	4.14 \pm 0.74	3.71 \pm 0.42	7.29 \pm 1.04*
	Tail	7.57 \pm 0.72	7.31 \pm 1.12	9.0 \pm 2.04	5.72 \pm 0.4*	25.71 \pm 2.44***
141	Head	2.33 \pm 0.58	1.6 \pm 0.34	2.2 \pm 0.32	1.5 \pm 0.37	3.5 \pm 0.49
	Mid. Piece	1.33 \pm 0.19	1.71 \pm 0.29	2.86 \pm 0.63*	1.43 \pm 0.2	1.85 \pm 0.55
	Tail	5.52 \pm 0.59	7.55 \pm 1.15	6.18 \pm 1.62	5.08 \pm 0.52	17.36 \pm 4.27*
148	Head	-	-	-	-	-
	Mid. Piece	3.57 \pm 0.57	3.42 \pm 0.35	6.0 \pm 0.53**	2.72 \pm 0.52	1.58 \pm 1.91**
	Tail	6.57 \pm 0.57	6.72 \pm 1.06	11.14 \pm 1.72*	7.71 \pm 0.75	16.28 \pm 1.86***

* P < 0.05

** P < 0.01

*** P < 0.001

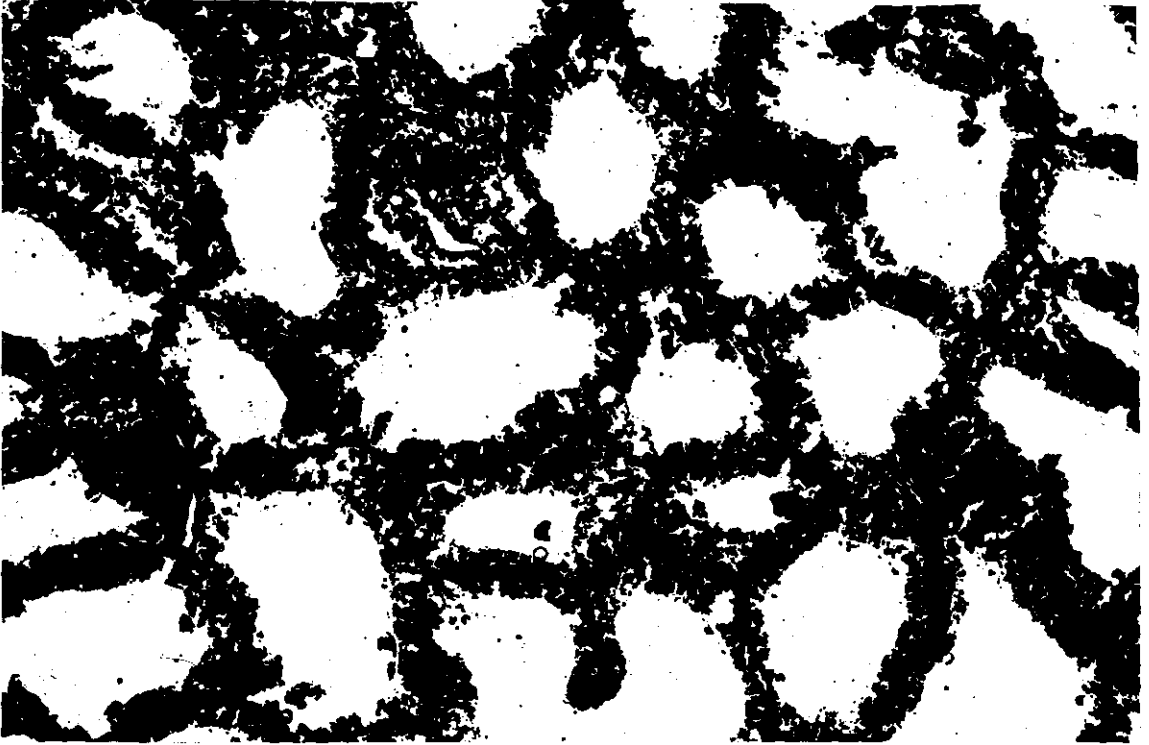


Fig. 1. Tests of Bovans cockerels medicated with Halofuginone hydrobromide (3 p.p.m.) for 120 successive days showing insufficient spermatogenic process with empty lumenae of seminiferous tubules from spermatid and sperms (H & E X 40).



Fig. 2. Tests of Bovans cockerels medicated with Maduramicin ammonium (10 p.p.m.) for 120 successive days showing insufficient spermatogenic process in the seminiferous tubules with empty lumenae from sperms (H & E X 40).

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تأثير إعطاء الهالوفوجينون والماديوراميسين لفترات طويلة على جودة السائل المنوى وهرمون التستستيرون فى مصل ديوك بوفانز

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تم دراسة تأثير اثنين من مضادات الكوكسيديا (هالوفوجينون هيدروبروميد ، ماديوراميسين أمونيوم) فى الديوك بخلطها فى العليقة من عمر يوم حتى ١٢٠ يوماً على معدل هرمون التستستيرون وجودة السائل المنوى والتغيرات الهستوباثولوجية فى أنسجة الخصيتين.

أجريت هذه الدراسة على خمسة وثلاثين كتكوتاً من سلالة بوفانز عمر يوم، وقسمت إلى خمس مجموعات متساوية تحتوى كل مجموعة على سبعة ذكور. المجموعة الأولى تم تغذيتها بعليقة خالية من مضادات الكوكسيديا (المجموعة الضابطة). المجموعتان الثانية والثالثة أعطيتا هالوفوجينون هيدروبروميد مضافا إلى العليقة بنسبة ١,٥، ٣ جزء فى المليون على التوالي. المجموعتان الرابعة والخامسة أعطيتا ماديوراميسين أمونيوم مضافا إلى العليقة بنسبة ٥، ١٠ جزء فى المليون على التوالي.

تم أخذ دم من وريد الجناح لكل طائر عند عمر ١٢٠، ١٢٧، ١٣٤، ١٤١ و ١٤٨ يوماً لفصل المصل. تم أخذ عينات من السائل المنوى من الديوك أسبوعياً عند عمر ١٢٠-١٤٨ يوماً. تم أخذ عينات من الخصيتين فى نهاية التجربة عند عمر ١٤٨ يوماً للفحص الهستوباثولوجى.

وقد لوحظ أن هالوفوجينون هيدروبروميد فى ديوك بوفانز عند جرعة ٣ جزء فى المليون أحدث انخفاضاً فى حجم السائل المنوى وعدد الحيوانات المنوية ولكن زيادة تشوهات الحيوان المنوى وتغيرات مرضية واضحة فى أنسجة الخصيتين.

ماديوراميسين أمونيوم (٥، ١٠ جزء فى المليون) عند إعطائه إلى ديوك بوفانز، عند جرعة ١٠ جزء فى المليون أحدث انخفاضاً فى حجم السائل المنوى وعدد الحيوانات المنوية ومستوى هرمون التستستيرون فى المصل.

ومن النتائج السابقة أنه يمكن إعطاء ماديوراميسين أمونيوم بجرعة ٥ جزء فى المليون فى الديوك لحمايتها من الكوكسيديا بدون آثار ضارة على خواص السائل المنوى أو مستوى هرمون التستستيرون بالمصل.