PHYSICAL SEMEN CHARACTERISTICS OF CALIFORNIA RABBIT BUCKS ADMINISTRATED WITH DIFFERENT CHROMIUM LEVELS

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ABSTRACT

The experimental work was carried out in the Rabbit Production Farm, Agricultural Researches and Experiments Station, Faculty of Agriculture, Mansoura University, during the period from February to Augusts 2006. A total of 12 sexually mature California bucks having 6-8 months of age and 3.0-3.5 kg live body weight) was used in this study. Bucks were healthy, free of diseases and external parasites. The experimental bucks were divided into three groups (4 males in each group) according to treatment. Bucks in the 1st Group served as control. However, group 2 and 3 were given chromium picolinate (9 and 18 µg/kg body weight) respectively. The bucks were given the dose orally every day as a suspend buck. To determine mass motility, progressive motility, Sperm cell concentration, Percentage of dead spermatozoa**,** percentage of abnormal spermatozoa was recorded. Results show that ejaculate volume of rabbit bucks significantly ($P<0.05$) decreased in both treated groups as compared to control group. This reduction was higher with low than high Cr dose, being about 12.5 and 8.6%, respectively. The obtained results revealed a negative effect of Cr treatment on ejaculate volume of rabbit bucks. Mass motility or percentage of progressive motility was significantly ($P<0.05$), being positive for the low level (9 µg/kg) and negative for the high level (18 µg/kg) of Cr. Level of 9 µg/kg significantly ($P<0.05$) increased score of mass motility by about 24% and progressive motility by about 34%, however, level of 18 µg/kg significantly ($P<0.05$) decreased score of mass motility by about 16% and progressive motility by about 22% as compared to controls. Dead sperm percentage was significant ($P<0.05$), being lower by about 17% for the low level (9 µg/kg) and higher by about 4% for the high level as compared to the control group (18 µg/kg) of Cr. Percentage of sperm abnormality was affected significantly ($P<0.05$) by the low Cr level, but was not affected by the high level of Cr. Sperm abnormality percentage significantly ($P<0.05$) reduced by about 48% as compared to the control group. Sperm cell concentration ($x10^6$/ml) significantly ($P<0.05$) increased by about 37% in low Cr group, but was not affected by the high level of Cr. However, total sperm output ($x10^6$/ejaculate) significantly ($P<0.05$) increased by about 16.5% in low Cr group and reduced by about 10% in high Cr group as compared to the control group.

INTRODUCTION

The domestic rabbit is emerging as a viable livestock species. It is due to its high prolificacy and growth rate and its better meat quality than for other farm animals. There is a great attention for using different types of growth promoters and anabolic agents in rabbit production in Egypt.

Chromium (Cr) is essential for both human and animals and has been on the list of the mineral elements regarded as essential since 1959 (McDonald et al. 1992). In recent years, there was considerable research interest in the utilization of Cr in animal feed. The beneficial effects of Cr in human health were well documented for its role as an integral component of the glucose tolerance factor (GTF), which potentates the action of insulin, one of the most important anabolic hormones (Burton et al., 1993; Hossain, et al.

1998 and Piva et al., 2003). GTF consists of one 3+ atom of Cr bound to several molecules of niacin and amino acids found in glutathionine (glutamic acid, glycine and 3+ cysteine). Without Cr at its core, GTF is inactive (Holdsworth and Neville, 1990).

It has been reported that Cr supplementation to diets of healthy rats (Anderson, et al. 1996) and humans (Anderson, 1994) improves glucose tolerance and insulin binding, therefore normalizing blood glucose levels. Also, dietary Cr supplementation has a positive effect on growth rate and feed efficiency in poultry [Cupo and Donaldson, 1997; NRC, 1997 and Sahin et al., 2001). Besides, Cr plays essential roles for normal metabolism of carbohydrates, proteins and lipids in human and livestock (McDowell, 1992). Most authors agree that Cr supplementation during periods of increased stress has a positive effect on weight gain. In male, a positive effect of Cr supplementation on weight gain (Chang and Mowat, 1992 and Kegley et al. (1997).

Supplementation of Cr has decreased the total cholesterol in blood of rabbits fed on a Cr-deficient diet (Abraham et al., 1982 a&b). An increase of HDL-cholesterol (Riales and Albrink, 1981) and a decrease in total cholesterol, LDL-cholesterol and triacylglycerols (Lefavi et al., 1993) have been observed in humans after Cr supplementation.

One of the theories (Lindemann, 1996) assumes reproduction can be affected by changing sensitivity to insulin. Most attention has been devoted to studying the effect of Cr on reproduction in pigs. Cr supplementation to sows has a positive effect on reproduction cycle (Lindemann et al., 1995 a & b), being in depends on doses of Cr (Lindemann et al., 2004).

Few reports studied the effect of Cr on reproductive performance of male. Some of these reports obtained a negative impact of Cr on testis histoarchitectonics, especially on Leydig cells (Li et al., 1999; Li et al., 2001 and Afonne et al., 2002) and others have explored that diet containing <100 µg/kg had negative effect on semen quality (Anderson and Polansky (1981). Therefore, the current study aimed at investigating the effect of administration of two oral doses (9 and 18 µg/kg LBW) from Cr on semen characteristics of rabbit White New Zealand rabbit bucks.

MATERIALS AND METHODS

The experimental work was carried out in the Rabbit Production Farm, Agricultural Researches and Experiments Station, Faculty of Agriculture, Mansoura University, during the period from February to Augusts 2006.

Animals and feeding system:

A total of 12 sexually mature California bucks having 6-8 months of age and 3.0-3.5 kg live body weight) was used in this study. Bucks were healthy, free of diseases and external parasites. Both testes were almost equal in size and moved freely up and down within the scrotal pouches. Rabbits were housed separately in individual wired-cages (50 x 50 x 35 cm) prepared with fresh water. Diets were offered *ad libitem* during the

experimental period in pelleted form to cover the nutrient requirement of rabbits during the experimental period as recommended by NRC (1977).

The averages of air temperature and relative humidity % in Mansora city, Dakahlyia governorate during different months of summer and autumn seasons are shown in Table (1).

Table (1): Averages of air temperature (^{0}C) and relative humidity (%) during different months of the experimental period.

Season	Month	Air temperature (^{0}C)			Relative humidity(%)		
		Min.	Max.	Mean	Min.	Max	Mean
Summer	July	22.6	33.5	28.1	43.0	96.0	70.0
	August	24.3	35.7	30.0	46.0	96.0	71.0
	September	20.8	33.5	27.1	37.0	96.0	67.0
Autumn	October	15.6	27.2	21.4	35.0	94.0	65.0
	November	12.5	24.1	8.30	36.0	94.0	65.0
	December	10.7	20.1	5.40	50.0	89.0	70.0

Treatment groups:

The experimental bucks were divided into three groups (4 males in each group) according to treatment. Bucks in the 1st Group served as control. However, group 2 and 3 were given chromium picolinate (9 and 18 µg/kg body weight) respectively.

the bucks were given the dose orally every day as a suspend buck. Average duration of 1 cycle of the seminiferous epithelium is 10 days and the spermatozoa pass through the epididymis in 4 to 7 days (Amann and Lambiase, 1969).

Semen collection and evaluation:

Pre-experimental period, bucks in all treatment groups were trained to collect semen by using an artificial vagina. During the experimental period (July), semen was collected every week from all bucks by means of an artificial vagina. The jelly mass was discarded from each ejaculate after collection, then semen volume/ejaculate was measured using gradual semen collection tube to nearest 0.1 ml.

To determine mass motility, a drop of fresh semen was placed on the warm and clean glass slide and examined using a microscope with warmed (37^{o}C) stage at low magnification (x10 power), mass motility was judged on a score from 0 to 5 according to Khalifa (1997). While, progressive motility of spermatozoa was determine in semen (one drop) diluted with 2-3 drops from warm sodium citrate solution (2.9%) on a warm slide (37^{o}C) covered by a warm cover slip. Progressive motility was examined under high magnification (x40 power). Percentage of progressive motility was recorded by spermatozoa observed moving in straight line across the field of vision with a normal vigorous swimming motion to the nearest 5% after viewing several microscopic fields.

Sperm cell concentration was determined by direct cell count using Hemocytometer and expressed in sperm count/ml fresh semen. Percentage of dead spermatozoa was immediately calculated after collection in a smear of fresh semen stained by eosin-negrosin mixture (1.65 g eosin, 10 g negrosin and 2.9 g sodium citrate diluted in 100 ml distilled water). The

percentage of dead spermatozoa was calculated from a total number of 200 spermatozoa counted in different microscopic fields. However, percentage of abnormal spermatozoa was recorded in the same semen smear of dead sperm count. Different types of sperm abnormalities were estimated in a total count of 200 sperm in various microscopic fields.

Total (TSOP), motile (MSOP) and live (LSOP) outputs per ejaculate were determined as the following:

TSOP/ejaculate=Ejaculate volume (ml) x sperm cell concentration ($x10^6$/ml)

MSOP/ejaculate = TSOP x progressive motility (%)

LSOP/ejaculate = TSOP x live sperm (%)

Statistical analysis:

Data were statistically analyzed by the computer program of SAS (2001) using the general linear models (GLM). The significant differences among group means were tested at a level of $P<0.05$ using new multiple rang test (Duncan, 1955).

RESULTS

Physical semen characteristics:

Ejaculate volume:

Results in Table (2) show that ejaculate volume of rabbit bucks significantly ($P<0.05$) decreased in both treated groups as compared to control group. This reduction was higher with low than high Cr dose, being about 12.5 and 8.6%, respectively.

Table (2): Effect of Cr level on physical semen characteristics of rabbit bucks.

Semen characteristics	Experimental group		
	Control	9 µg/kg	18 µg/kg
Ejaculate volume (ml)	0.675 ± 0.017^a	0.594 ± 0.028^b	0.617 ± 0.029^b
Mass motility (score 1-5)	3.08 ± 0.047^b	3.81 ± 0.067^a	2.58 ± 0.092^c
Progressive motility (%)	45.55 ± 0.97^b	61.11 ± 0.87^a	35.55 ± 0.76^c
Dead sperm (%)	12.72 ± 0.30^b	10.58 ± 0.28^c	13.30 ± 0.32^a
Sperm abnormality (%)	13.52 ± 0.38^a	7.02 ± 0.33^b	13.16 ± 0.31^a
Sperm concentration ($x10^6$/ml)	167.2 ± 1.9^b	228.9 ± 7.7^a	161.7 ± 2.9^b
Total sperm output ($x10^6$/ml)	112.9 ± 3.2^b	131.6 ± 4.8^a	101.7 ± 6.2^c

a, b and c: Group means denoted within the same row with different superscripts are significantly different at P<0.05.

As affected by collection week, semen volume showed different trend of change in experimental groups (Fig. 1). During the first 5 weeks, each of treated groups showed slightly higher ejaculate volume than the control group. The marked effect of Cr treatment on accessory sex glands appeared after the 5^{th} week in term of gradual reduction in semen volume in both treated groups versus nearly constant semen volume in the control group (Fig. 1).

The obtained results revealed a negative effect of Cr treatment on ejaculate volume of rabbit bucks

Sperm viability:

The effect of Cr on sperm viability in term of score of mass motility or percentage of progressive motility was significant ($P<0.05$), being positive for

the low level (9 µg/kg) and negative for the high level (18 µg/kg) of Cr. Level of 9 µg/kg significantly (P<0.05) increased score of mass motility by about 24% and progressive motility by about 34%, however, level of 18 µg/kg significantly (P<0.05) decreased score of mass motility by about 16% and progressive motility by about 22% as compared to controls (Table 2).

As affected by collection week, the effect of Cr treatment on score of mass motility and progressive motility percentage was pronouncedly observed at the 5th collection week. Both semen characteristics increased in low Cr group and decreased in high Cr group as compared to the control group, which showed nearly constant values from the 5th up to 9th week (Figs. 2 and 3).

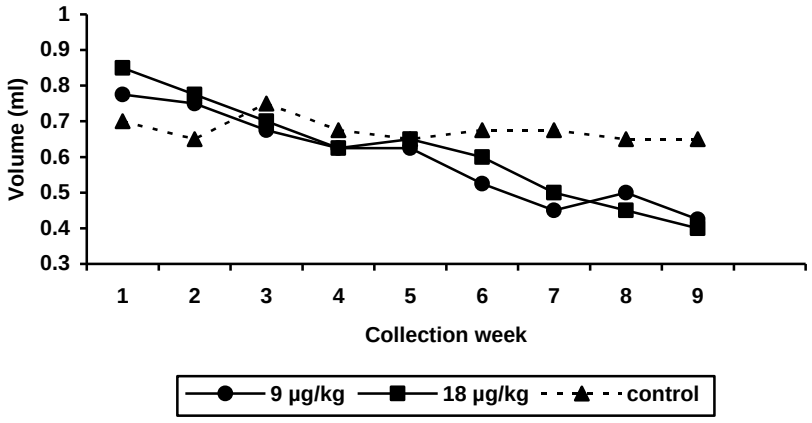

Figure (1): Changes in semen volume of rabbit bucks in different experimental groups throughout collection weeks.

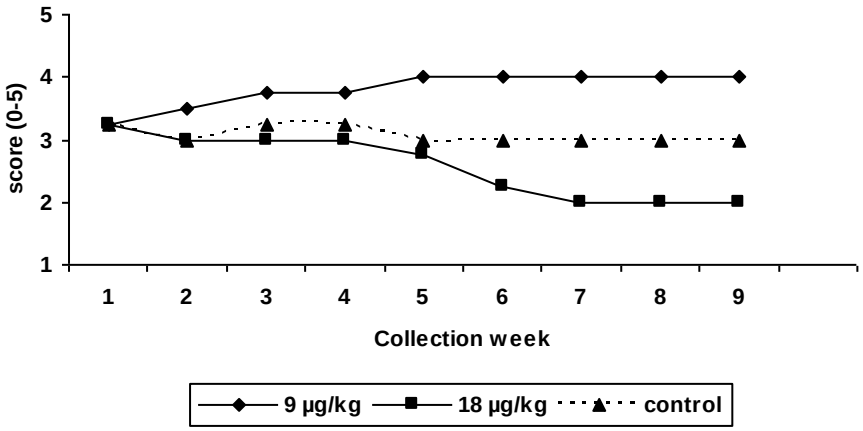

Figure (2): Changes in sperm mass motility of rabbit bucks in different experimental groups throughout collection weeks.

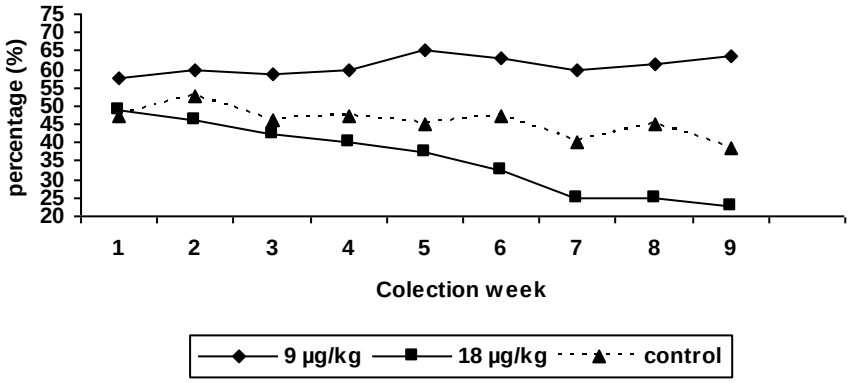

Figure (3): Changes in sperm progressive motility percentage (%) of rabbit bucks in different experimental groups throughout collection weeks.

Dead sperm percentage:

The effect of Cr on dead sperm percentage was significant (P<0.05), being lower by about 17% for the low level (9 µg/kg) and higher by about 4% for the high level as compared to the control group (18 µg/kg) of Cr (Table 2).

It is of interest to note that the effect of Cr treatment on increasing in score of mass motility and progressive motility percentage in low Cr group was associated with pronounced decrease in dead sperm percentage starting at the 5th collection week. However, dead sperm percentage in high Cr and control groups showed gradual increase at the same collection weeks, being almost higher in high Cr group than the control group (Fig. 4).

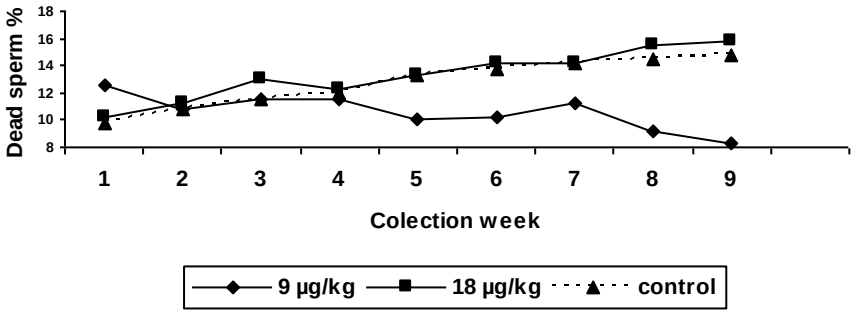

Figure (4): Changes in dead sperm percentage (%) of rabbit bucks in different experimental groups throughout collection weeks.

Sperm abnormality percentage:

Percentage of sperm abnormality was affected significantly (P<0.05) by the low Cr level, but was not affected by the high level of Cr. Sperm abnormality percentage significantly (P<0.05) reduced by about 48% as compared to the control group (Table 2).

It is worthy noting that the effect of Cr treatment on decreasing sperm abnormality percentage in low Cr occurred at the 1st collection week. However, abnormal sperm percentage in high Cr and control groups showed gradual increase throughout collection weeks, being nearly similar in high Cr and the control groups (Fig. 5).

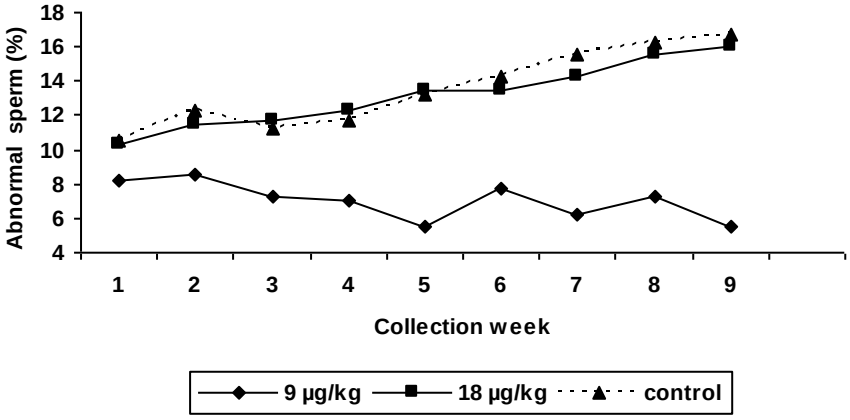

Figure (4): Changes in abnormal sperm percentage (%) of rabbit bucks in different experimental groups throughout collection weeks.

Sperm output:

Sperm cell concentration ($x10^6$/ml) significantly ($P<0.05$) increased by about 37% in low Cr group, but was not affected by the high level of Cr. However, total sperm output ($x10^6$/ejaculate) significantly ($P<0.05$) increased by about 16.5% in low Cr group and reduced by about 10% in high Cr group as compared to the control group (Table 2).

DISCUSSION

The present values of ejaculate volume are within the normal range reported on NZW rabbit bucks (Daader *et al*., 1997 and Abdel-Khalek et al., 2006). Accessory sex glands (seminal vesicles, prostate and Cowper's glands) are responsible for seminal plasma production, which represented the majority of semen volume (Abdel-Khalek *et al*., 1999). Hence, the lower ejaculate volume for both Cr groups was mainly attributed to marked influence of Cr treatment on decreasing activity of these glands. On contrast to the present trend of differences in ejaculate volume, the results available in the literature indicated no significant alterations were reported in semen volume of workers (Kumar et al., 2005) and (Li et al., 2001) exposed to Cr. The difference obtained and reported results may be related to differences in species and/or level of Cr treatment.

Interestingly to observe an opposite trend on sperm viability when Cr level doubled from 9 µg/kg (positive effect) to 18 µg/kg (negative effect). Most results in the literature indicated the results obtained on the high Cr level on sperm viability. In this respect, Danadevi et al. (2003) found rapid linear

reduction in sperm motility percentage in exposed workers compared to controls. Also, Li et al. (2001) found that Sperm motility decreased from 81.92±0.41% for the control group to 69.71±0.93% for the exposed workers. Recently, Subramanian et al. (2006) reported that Cr treatment decreased sperm forward motility of monkeys Increasing the influx of Ca^{++} from blood into the sperm cells leads to the increase of the metabolic activation of the sperm and consequently, increases in sperm motility. The Ca^{++} has been involved in the activation of many enzymes necessary for maturation, metabolism, sperm motility and membrane properties of spermatozoa (Morton *et al*., 1974) and calcium connection with other divalent metal ions occurring in semen have a significant regulation of sperm adenylate cyclaes activity which influences sperm metabolism and motility (Farag *et al*., 1983). Interactions between Cr, Ca and Mg have been reported by Moonsie-Shageer and Mowat (1993). Furthermore, reducing the specific activities of antioxidant enzymes, super oxide dismutase and catalase, and the concentration of reduced glutathione in both seminal plasma and sperm in a dose- and duration-dependent manner was reported by Subramanian et al. (2006) on monkeys treated with Cr.

The present results regard to increasing dead sperm percentage as affected by high Cr level supported the hypothesis of Subramanian et al. (2006), who show that chronic chromium exposure induces a reversible oxidative stress in the seminal plasma and sperm by creating an imbalance between reactive oxygen species and antioxidant system, leading to sperm death.

The present results indicated no significant effects of high Cr level on sperm abnormality, which may suggest normal spermatogenesis in particular spermiogenesis. Onyenmechi and Afonne et al. (2002) showed that Chromium had no effect on testis weight. Contrarily, Kumar et al. (2005) noticed significant increase in numbers of morphologically abnormal sperms in the exposed group mice with respect to the control. Further analysis of the data indicated that about 53% of the exposed mice showed less than 30% normal forms as compared to 10% in control mice. Also, Li H et al. (2001) reported that exposure of rats to Cr significantly increased the sperm abnormality from 2.75±0.06% in the control group to 6.68±0.32% in the exposed group at a CrO3 dose of 10 mg/kg body weight and to 7.6±0.15% at a CrO3 dose of 20 mg/kg body weight.

In contrast to the insignificant effect of high Cr level on sperm cell concentration, Onyenmechi and Afonne et al. (2002) showed that Chromium significantly decreased epididymal sperm number. Danadevi et al. (2003) found that sperm concentration of exposed workers was 14.5 $x10^6$/ml as compared to 62.8 $x10^6$/ml for the controls. Sperm cell concentration showed a negative correlation with blood chromium content in workers. Also, Li et al. (2001) found that sperm count of exposed workers was higher (47.05 $x10^6$/ml) than those in the control group (88.96 $x10^6$/ml). Furthermore, Li et al. (2001) reported that feeding Chromium (CrO_3) to rats significantly reduced the epididymal sperm counts from 87.40 $x10^6$/g epididymis in control group to 21.40 $x10^6$/g epididymis at a level of 10 mg/kg body weight and to 17.48 $x10^6$/g epididymis at a level of 20 mg/kg body weight. However, in agreement

with the present results, Recently Subramanian et al. (2006) reported that Chromium treatment decreased sperm count.

Generally, the deterioration rate in physical semen characteristics could be attributed to toxic effect of high levels of Cr. On the other hand, the significant improvement in physical semen characteristics of low Cr level as compared to the control group may be associated with the general effects of Cr on reducing cholesterol level that increased blood flow into testicular artery, and in turn activates spermatogenesis. For example, rabbits fed on a Cr-deficient diet had increased total cholesterol and aortal lipid concentrations and showed increased plaque formation (Abraham et al., 1982a,b). Cr supplementation has decreased the total cholesterol in their blood and increased HDL-cholesterol (Riales and Albrink, 1981) and a decrease in total cholesterol, LDL-cholesterol and triacylglycerols (Lefavi et al., 1993) have been observed in humans after Cr supplementation.

As affected by collection week, different trends of change in semen characteristics of experimental groups (Fig. 1-5). It is worthy noting that the marked effect of Cr treatment appeared after the 5th week

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الخـواص الفيزيائيـة للسـائل المنـوي لـذكور أرانـب الكاليفورنيـا مـع المسـتويات المختلفة من الكروم.

تـــــرك محمـــــد ابـــــراهيم دره , أمينـــــة عبـــــد المطلـــــب الســـــروي , فوزي صديق عبد الفتاح اسماعيل, أحمد السيد ناصف

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اجريت هذه الدراسة بمزرعة الدواجن كلية الزراعة جامعة المنصورة في الفترة من فبراير الى أغسطس2006. تم استخدام 12 ذكر كاليفورنيا بالغ عمر من 6 الى 8 شهور و متوسط وزن 3-3.5 كجم . الذكور كانت بصحة جيدة خالية من الأمراض و الطفيليات الخارجية. قسمت الذكور الى ثلاث مجموعات بمعدل ربعة ذكور لكل مجموعة و عوملت ذكور المجموعة الأولى ككنترول و جرعت المجموعتان الثانية و الثالثة بيكلونات الكروم بمعدل 9, 18 ميكروجرام لكل كيلوا جرام وزن حى على الترتيب عن طريق الفم. تم تقدير الحركة الجماعية و الفردية و تم حساب عدد الحيوانات المنوية الميتة و الحية و الشواذ و تركيز الحيوانات المنوية لكل ملليلتر و كذلك قدر عدد الحيوانات المنوية لكل قذفة. النتائج أوضحت ان حجم القذفة انخفض بدرجة معنوية ($p<0.05$) في كل من المعاملتان الثانية و الثالثة مقارنة بالكنترول و هذا الإنخفاض كان أكبر في المعاملة ذات الجرعة الأعلى عنها في المعاملة ذات الجرعة المنخفضة و كان تأثير الكروم بشكل كلى ايجابي عند الجرعة المنخفضة من الكروم و سلبيا عند الجرعة الأعلى من الكروم لكل من الحركة الكلية و الحركة الفردية و تأثرت نسبة الحيوانات المنوية الشاذة بالمعاملة بالكروم في الجرعة المنخفضة بينما لم يكن للجرعة الأعلى تأثير عليها حيث انخفضت نسبة الشواذ بمعدل 48% عن الكنترول في الجرعة المنخفضة من الكروم. و زاد تركيز الحيوانات المنوية في القذفة بحوالي 37% عند المعاملة بـ 9 ميكروجرام بيكلونات الكروم لكل كيلوا جرام وزن حى الا انها لم تتأثر عند المعاملة 18 ميكروجرام بيكلونات الكروم لكل كيلوا جرام وزن حى. وازداد العدد الكلي للحيوانات المنوية بالقذفة زيادة معنوية ($p<0.05$) بمعدل 16.5% في المعاملة بـ 9 ميكروجرام بيكلونات الكروم لكل كيلوا جرام وزن حى و انخفض بحوالى 10% للجرعة العالية من الكروم بالمقارنة بالكنترول