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CHANGES IN TESTOSTERONE, CORTISOL LEVEL AND SEMINAL CHARACTERISTICS IN RELATION TO COLLECTION REGIMEN IN RABBITS

(With 1 Table & 2 Figs.)

By

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علاقة تغيرات مستوى هرمون التستوستيرون والكورتيزول وخصائص السائل المنوي بأنظمة جمع المني في الأرانب

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أجري هذا البحث لدراسة تغيرات مستوى الهرمون الخصوي (التستوستيرون) وهرمون الكورتيزول وبعض الصفات الطبيعيه والكيميائيه للسائل المنوي الناتجه عن استخدام أنظمة مختلفه في جمع السائل المنوي . استخدم في هذه الدراسه ستة ذكور بالغه من أرانب البوسكات وقد تم جمع السائل المنوي منها على مدى ثلاثة أسابيع. في الأسبوع الأول تم أخذ قذفه واحده من كل ذكر (نظام A) ، وفي الأسبوع الثاني تم أخذ قذفتين متتاليتين (نظام B) ، وأما في الأسبوع الثالث فقد تم أخذ عدة قذفات متتاليه من كل ذكر وذلك حتى مرحلة الانهاك (نظام C) . ومن جهة أخرى فقد تم أخذ عينات من الدم قبل وبعد عملية القذف وذلك لقياس تغيرات مستوى الهرمونات باستخدام النظائر المشعه . وأظهرت الدراسه زيادة معنويه في مستوى الهرمون الخصوي في نظام B بعد القذف بالمقارنه مع مستواه قبل القذف (٠ر٣٨ - ١ر١٨ ng/ml) وزيادة غير معنويه لنفس الهرمون في كل من نظام A (٠ر٣٨ - ٠ر٧٠ ng/ml) ونظام (C) (٠ر٧٥ - ٠ر٦٧ ng/ml) بعد القذف بالمقارنه مع تركيزهما قبل القذف وكذلك زيادة غير معنويه في مستوى هرمون الكورتيزول بعد القذف (٢٧ر٨ - ٥١ر٦ - ٥٢ر١ nmol/L) بالمقارنه بمستواه قبل القذف (٣٥ر٣ - ٤٠ر١ - ٤٨ر٦ nmol/L) . كذلك انخفاض حجم القذفه وتركيز الحيوانات المنويه السليمه وتركيز الفركتوز في بلازما السائل المنوي من جراء تكرار القذف.

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SUMMARY

This research was designed to study the changes in testosterone, cortisol levels and some physical and chemical characteristics in rabbit's semen following different regimens of semen collection. Six adult male Bouscat rabbits were used in this experiment. The animals were ejaculated for three succesive weeks at frequencies of: Once a week (regime A), twice a week (two succesive ejaculates, regime B) and finally the males were ejaculated until exhausion (regime C). Blood was collected before and after ejaculation for hormone assays using RIA. The results showed the following). Significant increase in testosterone levels in regime B (0.38 and 1.18 ng/ml) and non-significant increase in both regimes A (0.48 and 0.70 ng/ml) and C (0.57 and 0.67 ng/ml) before and after ejaculation respectively. A non-significant increase in cortisol levels after ejaculation (37.8, 51.6 and 52.1 nmol/L) compared to levels before ejaculation (35.3, 40.1 and 48.6 nmol/L) for regimens A, B and C respectively. Decrease in semen volume, sperm concentration, normal sperm and fructose levels as a result of repetitive ejaculation.

INTRODUCTION

Now, it is well documented that testicular androgens play an important role for the stimulation and maintenance of spermatogenesis, accessory glands and sexual behavior in males. Sexual stimulation that occurs with mating is associated with a series of physiological changes in the animal's hormonal system. It has been found that copulation increased plasma testosterone in male rabbits (HALTMEYER and EIK-NES, et al. 1969) and LH, prolactin and testosterone in rats (KAMEL, et al. 1977).

However, KAMEL and FRANKEL (1978) reported that FSH levels did not change during mating. After 3 consecutive semen collections taken at one hour intervals, WEATHERSBEE, et al. and LODGE (1976) indicated that serum testosterone and estrogen concentrations in bull were highly variable.

Recent studies showed the involvement of glucocorticoids in male's sexual behavior. Cortisol levels increased directly in stallions after mating (TAMANINI, et al. 1983) and after sexual stimulation, with or without

ejaculation, (RABB, et al. 1989). Cortisol is known to be secreted under stressful conditions and induce suppression in testicular function which in turn affect testosterone level, (SAPOLSKY, 1985). Therefore, it seems of interest to study such hormonal changes in mammals. Rabbits were chosen in this experiment because males used to copulate very rapidly and repeatdly when introduced to a receptive female.

The purpose of the present study was to determine the changes in testosterone and cortisol levels and semen characteristics in relation to different regimens of ejaculation.

MATERIAL and METHODS

This experiment was carried out at the Department of Theriogenology and the Poultry Research Farm, Department of Animal Production, Assiut University, in order to study the changes in testosterone, cortisol and semen charateristics in relation to different regimens of semen collection in rabbits.

Experimental Animals and Sampling:

Six adult male Bouscat rabbits (One year old) were used in this study. The Males were housed in standard wood cages and feed and water were available ad libitum throughout the experimental period.

Semen collection: Bucks used in this experiment were trained to serve on artificial vagina (With suitable temperature of about 45 C, pressure and lubricant). Semen was collected by allowing the buck to mount a receptive female, meanwhile, the prepared artificial vagina was held in a suitable position for the male to ejaculate into it. One ejaculate was collected and not included in the study one week before putting the animals in the planned regime. The rabbits were then ejaculated every Tuesday for three week periods at frequencies of: Once a week (regime A), twice (regime B) and until exhaustion (regime C) for the first, the second and the third week respectively. During the third week, each male was given 5 minutes after the last ejaculation (The number of moutings accompanied by thrust vaired from animal to the other) to assure that the male became sexually unable to copulate.

Semen volume without gel mass was determined directly from the graduated collection tube and concentration was determined haemocytrometrically. Other physical characteristics, like, live and dead sperm, normal and abnormal (head, neck and tail) sperm were evaluated in smears

stained with alkaline methyle violet, and nigrosin-eosin stains. Finally, fructose concentration was determined in most of the collected samples according to MANN, (1946). In some samples and especially after the third thrust, the amount of semen was not enough for performing all the physical and chemical evaluations.

Blood collection: blood samples from the bucks were collected weekly from the ear vein before and after ejaculation according to the previous mentioned regimes. Blood was allowed to clot then centrifugated and the serum was aspirated and kept frozen in glass vials at -20 until the time of hormone assays. All samples were collected at a fixed predetermined time of day to avoid any circadian changes in the level of hormones.

Hormone Analyses; Hormones were measured by using radio-immuno-assay kits (Diagnostic Products Corporation, Los Angeles, CA). Testosterone was measured by a single antibody assay, which was highly specific for testosterone. The assay was accurate, precise (inter and intra assay C.V = 10.8 and 7.5 respectively) and parallel's. Sensitivity was 0.04 ng/ml. The cortisol assay was accurate, precise (C.V. 5%) and parallel with a sensitivity of 5.5 nmol/L.

Statistical Analysis: Data were analyzed using the General Linear Model procedure (GLM).

RESULTS

The results of this experiment are given in Table (1) and Fig. (1 & 2). A one way analysis of variance showed no significant differences in the levels of testosterone hormones before and after semen collection for both regime A and C. Regime B showed significant difference ($P<0.02$) where the level of hormone was higher (1.18 $\pm$ 0.14 ng/ml) after ejaculation, compared to level before ejaculation (0.38 $\pm$ 0.26). The average levels for regime A was (0.48 $\pm$ 0.12 and 0.70 $\pm$ 0.26 ng/ml) and for regime C (0.57 $\pm$ 0.26 and 0.67 $\pm$ 0.33 ng/ml) before and after ejaculation, respectively. It is worth to mention that the time lapse between the sampling of blood was about 5 minutes for regimen A, 10 minutes for regimen B and 16.33 $\pm$ 4.0 minutes for regimen C.

Although the cortisol level was higher after semen ejaculation than before the collection, yet there were no significant differences in the levels of cortisol in all the different regimes. The averages of hormone levels before

and after ejaculation were (35.3 ± 4.3, 37.8 ± 6.3; 40.1 ± 4.5, 51.6 ± 6.1 and 48.6 ± 6.5, 52.1 ± 3.2 mmol/L) for regimes A, B and C, respectively.

The results of semen characteristics are presented in Table (1). The volume of the ejaculate was not sufficient to carry all the tests specially after the third ejaculate. Also some of the thrusts (specially after the fourth mount) was not accompanied by ejaculation. The average number of thrusts is 5.66 ± 1.2 (range 4–7) while the average number of ejaculations is 3.33 ± 0.82 (range 2–4). Some of these characteristics showed significant differences. In regime B and C, fructose concentrations were higher ($P<0.02$) in the first than the second ejaculate. In regime C, highly significant differences $P<0.005$ among semen volumes and significant difference $P<0.03$ in the sperm concentration / ejaculated were observed.

DISCUSSION

Despite the insignificant differences in the concentration of testosterone and cortisol in this experiment, it has been observed that the patterns of response in both hormones were relatively uniform regardless of the regime used for ejaculation. Levels of both hormones were higher after ejaculation in all the different regimens (Fig. 1 and 2).

Our results showed an increase in plasma testosterone after semen collection. These results are similar to those obtained by HALTMEYER and EIK-NES (1968) who found a marked increase in plasma testosterone in rabbits following copulation with receptive female. The insignificant increase reported in this study may be due to the time difference in blood collection. In HALTMEYEER and EIK-ENS experiment, blood was collected 45 min. after copulation, while in our experiment, blood has been collected in a few minutes directly after the first ejaculate (Regrime A) which probably was completed quickly before the rise in serum testosterone is established. Therefore, the time course with regard to this increase must be considered. The importance of bleeding time was more pronounced in regime B, in which males were ejaculatied two times, consequently longer time taken until bleeding and more elevation of testosterone was observed and significnat difference was found (Fig. 1). In rats KAMEL and FRANKEL (1978) reported that testosterone rose and peaked at 30–60 minutes after mating. In regime C, although the time required for the male to be exhausted from ejaculation was much longer than regime A and B, the level of testosterone after the last

ejaculation was less than the two previous regimens. This decrease in testosterone level could be due to the stress as a result of repeated ejaculations. Testosterone secretion has been shown to decrease under stress in several species (in rabbits, MOOR et al., 1977; in rats, POLLARD, et al. 1980 and in stallions, RABB et al, 1989).

The results of the present study showed no significant difference between cortisol levels before and after ejaculation in regime A, B and C, respectively. Many investigators, reported that exposure to stressful conditions induce release of cortisol in different species. (In stallion, TAMANINI et al., 1983 and RABB et al., 1989; in baboon, SAPOLSKY 1985 and in pigs. NYBERG et al., 1988). The insignificant differences in cortisol levels reported herein may be due to the handling procedure; (Transefer the animals from the cages to the injection box, insertion of the needle ... etc) which may cause the elevation of hormone especially before ejaculation, (for further detail about handling procedure, see KAMEL and FRANKEL, 1978). WEATHERSBEE, et al. (1976) mentioned that, each bull in their study was exposed on a separate day to the handling and bleeding technique in addition to manipulating the caunnula before starting the experiment. In summary, both the time course for sampling and handling procedure must be taken into consideration in such these experiments.

The results of semen characteristics (Table 1) showed that semen volume was large in the first ejaculates and decreased in each successive ejaculate (in Regime B and C). Some semen characteristics also decreased with volume i.e. Conc./ejaculate, normal sperm, live sperm and fructose, the others did not show any trend. It has been observed that the sperm concentration/ml was higher in the second ejaculate than the first one. These results are comparable with other studies by DESJARDINS, et al. (1968). These observations suggested that the first ejaculation provided stimulation for added sperm in the subsequent ejaculates. Also, fructose concentration showed decline in each successive ejaculate which may indicate that seminal vesicles require much time to replenish its secretion (PURVIS, et al. 1986).

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Table 1: Changes in semen characteristics in rabbits after three different regimens of ejaculation.

Regimen	Ejacul.	Volume	Concentration X 10^6 Ejaculate	Concentration X 10^6 /ml	Live sperm	Dead sperm	Abnormal sperm: Head	Abnormal sperm: Neck	Abnormal sperm: Tail	Normal sperm %	Fructose mg%
A	1	0.67±0.07	184.0± 42.9	268 ± 59.8	91.6 ± 4.1	8.3 ± 4.1	5.7 ±0.8	4.9 ±1.9	7.8 ± 0.9	81.7 ±2.8	418 ± 85.8
B	1	0.63±0.13	273.0± 90.1	361 ± 80.7	86.0± 5.5	14.0 ± 5.5	12.5 ±1.8	1.3 ±0.4	7.0 ± 1.3	79.2 ±1.8	316 ± 59.6 *
	2	0.47±0.06	149.3±308	455 ± 83.7	71.0 ±10.1	29.0 ±10.1	16.2 ±2.3	2.5 ±0.5	6.0 ± 0.7	75.3 ±2.9	76 ± 15.1 *
C	1	0.73±0.11 **	172.5± 42.2 *	273 ± 73.9	76.6 ± 6.5	23.3 ± 6.5	7.7 ±1.5	2.0 ±0.3	5.7 ± 1.9	88.2 ±2.2	409 ± 55.1 *
	2	0.52±0.09 * *	176.8± 61.3 *	383 ± 98.6	81.7 ± 6.9	20.7 ± 6.1	9.0 ±1.4	2.7 ±0.7	3.8 ± 0.8	84.5 ±2.9	107 ± 5.3 *
	3	0.20±0.05 * *	25.0± 10.3 *	162 ± 58.2	67.5 ±22.5	32.5 ±22.5	8.5 ±3.5	1.5 ±3.5	18.0 ± 0.0	49.5 ±6.3	n.m.
	4	0.10±0.0 **	4.0± 1.00 *	40 ± 1.00	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.

** Significant at < 0.005 level
* Significant at < 0.03 level
n.m. The amount of semen was not enough to measure these traits.

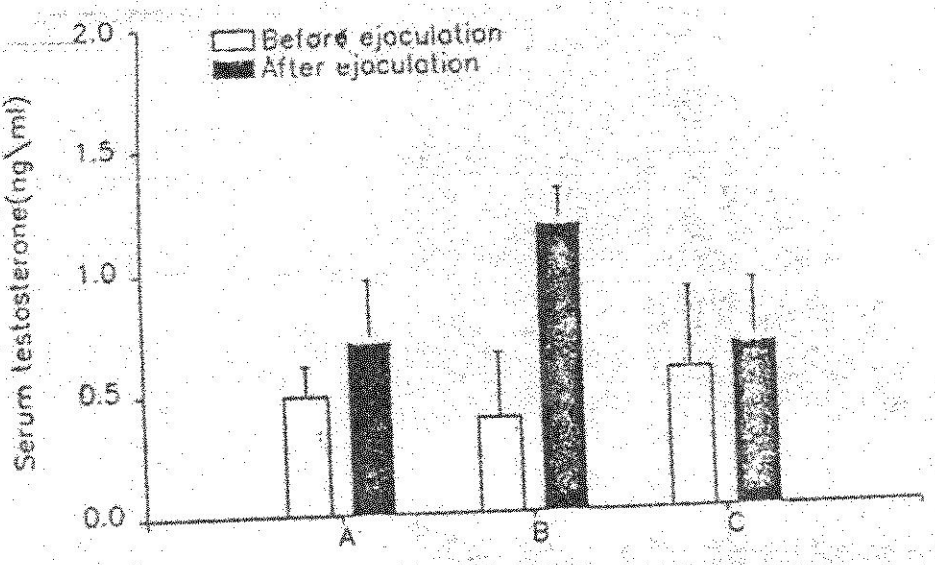

Fig.1 Changes in serum testosterone concentrations in rabbits before and after three different regimens of ejaculation

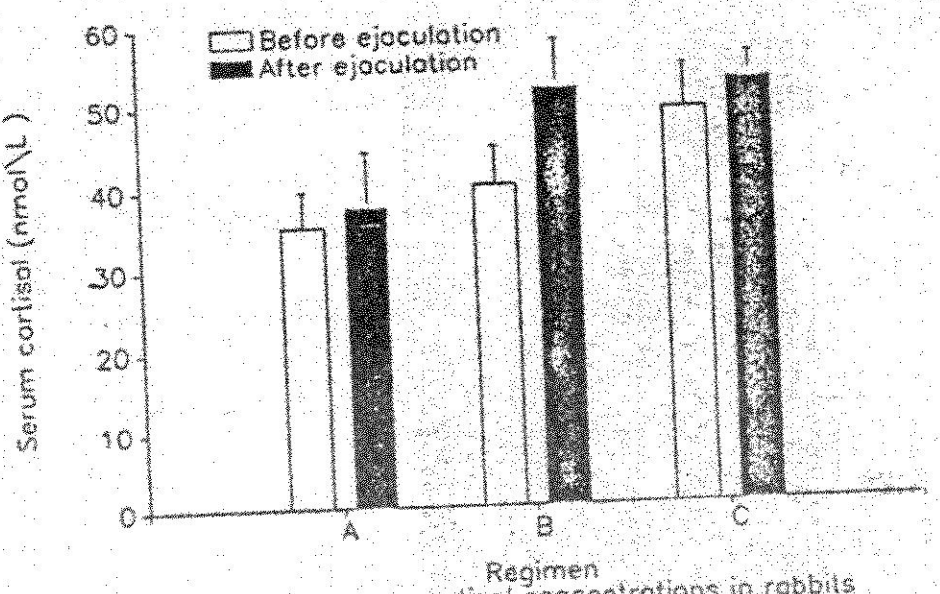

Fig. 2 Changes in serum cortisol concentrations in rabbits before and after three different regimens of ejaculation