

Dept. of Animal Production,
Fac. of Agriculture, Assiut University,
Head of Dept. Prof. Dr. F. El-Hommosi.

**THE EFFECT OF INDUCED CRYPTORCHIDISM ON
SEMEN PICTURE, SERUM TESTOSTERONE,
VIT. C., VIT. A. AND
B-CAROTENES IN BUFFALO BULLS**
(With 3 Tables and One Figure)

By

F.M. ALLAM and S.H. SHEHATA *

*: Dept. of Theriogenology, Fac. Vet. Med., Assiut Univ.
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تأثير الخصيه المعلقه على صورة السائل المنوي وتركيز التستوسترون
وفيتامين C وفيتامين A وبيتاكاروتين في طلائق الجاموس

فاروق علام، شحاته حسن

أجري هذا البحث على ٨ طلائق جاموس عمر سنه ، ٤ منهم خضعوا لجراحة تعليق الخصيه ، والأربعة الآخرين إعتبروا كونترول. تم الحصول على ١٠٤ عينه دم خلال مرحلتي البحث ٦ شهور (الثلاثة شهور الأولى عند عمر ١٢-١٥ شهر طلائق ناميه ثم عند عمر ٢٠-٢٣ شهر طلائق بالغه). تم قياس تركيز التستوسترون وفيتامين C وفيتامين A وبيتاكاروتين . تم مراقبة السلوك الجنسي بداية من عمر ١٨-٢٣ شهر. كذلك تم متابعة الزيادة اليوميه في نمو الطلائق خلال فترتي البحث. وباستخدام المهبل الصناعي تم الحصول على عينات من السائل المنوي خلال متابعة السلوك الجنسي. وجد تركيز التستوستيرون كان عالياً (معنويه أكثر من ١%) خلال مرحلة البلوغ عن المرحله الناميه ولم تسجل تركيزات الدم فروقاً معنويه. نشطت الطلائق ذات الخصيه المعلقه والطلائق الكونترول جنسياً بداية من الشهر ١٨ من العمر مع تقييم عالي لصفات العينات المنويه بالطلائق الكونترول عند مقارنتها بطلائق ذات الخصيه المعلقه.

SUMMARY

Eight buffalo bulls of one year old were used in the study. Four of them were subjected to experimental cryptorchidism by surgical operation. The other four bulls were kept as control (intact). A total number of 104 blood samples were taken as long as six months of the experiment (growing 12-15 and adult 20-23 month old). Blood serum were subjected for testosterone, Vit.C., Vit. A and B-carotenes determination. Sexual behaviour up to eighteen months old till twenty three months old were evaluated for all bulls. Daily body gain was recorded for all animals as long as the experiment. Semen samples were collected during sexual behaviour evaluation from all bulls. Our results recorded a significant increase ($P > 0.01$) in serum testosterone during the adult period rather than the growing period. However, non significant differences were recorded for serum Vit. C, Vit A and B-carotenes between cryptorchid and intact bulls. Animals started to show sexual interest starting from 18 months old for both cryptorchid and intact bulls. Semen evaluation was high in intact bulls than in cryptorchidism ones.

Keywords: Cryptorchidism-semen-serum Parameters-buffalo-bulls

INTRODUCTION

During the last decades in beef production, there is an interest in keeping cryptorchid bulls because of their higher rate in body gain than normal bulls. LAUCH (1979) found that bulls with shortened scrotum were easier in care, therefor, they can grow faster than the females due to their sexual calmness. Serum testosterone levels remain normal or slightly below in bilateral cryptorchid bulls (BASS *et al.*, 1976 and SCHAMBACHER 1978). Moreover, KARG *et al.*, (1976) and BAMBERG *et al.*, (1976) observed that the period and the time of the year had increased the testosterone level and also influence uncertained blood values. KOLB *et al.*, (1991) reported that there was no influence of experimental cryptorchidism or castration on the concentration of B-carotene or ascorbic acid but only seasonal effect on the mentioned values. The objective of the present work was to study the effect of artificial cryptorchidism of one year old bulls on the concentrations of serum testosterone, Vit C, Vit A and B-carotenes. Moreover, to study the animal growth rate and sexual behavior.

MATERIAL and METHODS

Four buffalo bulls approximately of one year old were made artificially unilateral cryptorchid by the surgical procedure described by *SCHANBACHER (1978)*. The bulls were premedicated by RamBun (Bayer and procaine hydrochloride (3%) as a local anesathia was injected at the site of operation. The right hind leg was raised to allow for surgical approach. A total incision was made high on the scrotal skin under aseptic conditions. The right testicle was separated from the scrotum and pushed through inguinal canal and prevented later from descent of the abdominal test. Topical application of antibiotic powder was sprayed within the scrotum and about the incision side to reduce the chance of the secondary infection. The wound was closed by using number 2 chromic cat gut. The reproductive function of 4 cryptorchid bulls and additional 4 intact bulls of the same age were assessed as a control. Blood samples were collected biweekly from all bulls as long as three months (age 12-15 mo.). Blood samples were continued another three months (20-23 mo.) for operation period of maturity. Blood samples were collected by jugular vein puncture using sterile, dry and wide gauge needle and allow to flow freely and gently on the wall of sterile clean test tube. blood was allowed to clot at room temperature, then centrifuged at 3000 rpm for 20 min. Clean non hemolyzed serum transferred into sterile vial and stored at -20 degree C until analysis. Serum testosterone was determined by RIA technique using test kits supplied by Orio Diagnostic (Finland). B-carotene and Vit A were determined as described by *BRADLEY and HORNBECK (1973)*, while Vit C was determined according to *GIBSON et al (1966)*. At the age of 18 months old, sexual behavior for all animals were evaluated. Moreover, another blood and semen samples were collected artificial vagina and evaluated for mass activity, individual motility, viability and sperm count using the Haemocytometer. According to *MILLOVANOV (1962)*, *LAING (1979)* and *BEARDEN and PUQUAY (1980)*. During the experiment all bulls were kept in the same management and nutritional conditions. in each period animals were individually fed roughage and concentrate diets and daily requirements were calculated according to *GHONEIM (1967)*. The data obtained were statistically analyzed by one way analysis of variance using general model (GLM) procedures of SAS (1987) for personal computer.

RESULTS

The serum testosterone, Vit C, Vit A and B carotene levels at the age of 12-15 mo. and 20-23 mo. are present in table 1. From the table it is clear that serum testosterone and Vit A were similar in both intact and cryptorchid bulls. However, Vit C and B Carotene were significantly higher than in cryptorchid bulls at the age of 12-15 mo. Moreover, Serum testosterone and Vit A recorded a significant increase ($P < 0.01$) at the age of 20-23 mo. when compared with the age of 12-15 mo. concerning the daily body weight gain, our results recorded 970 ± 0.09 gram in cryptorchid bull and 960 ± 0.08 gram in intact bull as a daily body weight during the age of 12-15 mo. Moreover, at the second age, the daily body weight gain recorded 360 ± 0.06 and 363 ± 0.10 in cryptorchid and intact bulls, respectively.

The sexual behaviour in relation to age and testosterone concentration individually for both intact and cryptorchid are present in table 2. From the table, sexual behaviour in bulls appears at the age of 20 mo. when serum testosterone reach 3 ng/ml. This sexual characterized by nasogenital act and some trials to mount the female. At the age of 21 mo., all bulls exhibit some interest to false mounting with licking the vulva lips. This false mounting represented by incomplete mounting of the female. At the age of 21.5 mo., some bulls mate successful mounting with intromission, while 3 bulls did it without intromission at the age 22 mo. all bulls mate complete mounting, with complete intromission with reaction time less than 10 min. except 4 bulls did it with reaction time between 10-20 min. When the animals reached 22.5 mo. all bulls exhibited good interest to mount with reaction time less than 10 min.

Concerning semen collection we tried to collect from bulls of 18 mo. old but this trials failed up to 22 mo. old. While the two cryptorchid and the two intact bulls (22.5 mo.) have succeeded in the treatment with an artificial vagina. The physical and microscopically evaluation of semen samples are presented in table 3. From the table, it is clear that mass activity, individual motility, alive spermatozoa and total sperm cell count were higher in intact bulls than in cryptorchid bulls. However, no differences were recorded in physical characteristics between intact and cryptorchid bulls semen (Volume, colour and pH).

DISCUSSION

In this study serum testosterone (T), Vit.C, Vit. A and B carotene were assayed in 8 buffalo bulls as long as growing period (12-15 mo. old) and adult period (20-23 mo. old). Four of those buffalo bulls were experimentally cryptorchid (CRYPT) induced at the age of 12 mo. old. serum testosterone and Vit.A recorded similar values at the growing period for both CRYPT and intact bulls. Moreover, Vit. C and B-carotene recorded a significant increase in intact bull than in CRYPT in this period of age (table 1). Serum testosterone levels recorded a significant increase in adult period with similar in both CRYPT and intact bulls when compared with T values at the growing period. These results coincide with that recorded by *BAMBERG et al* (1976), *SHARMA et al* (1988) and *LUNSTRA* and *SCHANBACHER* (1978). They reported that there is no significant differences in serum T values between CRYPT and intact bulls and in rams in long term cryptorchidism. As known that Leydig cells are the principle androgen producing cells in males (*ZIRKIN et al*.,1980), it was found that elevated temperature of the testes maintains serum T near normals as result of increased T synthesis by leydig cells (*GOSBODERWICZ and LE-DEMARE, 1962 and HOCHEREAU-DE RIVERS et al.*, 1979). Moreover, *LUNSTRA and SCHANBACHER* (1988) found an increased rate of T synthesis per Leydig cells in CRYPT rams, but it is unknown if this achieved through increased temperature, increased LH or some other mechanisms. Some reports indicated that testicular interstitial fluids contains factor that stimulate T production by isolated Leydig cells (*SHARP and COOPER 1984 and RISBRIDGER et al.*, 1986). So, the concentration of interstitial fluid proteins increase with testicular damage such as that occurring in cryptorchidism, these proteinoccus factors, rather than LH, may be stimulating CRYPT Leydig cells enlargements and increased T secretion (*LUNSTRA and SCHANBACHER 1978*).

Concerning Vit C and B-carotene, the obtained results did not record significant differences between intact and CRYPT bulls, but B carotene recorded a higher values in the growing period of bulls and Vit C was slightly higher in intact than in CRYPT bulls. *KOLB et al.* (1991) reported that there was no influence of experimental cryptorchidism or castration on the values of Vit C and B-carotene. The lower values of B-carotene obtained in this study may be due to its importance to maintain semen quality and quantity (*KOLB, 1991*). The body weight, gains recorded 970+0.09 gram and 363 + 0.1 gram for CRYPT and intact during the growing period. However, it was

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regulatory function in cryptorchid bull. The value of alive sperms recorded for cryptorchid bull in our study are within the mentioned by *GODFREY et al. (1990)*.

Our conclusion is that CRYPT animals were quite in care with some increase in (non significant) in body gain during the growing period. in addition, there was no influence of experimental of cryptorchidism on the blood serum values. So, Cryptorchid animals could make a model for studying the regulation of gonadotrophin secretion (*SCHANBACHER, 1978*).

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360±0.06 gram and 363±0.1 gram for CRYPT and intact bulls, respectively at the adult period. Non significant difference could be observed between the two groups throughout the period of study. Only one CRYPT bull recorded average daily gain (final body weight 480 Kg) and one intact bull gives 603 gram daily gain (final body weight 442 kg). final body weight of one year old CRYPT bulls found by *KOLB (1991)* was 417 kg and intact bulls was 410 kg when compared with our results (335 and 305 kg for CRYPT and intact bulls) at the same period. Moreover, the obtained results in their respect are convenient for those of *SHARMA et al., (1988)*.

At the age of eighteen months old we started to evaluate sexual behaviour of bulls but these observations were recorded at 20 mo. old (table 2). Different sexual behaviour with varying libido were observed for both groups. The cryptorchid bull exhibited sexual behavioral characteristics by nasogenital investigation at 20 mo. old, however, one intact bull showed the same trend. This may be due to their higher level of T. As long as one month after 20.5-21.5 mo. old there is no differences in the sexual but reaction time increased (from 10-20 min). Moreover, the CRYPT group was calm and docile in its sexual behaviour than the intact group. There are few available literature that explain the interaction between sexual behaviour and T concentration. several authors reported an increase in the level of T. Following sexual stimulation (*SMITH et al., 1973; WEATHERS BEE and LODGE 1976 and ILLIUS et al., 1976*). However, *OSMAN et al., (1990)* said that bulls of different breeds behave in similar way with regard to serum testosterone level after mounting of sexual exhaustion. *BORG et al., (1991)* mentioned that reproductive activity had no effect and established association between average T during exposure to female and mounting.

Concerning semen collection and semen quality, the obtained results are present in table 3. The physical character of the obtained semen (colour and pH) did not varied between intact and CRYPT bulls. However, the volume of the ejaculate, mass activity, individual motility, percentage of alive spermatozoa and total sperm cell count showed a marked decrease in their values in cryptorchid bulls. so, decreased semen quality indicate the close relationship between the endocrine and the exocrine function of the bovine testes. in CRYPT animal the extra tubular tissue of the testes occupied approximately two thirds of the testes and exhibit an increase in the amount of connective tissue matrix and collagen (*LUNSTRA and SCHANBACHER, 1988*). So, the lower sperm cell count and viability of semen may be due to some degenerative changes that happen in the testis due to loss of its thermo-

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Table (1): Serum levels of testosterone, Vit.C, Vit. A and β -carotene.

Age	Animal	N	Testosterone mg/ml	Vit.C mg/L	Vit.A mg/L	β -carotene mg/L
12-15 months	Intact	24	1.092 \pm 0.12	7.30 \pm 0.73	2.77 \pm 0.20	6.08 \pm 1.75*
	Crypt	24	0.857 \pm 0.24	6.43 \pm 0.63	2.62 \pm 0.16	5.43 \pm 2.71
20-23 months	Intact	28	10.98 \pm 0.75*	6.96 \pm 0.38	3.846 \pm 0.21*	4.015 \pm 0.16
	Crypt	28	9.15 \pm 0.96*	6.35 \pm 0.60	3.832 \pm 0.16	4.018 \pm 0.16

* Significant at (P< 0.01)

Table (2): Individual serum T. at different ages in relation to sexual behavior in buffal bulls.

Bull	Bull No.	Age in Months													
		20		20.5		21		21.5		22		22.5		23	
Type	No.	T*	Sex*	T	Sex	T	Sex	T	Sex	T	Sex	T	Sex	T	Sex
Intact	1	3	A	4	B	4.2	b	14	C			13	D ₁	E	-
	2	2.5	-	12	B	14	B	5	C	5	D ₂	15	ExO	13	ExO
	3	2	-	3.5	B	3.1	B	5	C ₁	13	D	16	ExO	14	ExO
	4	2	-	7	B	11	B	15	C ₁	13	D ₁	10	E	10	-
Crypt	1	4	A	4	B	14	B	5	C	15	D	15	ExO	16	ExO
	2	3	A	14	B	2.5	B	3.2	C	2	D ₂	5	E	2.5	-
	3	5	A	3.5	B	4.2	B	15	C ₁	15	D ₂	5	E	8	-
	4	5	A	15	B	11.2	B	4	C	14	D	13	ExO	15	ExO

A: Exhibit sexual behavior (Trait to mount with nasogenital act.)

B: Exhibit some interest to mount heifers with licking the vulva.

C: Successful mounting

C₁: Without intromission.

D: Complete mounting with complete intromission.

D₁: Reaction time 10 min.

D₂: Reaction time 20 min.

E: Good interest with reaction time less than 10 min.

O: Semen collection

T*: Concentration in ng/ml

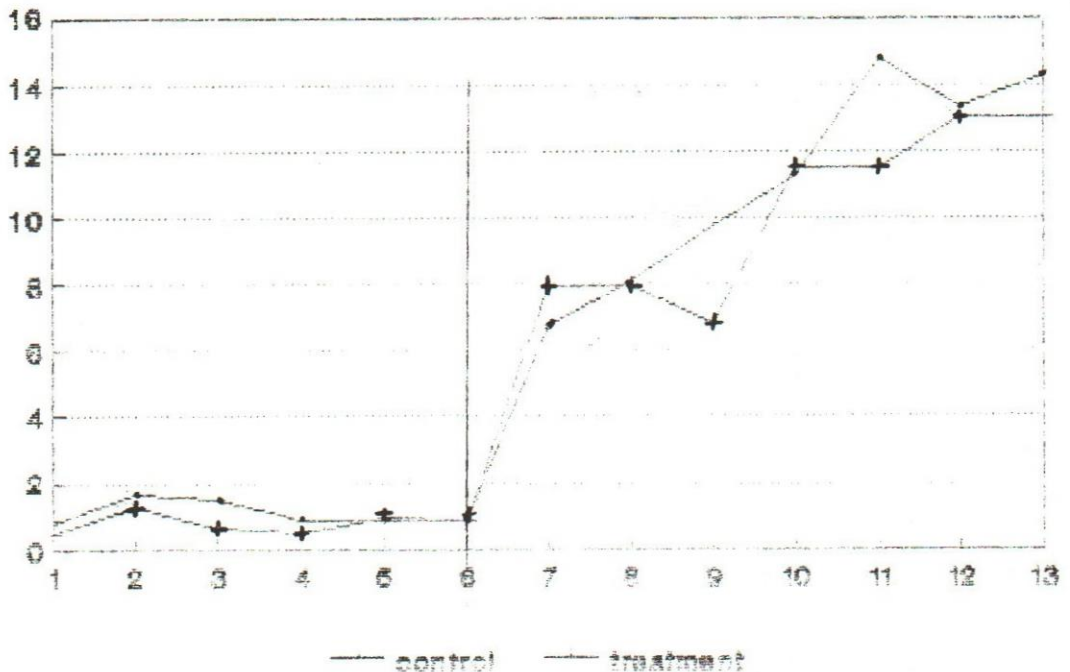
Sex*: Sexual behavior.

Table (3): Semen evaluation for intact and cryptorchid buffalo bulls.

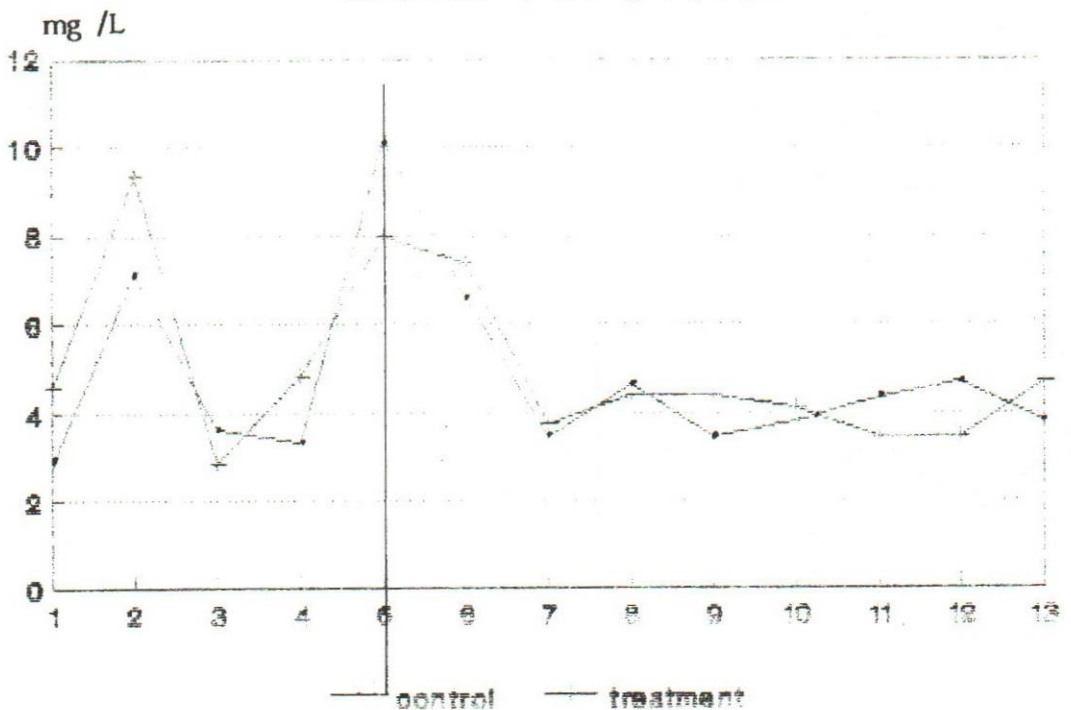
Bulls	n	Volume	Color	pH	Mass Activity	Individual motility	Viability	Sperm Cells
Intact	2	3-5	Whit Creamy	6.6	+++	60%	42.7	730000
Crypt	2	2.5-4	Gryish	6.5	+	40%	72.2	490000

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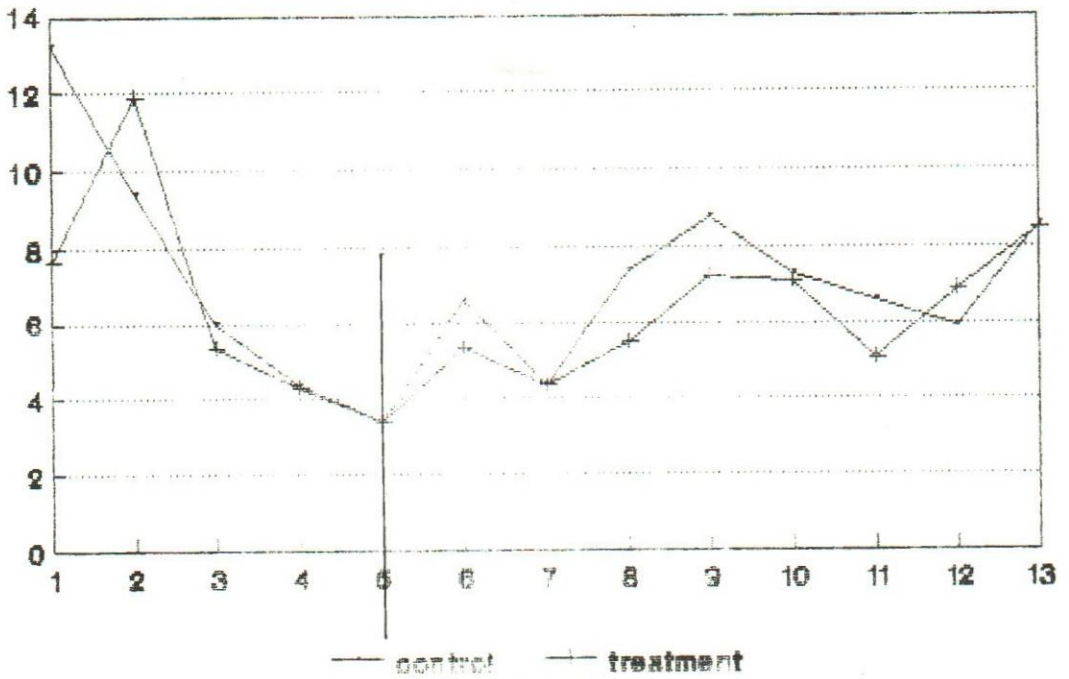
Testosterone



beta carotene



vit.C



vit.A

