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## علاقة فيتامين أ بالكفاءة التناسلية لذكور الجمال

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أجريت هذه الدراسة على مدار عام كامل لبيان تأثير فيتامين أ على الكفاءة التناسلية في ٧١ من ذكور الجمال التي تراوح أعمارهم من ٤ سنوات الى أكبر من ١٥ سنة • تم تحليل عينات الدم في هذه الحيوانات لقياس كل من فيتامين أ والكاروتين • كما تم قياس المعدلات المختلفة لانتاج الحيامن ومستوى الهرمون الذكري (اليتستيسيترون) بالإضافة الى الفحص الهستوباثولوجي لخصية هذه الحيوانات •

كان متوسط تركيز فيتامين أ والكاروتين في دم الجمال هو ٦٠٧٦، ٦٩٧٥ ميكرو جرام % على التوالي •

اثر عمر الحيوان وكذلك موسم السنة تأثيرا معنويا على قيم فيتامين أ ولم يلاحظ ذلك بالنسبة لقيم الكاروتين • فقد أظهرت التحاليل الاحصائية أن أعلى تركيز لقيمة فيتامين أ كان في الجمال التي تتراوح أعمارهم من ٨-١٢ سنة (٩٢٦٧ ميكرو جرام %) وأثناء موسم العليقة الخضراء (٦٨٠٧ ميكرو جرام %) • على النقيض من ذلك كان أقل مستوى لقيم فيتامين أ في الحيوانات التي تزيد أعمارها عن ١٥ سنة (٤٠١٩ ميكرو جرام %) وفي خلال موسم الجفاف (٥٣٣٠ ميكرو جرام %) •

كان هناك ارتباط وثيق بين أعلى تركيز لحيامين أ وأكبر حجم للخصية وأعلى معدل لانتاج الحيامن لكل جرام خصية ومخزون الخصية من الحيامن وتركيز الهرمون الذكري • بالإضافة لذلك فان التحاليل الاحصائية قد أظهرت ارتباط معنوي بين فيتامين أ وكل من هذه المعالم •

أظهر الفحص الهستولوجي وجود استحالة في الخلايا المكونة لخصية الجمال التي

تعاني من نقص فيتامين أ وقد شمل البحث وصف لصور هذه العلاقة •

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**VITAMIN A AND MALE REPRODUCTION IN THE CAMEL  
(CAMELUS DROMEDARIUS)**  
(With 3 Tables and 2 Figures)

By

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**SUMMARY**

The effect of vitamin A on male reproduction was investigated in 71 camels, 4 to 15 years of age, for one year. Blood samples were analysed for vitamin A and B-carotene concentrations. Sperm production rates, testosterone values and testicular histopathology were figured out for respective animals.

The mean blood concentrations of vitamin A and B-carotene in all camels were 60.76 Ug% and 69.75 Ug% respectively. Though age significantly influenced ( $P < 0.01$ ) the vitamin A values, it had no statistically significant effect on B-carotene. Maximum (92.67 Ug%) and minimum (40.19%) vitamin A values were found in sera of camels 8.5-12 and 15 years of age respectively.

Season has a significant ( $P < 0.01$ ) effect on the concentration of vitamin A. Values of vitamin A were higher (68.07 Ug%) during the green season (November to May), than (53.30 Ug%) during the dry season (June to October).

Higher levels of vitamin A were associated with larger testes and maximum values of sperm production per gram parenchyma tissues, gonadal sperm reserve, daily sperm production and testosterone concentration. Statistical analysis revealed a significant coefficient of correlation between vitamin A and these criteria.

Histological examination of the testes of vitamin A deficient camels showed testicular degeneration. Detailed description for the forms of degeneration was encountered.

**INTRODUCTION**

Vitamin A has a profound influence on reproduction in both male and female animals. Clinical signs of male infertility related to vitamin A deficiency include delayed onset of puberty (BONSEMBIANTE *et al.*, 1983), suppressed libido (PALLUDAN, 1963; HUANG *et al.*, 1983), reduced testes size (RAO and RAJA, 1977 a & b) and inferior semen characteristics (GOLYARKIN, 1981; KUPFER *et al.*, 1986). Advanced deficiency causes degeneration of the seminiferous tubules and testicular atrophy (HUANG and HEMBREE, 1976; RAO and RAJA, 1977 c; UNNI *et al.*, 1983).

Despite the extensive studies published on the role of vitamin A on reproduction in the boar (HJARDE *et al.*, 1961; PALLUDAN, 1961, 1963, 1966; RAO and RAJA, 1977 a,b,c),

S.T. ISMAIL, *et al.*

bull (EVANS, 1932; ERB *et al.*, 1947; MULLER *et al.*, 1977; LANGE, 1977; GOLYARKIN, 1981; AFIEFY *et al.*, 1984; KUPFER *et al.*, 1986), ram (LINDLEY *et al.*, 1949; RIVERÒS and CORUALAN, 1979) and laboratory animals (MAYER and GODDARD, 1951; HOWELL *et al.*, 1963; HUANG and HEMBREE, 1979; MITRARAND *et al.*, 1979; HUANG *et al.*, 1984; HUANG *et al.*, 1985), similar information are still lacking in the camel.

Therefore, the purposes of the present study were: 1) to supply the standard serum values of vitamin A and its precursor B-carotene for young, mature and aged male camels, 2) to determine the effect of season and nutrition on the sera levels of vitamin A, and 3) to investigate the relationships between vitamin A and each of testes size, sperm production rates, testosterone and testicular histopathology.

## MATERIAL and METHODS

### Animals :

The present study was carried out on 71 male camels slaughtered in the Cairo and Giza abattoirs over a period of one year. The camel's age ranged from 4 to over 15 years.

### Collection of samples :

1. Blood samples were collected by venepuncture from the jugular vein before slaughtering the camel. Following clotting of the blood, serum harvested by centrifugation was used for the determination of vitamin A and B-carotene. Using EDTA as anticoagulant, plasma was obtained by centrifugation at 3000 rpm for 15 minutes and stored at -20°C until assayed for testosterone.

2. Testes and epididymides were recovered immediately after slaughter and examined to determine their weights and sperm production rates. Tissue sections were also prepared to evaluate the testicular histopathology.

### Samples assay :

1. Vitamin A and Carotene: Determination of vitamin A and carotene in serum was carried out using the principals of McCORMIC (1986). Proteins were precipitated with ethanol, then carotene was extracted with ether and measured by a Perkin Elmer 550 spectrophotometer at a wave length of 440 nm. After evaporating the solvent, vitamin A was determined in the residues which dissolved in chloroform followed by addition of Carr Price reagent. Vitamin A was measured at 620 nm wave length.

2. Testosterone: Plasma testosterone was radioimmuno-assayed in duplicate, using Kit (SEVONO BIODATA, DIAGNOSTICA, CODE 1603).

3. Sperm production rates: Testicular and epididymal sperm reserves were counted in tissue homogenates (AMANN and ALMQUIST, 1961). Daily sperm production was determined from the quantitative histology technique using the formula proposed by AMANN and ALMQUIST (1962).

4. Testicular histopathology: Three pieces taken from each testis were fixed in Bouin's solution and processed to obtain 5-7 micron-thick paraffin sections (McENTEE, 1977). The prepared tissue sections were stained with Harris Haematoxylin and eosin for detection of any testicular abnormality.

### Statistical Analyses :

The data were grouped (mean  $\pm$  SD) according to age and season. The effect of age

## VITAMIN A AND MALE REPRODUCTION IN THE CAMEL

was determined by one-way analysis of variance. Differences between green and dry seasons were evaluated by the student's test. Coefficients of correlation were tried between vitamin A and carotene values from one hand and other parameters studied on the other hand. All analyses were done according the SNEDECOR and COCHRAN (1976).

### RESULTS

The mean concentrations of vitamin A and B-carotene in blood sera of male camels 4 to 15 years of age, are given in Table 1. The overall concentrations of vitamin A and B-carotene for all camels were  $60.76 \pm 6.77$  Ug% and  $69.75 \pm 8.51$  Ug% respectively. Age markedly ( $P < 0.01$ ) influenced vitamin A concentrations, while carotene values showed non-significant changes among the age groups studied (Table 1). Maximal vitamin A values ( $92.67 \pm 9.17$  Ug%) were found in sera of male camels 8.5-12 years of age. Similarly, testes weight, sperm production per gram testicular parenchyma, gonadal sperm reserve and daily sperm production were significantly higher in the same group. Lowest concentrations of vitamin A ( $40.19 \pm 5.69$  Ug%) were also associated with minimal values of sperm production rates and testosterone in camels over 15 years of age.

Seasonal variations in vitamin A and B-carotene concentrations are shown in Table 2. Values of vitamin A were significantly ( $P < 0.01$ ) higher ( $68.07 \pm 12.47$  Ug%) during the green season (November-May) than ( $53.30 \pm 9.98$  Ug%) during the dry season (June-October). In accordance, testes weight, sperm production rates and testosterone concentration were significantly higher during green season.

The coefficient of correlation between vitamin A and each of the testes weight, gonadal sperm reserve, daily sperm production and epididymal sperm reserve were highly significant ( $P < 0.01$ ), while the correlation with testosterone was significant at ( $P < 0.05$ ) level of probability. However, the correlations between carotene and any of the parameters studied were non-significant (Table 3).

Histological examination of the testes revealed an association between the blood serum levels of vitamin A and the degree of testicular degeneration in the same group of camels. The significantly low concentration of vitamin A were always accompanied with marked pyknosis of the spermiogenic cells, sloughing of the germinal cells and presence of multinucleated giant cells in the lumina of the seminiferous tubules (Fig. 1). In very low serum vitamin A values, seminiferous tubules were devoid of any spermiogenic cells and were lined only with Sertoli cells (Fig. 2).

### DISCUSSION

Among farm animals, the blood serum vitamin A level (g/100 ml) was found to be 42.60 to 72.31 in cattle (PANDEY and ROY, 1966; GHOSAL and DWARAKNATH, 1976), from 40.67 to 89.85 in sheep (DWARAKNATH and PAREK, 1971; GHOSAL and DWARAKNATH, 1976) and 37.5 in buffalo (PANDEY and ROY, 1966). In this study, the vitamin A concentration averaged  $60.76$  Ug/100 ml (range 40.19 to 92.67 Ug/100 ml) in the one humped camel. In Bikaneri camels, a lower value ( $45.72$  Ug/100 ml) was reported by GHOSAL and DWARAKNATH (1976). Breed, age, sex, seasonal temperature and availability of green feeders are factors responsible for such variations.

Vitamin A concentration increased with the advancement of age to reach a maximal value of  $92.67$  Ug/100 ml in 8.5-12 years old camel. It decreased thenceafter, to reach a lowest level of  $40.19$  Ug/100 ml in the camels over 15 years of age. Perusal of literature revealed no information on the relationship between age and vitamin A.

Among seasons, the markedly higher vitamin A concentration (68.07 U<sub>g</sub>/100 ml) during November to May than during June to October (52.30 U<sub>g</sub>/100 ml) could be due to the availability of the green fodders, the main source of vitamin A to the animals. The drying of the green fodders to feed animals during the dry period of the year (June to October), causes great losses (73-84 %) of its carotene content (COLUMBUS *et al.*, 1958). Also head stress which is bound to depress thyroid activity during summer affects vitamin A status by lesser conversion of ingested carotenoids into vitamin A and its subsequent absorption from the intestine (CHANDA and OWEN, 1952). Direct solar radiation and high environmental temperature can also adversely affect the vitamin A storage by increasing its mobilization (PAGE *et al.*, 1960).

The findings of a marked association between the serum vitamin A level and each of testes weight, sperm production rates and testosterone values among ages and seasons was confirmed by significant coefficients of correlation. In accordance, animals raised on vitamin A deficient diet were infertile and their testes were small and atrophic (ERB *et al.*, 1947; PALLUDAN, 1963, 1966; RAO and RAJA, 1977 a & b; HUANG *et al.*, 1983). Atrophy of accessory sex organs and epididymis was also noted in the vitamin A deficient animals (RAO and RAJA, 1977 b). Moreover, a greatly reduced semen volume was found in animals receiving vitamin A deficient rations (RIVEROS and CORVOLAN, 1979; AFIEFY *et al.*, 1984). Addition of carotene or vitamin A supplement to the animals resulted in a marked improvement in semen characteristics (MULLER *et al.*, 1977; GOLYARKIN, 1981; AFIEFY *et al.*, 1984; KUPFER *et al.*, 1986).

The influence of vitamin A on sexual behaviour of the animals was reported by many authors (PALLUDAN, 1963; JUNEJA *et al.*, 1966; APPLING and CHYTIL, 1981 and HUANG *et al.*, 1981 and HUANG *et al.*, 1983). They observed a marked drop in sexual behaviour of vitamin A deficient animals. In accordance, a significant correlation was reported between vitamin A and testosterone concentration in this study. Addition of vitamin A to rations of bulls, boars and rats significantly improved their libido (MULLER *et al.*, 1977; HUANG *et al.*, 1983). MAYER and ATRAUNT (1949) claimed that vitamin A deficiency interferes with the ability of testes to synthesis or release testosterone. In addition, JUNEJA *et al.* (1966) noted that vitamin A deficiency markedly adverse the biosynthesis of steroid of steroid hormones in the testes, adrenals and ovaries of the rats. Recent explanation for this association was given by HUANG *et al.* (1983, 1984, 1985) who reported that the decrease of testosterone secretion in vitamin A deficient animals is due to a hyporesponsiveness of the Leyding cells which may, in turn, be attributed to the cessation of spermatogenesis in such cases.

The most profound effect of vitamin A deficiency is reflected on testicular composition. Histological examination of the vitamin A deficient camels revealed varying degrees of testicular degeneration. Seminiferous tubules were devoid of any spermatozoa, spermatids and spermatocytes in sever deficiency. Only Sertoli cells and few spermatogonia persisted the deficiency. Similar observations were noted in other species (HODGSON *et al.*, 1946; PALLUDAN, 1963; RAO and RAJA, 1977 b; MITRARAND *et al.*, 1979; HUANG *et al.*, 1983; UNNI *et al.*, 1983). Following treatment of such animals with vitamin A, regeneration of testicular tissues occurred (HUANG and HEMBREE, 1976; RAO and RAJA, 1977 c).

The mechanism by which vitamin A affects spermatogenesis was early explained by PALLUDAN (1963) who reported that the endocrine disturbances associated with vitamin A deficiency was followed by degenerative changes in the reproductive organs. Recently, HUANG *et al.* (1984) found that a complete cessation of spermatogenesis was associated with a change in the secretion of both FSH and LH.

## VITAMIN A AND MALE REPRODUCTION IN THE CAMEL

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## VITAMIN A AND MALE REPRODUCTION IN THE CAMEL

Table (1): Association between vitamin A and age and their influences on testes weight, sperm production rates and testosterone values in the camel. ( $\pm$  SD)

Parameter	Age groups (years)					overall mean
	4-6 (n=15)	6.5-8 (n=16)	8.5-12 (n=15)	12.5-15 (n=14)	15 (n=11)	
Vitamin A (Mg %)	53.84 $\pm$ 7.45	65.55 $\pm$ 8.03	92.67 $\pm$ 9.17	48.35 $\pm$ 6.93	40.19 $\pm$ 5.69	60.76 $\pm$ 6.77
$\beta$ -carotane (Mg %)	82.18 $\pm$ 21.36	50.66 $\pm$ 7.71	65.79 $\pm$ 22.30	73.82 $\pm$ 22.58	74.02 $\pm$ 14.29	69.75 $\pm$ 8.51
Testes weight (gm)	59.40 $\pm$ 4.62	63.32 $\pm$ 6.13	82.99 $\pm$ 5.62	77.25 $\pm$ 3.83	76.75 $\pm$ 6.93	72.10 $\pm$ 3.00
Sperm prod./gm $\times$ 10 <sup>6</sup>	80.09 $\pm$ 8.19	81.08 $\pm$ 7.09	84.74 $\pm$ 5.98	64.91 $\pm$ 3.57	42.65 $\pm$ 3.47	70.37 $\pm$ 3.99
Gonadal sperm reserve $\times$ 10 <sup>9</sup>	3.90 $\pm$ 0.44	4.64 $\pm$ 0.60	5.47 $\pm$ 0.31	3.83 $\pm$ 0.33	1.94 $\pm$ 0.25	3.98 $\pm$ 0.25
Daily sperm prod. $\times$ 10 <sup>9</sup>	0.89 $\pm$ 0.10	1.06 $\pm$ 0.14	1.25 $\pm$ 0.07	0.83 $\pm$ 0.08	0.58 $\pm$ 0.06	0.92 $\pm$ 0.06
Epididymal sperm reserve $\times$ 10 <sup>9</sup>	1.88 $\pm$ 0.33	2.92 $\pm$ 0.46	2.58 $\pm$ 0.44	1.86 $\pm$ 0.52	1.77 $\pm$ 0.46	2.22 $\pm$ 0.21
Testosterone (ng/ml)	1.12 $\pm$ 0.23	1.89 $\pm$ 0.10	2.23 $\pm$ 0.65	1.22 $\pm$ 0.46	0.98 $\pm$ 0.73	1.47 $\pm$ 0.29



Table (2): Association between vitamin A and season and their influences on testes weight, sperm production rates and testosterone concentration in the camel ( $\pm$  SD).

Parameter	Green season (November to May)	Dry season (June to October)
Number of animals	33	38
Vitamin A ( $\mu$ g %)	68.07 $\pm$ 12.47	53.30 $\pm$ 9.98
$\beta$ -carotene ( $\mu$ g %)	73.11 $\pm$ 16.87	65.87 $\pm$ 17.82
Testis weight (gm)	80.01 $\pm$ 5.08	64.08 $\pm$ 4.23
Sperm prod./gm $\times$ 10 <sup>6</sup>	76.45 $\pm$ 7.64	64.24 $\pm$ 5.84
Gonadal sperm reservex10 <sup>3</sup>	4.35 $\pm$ 0.39	3.57 $\pm$ 0.41
Daily sperm prod. $\times$ 10 <sup>9</sup>	1.05 $\pm$ 0.09	0.79 $\pm$ 0.08
Epid.sperm reservex10 <sup>9</sup>	2.45 $\pm$ 0.42	2.01 $\pm$ 0.30
Testosterone (ng/ml)	1.89 $\pm$ 0.34	1.06 $\pm$ 0.36

Table (3): Coefficient of correlation between vitamin A and  $\beta$ -carotene and the different criteria studied. (n = 71).

Traits	Vitamin A	$\beta$ -carotene
$\beta$ -carotene	-0.284*	-
Testes weight	0.649**	-0.200
Gonadal sperm reserve	0.520**	-0.106
Daily sperm production	0.509**	-0.108
Epid.sperm reserve	0.551**	-0.138
Testosteron	0.279*	-0.172

\*\* significant at 1 % level. & \* significant at 5 % level.

The following table shows the results of the tests conducted on the various specimens of the material under investigation.

Specimen No.	Weight (g)	Volume (cc)	Density (g/cc)
1	10.0	10.0	1.00
2	10.0	10.0	1.00
3	10.0	10.0	1.00
4	10.0	10.0	1.00
5	10.0	10.0	1.00
6	10.0	10.0	1.00
7	10.0	10.0	1.00
8	10.0	10.0	1.00
9	10.0	10.0	1.00
10	10.0	10.0	1.00

The results of the tests conducted on the various specimens of the material under investigation are shown in the following table.

Specimen No.	Weight (g)	Volume (cc)	Density (g/cc)
1	10.0	10.0	1.00
2	10.0	10.0	1.00
3	10.0	10.0	1.00
4	10.0	10.0	1.00
5	10.0	10.0	1.00
6	10.0	10.0	1.00
7	10.0	10.0	1.00
8	10.0	10.0	1.00
9	10.0	10.0	1.00
10	10.0	10.0	1.00

The results of the tests conducted on the various specimens of the material under investigation are shown in the following table.

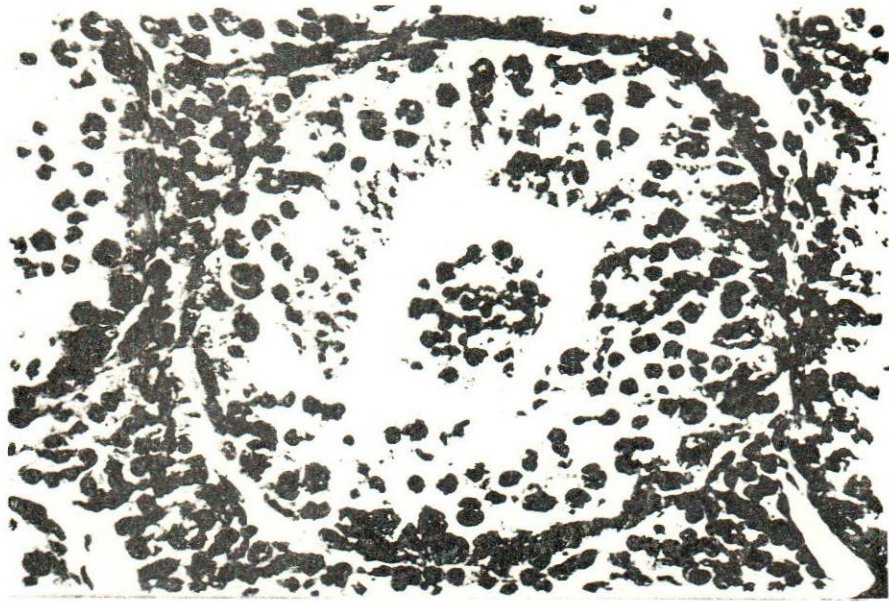


Fig. (1): Testes in camels of low vitamin A values.

Seminiferous tubules revealing pyknosis of spermiogenic cells and accumulation of detached germinal cells in the lumen ( X 400 ).

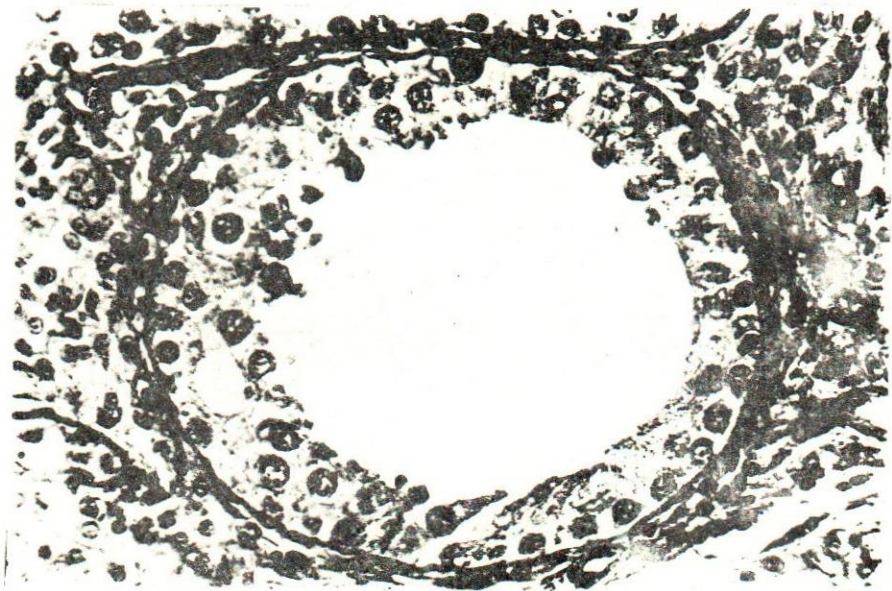


Fig. (2): Testes in camels of very low vitamin A values.

Seminiferous tubule lined only with Sertoli cells and few spermatogonia. Note the absence of most of the spermiogenic cells ( X 400 ).

