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EFFECTS OF VITAMIN A AND VITAMIN E ON SEMEN QUALITY AND FERTILITY OF FRIESIAN BULLS

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SUMMARY

Fifteen Sexually mature Friesian bulls were divided into three similar groups according to age and body weight. Bulls of the 1*st* group was the control. Each bull of the 2 *nd* group was given i.m. injection of 296 I.U. vitamin A/kg body weight once a week along the experimental period (August- Desember). Each bull of the 3*rd* group was injected i.m. with 15mg vitamin E/Kg concentrate ration once a week. Two succesive ejaculates were separately obtained from each bull twice weekly. Some physical and chemical characteristies of semen were determined. Blood samples were collected bi-weekly for estimation of vitamins and testosterone concetration. The fertility of the frozen semen was obtained from the results of pregnancy diagnosis through rectal palpation 2 mo. after the 1*st* service using 481 cows.

The results showed that bulls injected with vitamin A had the largest ejaculate volume, the highest motility and the hight sperm concentration. Live spermatozoa% was higher and abnormal spermatozoa % was lower in semen collected from bulls treated with vitamins. The treatment resulted in an increase of glutamic oxaloactic transaminase (GOT) and glutamic pyruvic transaminase (GPT) concentrations in both seminal plasma and spermatozoal extract. Vitamin E showed the highest value of fructose concentration in semen. Injection of vitamin A or E led to a considerable increase of testasterone blood serum. Injection of vitamins A and E improved conception rate by 36% and 30%, respectively. It might be concluded that supplementation of such vitamins is essential for the breeding bulls in order to improve semen quality and ameliorate the adverse effects of vitamin deficiency in the rations .

Keyowrds: Friesian bulls, vitamin, semen quality, fertility

INTRODUCTION

Most of vitamins, other than A and E are either contained in rations or synthesized in sufficient quantities within body tissues. It is well documented that vitamin A is essential for normal functioning of the epithelial tissues. The importance of vitamin A in male reproduction has been recognized for many years (Huang and Hembree, 1976; and Golyarkin, 1981). Although vitamin A and E requirements for growth, health and reproduction have been well defined for most mammalian species, literature contains insufficient specific recommendation of the optimum allowances

for the breeding bulls used in artificial insemination programs. Addition of vitamin A to rations of bulls, boars and rats significantly improved their libido (Huang *et al.*, 1983).

The shortage of green fodder resources during summer in semi-arid areas led to the lack of vitamins A and E, hence vitamin supplementation is so essential for the proper reproductive performance of the breeding bulls. The present work was conducted to study the effect of vitamin A and vitamin E injections in Friesian bulls on some physical and chemical semen characteristics, seminal enzymes, testosterone concentration in blood and the conception rate after using frozen semen of treated and untreated bulls.

MATERIALS AND METHODS

Experimental animals

Fifteen mature Friesian bulls were used in this study . They were divided into three similar groups (5 bulls in each group) according to their age (22-27 months) and body weight (av. 375 kg). Animals were housed individually under semi- open sheds and were weighed regularly one time every month after 16 hours fasting.

Feeding

A concentrate mixture was used in addition to rice straw. The mixture was composed of undecorticated contton seed cake 25%, Corn 15%, Coarse wheat bran 44%, extracted rice bran 8.5%, molasses 3%, limestone 3% and sodium chloride 1.5%. Chemical analysis of samples from concentrate mixture and rice straw was determined according to A.O.A.C. (1970).

Estimation of vitamin A and E in ration was performed according to Bernard Oser (1979). Individual feeding was practiced. Animals were fed according to the standard allowances recommended by N.R.C (1978). Daily amounts provided per head ranged from 4.5 to 6.0 kg concentrate mixture and from 4.0 to 5.5 kg rice straw. Bulls were not allowed to graze or to receive green fodder.

Experimental treatments

Bulls of the first group was used as control. Each bull of the 2nd group was given i.m. injection of vitamin A once a week along the experimental period (August-December). Bulls of the 3rd group were injected i.m. with vitamin E. The injected vitamin A was in the form of acetate which contained 500000 I.U. per one gram. One gram was dissolved in 10 ml distilled water, therefore 1 ml from this solution contained 50000 I.U vitamin A. Each bull from the second group was given 296 I.U. vitamin A/kg live body weight/ week as recommended by N.R.C. (1978).

The injected vitamin E was in the form of acetate in which one mg equals to one I.U. A total of 10 g equals to 10000 IU Alpha-tocophyrol acetate were dissolved in 25 ml corn oil, therefore each ml of this solution contained 400 IU vitamin E. Each bull of the 3rd group received 15 mg vitamin E per kg of concentrate ration as recommended by Menke and Huss (1975). It should be mentioned that bulls were injected with the assigned concentration of each vitamin in addition to the actually existed amoubts of these vitamins in rations (both concentrate mixture and rice straw).

Semen collection and analysis

Semen ejaculates were collected twice weekly from each bull by means of artificial vagina At each collection two successive ejaculates were separately obtained. A total number of 1200 ejaculates were used to evaluate the following semen physical characteristics:

- Ejaculate volume (in milliliters)
- Mass motility (estimated on a percentage score according to the procedure outlined by Melrose and Laing, 1970) .
- Live sperm percentage (according to the method of Hancock, 1951).
- Ahnormal sperm percentage (calculated according to Blom, 1950).
- Sperm-cell concentration ($x10^6$/ml) determined according to Herman and Madden, 1953).

Once a week equal volumes of semen from the 1st and the 2nd ejaculates of 300 samples were added to determine the following semen chemical characteristics :

-Fructose concentration (mg/100 ml) assessed according to the technique adopted by Mann (1964).

-Glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) activities (RFU/ml): GOT and GPT were determined extracellulary (in seminal plasma) and intracellularly (spermatozoal extract) using the method described by Reitman and Frankel (1957).

Collection and analysis of blood samples :

One hundred sixty five blood samples were collected at bi-weekly intervals before feeding, each sample was about 20ml. Samples were left to coagulate at room temperature then centrifuged at 3000 r.p.m. for 10 min. for separation of blood serum. Serum was analyzed for the following :

-Total carotenoids and vitamin A: estimated according to the method of Pett and Lepage (1940).

-Vitamin E: estimated according to the recommendation of ferric chloride dipyridyl method (Emmerier- Engel reaction) Bernard Oser (1979).

- Testosterone concentration: Assessment of total testosterone concentration was performed by the method of Jaffe and Behrman (1974) by using Coat-A-Count I 125 radioimmunoassay (RIA) kits purchased from Diagnostic Products Corporation, Los Angelos, California, USA.

Semen freezing technique and conception rate :

Two bulls from each group were randomly selected to freeze their semen. Only ejaculates of high quality were used. Conception rate (C.R) results using frozen semen were sued as an additional criterion of semen quality. The fertility of semen samples was obtained from the results of pregnancy diagnosis through rectal palpation 2 months after the first service using 481 clinically normal cows (each cow received one insemination by the same inseminator).

Statistical analysis :

Statisitical analysis was performed by the methoof least squares ANOVA using the general linear model procedures of SAS (1987) . Duncan multiple range test was

used to test the differences among means (Duncan, 1955). Simple correlation coefficients among physical and chemical characters were also calculated.

RESULTS AND DISCUSSION

A. Effect of Vitamin A and Vitamin E on Physical Characteristics of Bull semen

Ejaculate Volume (ml)

The overall means of ejaculate volum of the treated groups were significantly ($p<0.01$) different from the control.Bulls injected with vitamin A gave the largest ejaculate volume (Table 1). This increase might be attributed to the role of vitamin A in either maintaining the testes weight within the normal limits and/ or preservation of epididymis and accessory sex organs which contribute in producing seminal plasma. Differences among months in ejaculate volume were significant ($p<0.01$). The highest volumes were obtained in August.

Motility of spermatozoa (%)

The overall differences among treatment groups were significant ($p<0.01$). The highest initial motility was obsrved in semen collected from bulls treated with vitamin A (Table 1). Sperm motility chanaged significantly ($p<0.01$) with the month of semen collection, being highest during August.

Sperm Concentration ($x10^6$/ml)

Treatment had a significant effect ($p<0.01$) on sperm concentration. The best was that of vitamin A treated bulls which did not differ significantly from that of vitamin E treated bulls (Table 1). Vitamin A deficiency caused degeneration in the seminiferous tubules in bulls (Roberts, 1971; Hafez,1987 and Arthur, 1975).

Live spermatozoa

The overall means of live spermatozoa percentage were significantly higher ($P<0.05$) in groups 2 and 3 than the control. Vitamins A and E may maintain the viability and permeability of cell membranes of the spermatozoa. The present results are in agreement with those found by Otto (1965). Also Singh *et al.* (1989) found that vitamin E improved significantly live sperm percentage in buffalo bulls.

Abnormal spermatozoa (%)

Percentage of sperm abnormalities was significantly ($P<0.05$)lower in vitamin treated bulls when compared to the control group. Similar results were obtained by Afiefy *et al.* (1984); Kupfer *et al.* (1986) and Erdinc *et al.* (1990) who indicated that vitamin A supplementation led to a reduction in the percentage of abnormal spermatozoa. It is well-known that vitamin A deficiency in bulls resulted in pronounced increase in total sperm abnormalities. This is due to degeneration on the germinal epithelium of the testes (Hafez, 1987). The lowest percentage of abnormal spermatozoa was obtained during October, November and December.

Analysis of variance of semen physical characteristics of bulls treated with vitamin A and vitamin E is shown in Table 2.

Table 1. Overall means ± SE of physical characteristics of Friesian bull semen as affected by vitamin A and vitamin E treatments.

Character	Control (G1)	Vitamin A (G2)	Vitamin E (G3)
Ejaculate volume, ml	1.98± 0.03^{b}	2.44± 0.04^{a}	2.29± 0.04^{a}
Sperm motility, %	64.18± 1.0^{c}	73.25± 1.00^{a}	69.46± 0.00^{b}
Sperm concentration,x10^{6}/ml	773.5± 15.1^{b}	963.0± 16.0^{a}	907.5± 13.7
Live spermatozoa, %	74.89± 1.0^{b}	78.03±1.00^{a}	76.99± 1.0^{a}
Abnormal spermatozoa, %	14.85± 0.45	13.84± 0.26^{a}	13.72± 0.20^{a}

Means within the same row having the same superscript are statistically non-significant otherwise, they differ significantly at (P<0.01) for the first three characters and at (P<0.05) for the last two characters

Table 2. Analysis of variance of semen physical characteristics of bulls treated with vitamin A and vitamin E.

Source of variance	df	Ejaculate volume	Sperm motility	Sperm concent.	Live sperm	Abnormal sperm	Sperm output
Treatment	2	23.154**	1.8075**	484950**	0.2625**	0.0169**	73478319**
Bull (Treatment) or (E1)	12	1.185	0.2306	457726	0.0409	0.0021	4470947
Month	4	8.975**	0.1670**	359482**	0.0076**	0.0057**	24676642**
Treatment x Month	8	1.030	0.0378	312351	0.0063	0.0016	1942446
Bull x Month (E2)	48	0.744	0.0403	89411	0.0144	0.0008	1380949
Ejaculate number (Ejac No)	1	0.263	0.0016	1276	0.0030	0.0022	363406
Treatment x Ejac No	2	0.571	0.0038	30041	0.0008	0.0001	1664767
Treatment x Month	4	9.351**	0.0087	18639	0.0025	0.0003	7360860
Treatment x Monthx Ejac No	8	0.255	0.0026	32181	0.0013	0.0003	546845
Error (E3)	1177	0.510	0.0151	78415	0.0054	0.0004	915729

* Significant p<0.05 ** Significant p<0.05

B. Effects of vitamins A and E on chemical characteristics of semen:

1- Got and GPT activities

Treatment resulted in a significant (P<0.01) increase of GOT and GPT concentrations in both seminal plasma and spermatozoal extract. Such vitamins may exert their influence on prostate and epididymal cells. The overall means of GOT in seminal plasma were 107.4, 117.4 and 122.6 RFU/ml in groups 1,2 and 3, respectively, whereas the values of GOT in spermatozoal extract were 83.6,91.6 and 93.1 RFU/ml, respectively. In terms of GPT concentrations, the values in seminal plasma were 43.1,44.5 and 44.4 RFU/ml whereas the values of GPT in spermatozoal extract were 40.1, 41.7 and 41.8 RFU/ml, respectively.

2- Fructose concentration

Injection with vitamin E showed the highest value (335mg/100ml) followed by vitamin A (308/100ml), whereas the control was the least (265mg/100ml). Differences anong groups were significant (p<0.01). Increasing semen output / ejaculate caused high utilization of fructose in semen, therefore, the correlation coefficient (r) was negative (-0.158) and significant (p<0.05) (Table 3).

Table 3 Correlation coefficients of some semen characteristics and blood testosterone and vitamins (A, E).

Characters	Motility	Abnormal	Live	Sperm concn.	Sperm output	GOT-P	GOT-S	GPT-P	GPT-S	Fructose	Testos-terone	Vitamin E	Carotene	Vitamin A
Ejaculate volume	0.203**	-0.053	-0.033	0.049	0.633**	0.075	-0.107	-0.248**	-0.241**	- 0.142	0.252**	0.143	0.104	-0.033
Motility		-0.330**	0.511**	0.502**	0.456**	0.017	0.032	0.032	-0.003	- 0.100	0.248**	0.091	-0.134	0.095*
Abnormal			-0.355**	-0.272**	-0.269**	0.056	0.092	0.122	0.123	0.005	0.091	0.196**	-0.022	0.175**
Live				0.467**	0.331**	-0.226**	0.001	0.106*	0.080	0.058	-0.066	-0.414**	-0.127	0.02
Sperm concn.					0.759**	-0.127	- 0.078	- 0.150**	-0.112**	- 0.123*	-0.069	-0.120	-0.137	-0.088
Sperm output						-0.054	- 0.087**	- 0.274**	-0.245**	- 0.158**	0.036**	-0.018**	-0.009**	-0.113**
GOT - P							0.442**	- 0.118**	- 0.183**	0.577**	0.292**	0.403**	0.498**	-0.064**
GOT - S								0.553**	0.512**	0.442*	0.247**	0.257*	-0.120**	0.414**
GPT - P									0.890**	0.145	0.224**	0.176*	-0.665**	0.689**
GPT - S										0.134	0.193*	0.150	-0.667**	0.613**
Fructose											0.160	0.095**	0.307**	-0.161**
Testosterone												0.426**	-0.076	0.247*
Vitamin E													-0.038	0.171**
Carotene														-0.618**

* Correlation coefficient is significant at $P < 0.05$
** Correlation coefficient is significant at $P < 0.01$

GOT-P = Glotamic oxaloacetic transaminase in seminal plasma
GOT-S = Glotamic oxaloacetic transaminase in spermatozoal extract.
GPT-P = Glotamic pyruvic transaminase in seminal plasma.
GPT-S = Glotamic pyruvic transaminase in spermatozoal extract.

C. Blood serum analysis

1- Total carotenoisds, vitamin A and vitamin E :

Injection of vitamin A resulted in a significant ($p<0.05$) increase in serum content of carotene. The overall means of carotenids in blood serum of groups 1,2 and 3 were 88, 98 and 74 µt/100 ml, respectively. Group 3 had higher and significant ($P<0.05$) level of vitamin E in serum when compared to the other two groups. The values of vitamin E in serum of groups 1,2,and 3 were 19.7,20.2 and 21.8 I.U/100ml serum, respectively.

2- Testosterone concentration (ng/ml) .

Treatment resulted in a significant ($p<0.01$) effect on testosterone concentration in blood serum. The highest value was recorded for group 3 (2.21 ng/ml) followed by group 2 (1.64 ng/ml) wheras the control was the least (1.39 ng/ml).A significant ($P<0.01$) and positive correlations were found between testosterone and each of vitamin A and E concertrations in blood serum (table 3).

D- Conception rate (C.R.)

The highest CR (55%) was obtained for vitamin A followed by vitamin E (53%). The least CR. was that of the control (40%) this improvement of CR might be due to the high quality semen produced as a result of the treatment.

Conclusions

Vitamins A and E supplementation is essential for increasing the fertility of breeding bulls by improving semen quality and ameliorating the adverse effects of vitamin deficiency in the rations. Further studies are still needed to investigate the combined action of administration of both vitamin A and E together on reproductive performance of the breeding bulls. Additionally, histological examination of the testes of the vitamin injected bulls will assist in determining the stage of spermatogenesis that mostly affected by vitamin treatment.

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تأثير فيتامين أ وفيتامين هـ على خصائص السائل المنوى والخصوبة فى ذكور الفريزيان

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أُستخدم فى هذه الدراسة ١٥ طلوقة فريزيان ناضجة جنسياً ووزعت الحيوانات تبعاً لأوزانها وأعمارها فى ثلاث مجاميع متساوية. إستخدمت طلائق المجموعة الأولى كمجموعة مقارنة وفى المجموعة الثانية تم حقن كل طلوقة فى العضل بـ ٢٩٦ وحدة دولية من فيتامين أ لكل كجم وزن حى مرة كل أسبوع طوال فترة التجربة (أغسطس - ديسمبر) أما فى المجموعة الثالثة فقد تم الحقن فى العضل بـ ١٥ ملليجرام فيتامين هـ لكل كجم عليقة مركزة مرة فى الأسبوع.

تم جمع السائل المنوى بإستخدام المهبل الصناعى مرتين أسبوعياً من كل طلوقة حيث جُمعت قذفتين متتاليتين منفصلتين فى كل مرة جمع لدراسة بعض الصفات الطبيعية والكيماتية للسائل المنوى. كما أُخذت عينات من الدم مرة كل أسبوعين لتقدير تركيز كل من التستسترون والفيتامين.

وقد أُستخدمت ٤٨١ بقرة فريزيان لتقدير نسبة الخصب للسائل المنوى المجمد عن طريق تشخيص الحمل بعد شهرين من إجراء التلقيح. وقد أوضحت النتائج أن الطلائق التى حُقنت بفيتامين أ أعطت أكبر حجم للقذفة وأعلى تركيز. ووجد أن الحقن بالفيتامينات أدى إلى إرتفاع نسبة الحيوانات المنوية الحية وإنخفاض نسبة الحيوانات المنوية الشاذة. كما أن المعاملة بالفيتامينات أدت إلى زيادة تركيز كل من الإنزيمات الناقلة لمجموعة الأمين (GOT, GPT)وذلك فى بلازما السائل المنوى ومستخلص الإسبرمات. كما وجد أن فيتامين هـ أدى إلى زيادة تركيز سكر الفركتوز فى السائل المنوى. والحقن بفيتامين أ، فيتامين هـ أدى إلى زيادة تركيز هرمون التستسترون زيادة ملحوظة فى سيرم الدم كما أن الحقن بفيتامين أ، فيتامين هـ أدى إلى تحسين نسبة الخصب بـ ٣٦٪، ٣٠٪ على التوالى مقارنة بمجموعة المقارنة.

من هذه الدراسة يمكن إستنتاج أن الامداد بهذه الفيتامينات ضرورى لطلائق التربية لتحسين خصائص السائل المنوى لتلافى التأثير الضار الناتج عن نقص هذه الفيتامينات فى العلائق.