EFFECT OF BETA-CAROTENE INJECTION ON ESTRUS, VITAMIN A AND ESTRADIOL-17β CONCENTRATIONS IN PUBERTAL FARAFRA EWE LAMBS

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SUMMARY

To study the effect of Beta Carotene (BC) treatment on puberty and related phenomena as well as concentrations of vitamin A, and estradiol-17β hormone 48 Farafra ewe lambs were divided into two equal groups. The control group (G1) was injected intramuscularly with 0.1 mg arachis oil, while the treated group (G2) was injected with 0.1 mg BC + arachis oil / kg twice a week from weaning to puberty. Estradiol 17 β, progesterone (P_4), BC and vitamin A were assayed in blood sera taken from 6 animals/ group.

Results indicated that treatment with BC was accompanied by increasing estradiol-17β and vitamin A concentrations at puberty. Ewe lambs of G2 displayed ($P< 0.05$) more estrous cases post-puberty than G1. Both groups displayed short cycles at puberty (6.2 d for G2 vs. 7.4 d for G1) with no significant difference between the two groups. P_4 concentration at estrus of puberty was ≥ 1.0 ng/ml of both groups. Post-puberty BC and Vit.A concentrations had ($P<0.05$) positive correlation in the two studied groups. BC concentration in G2 was higher ($P< 0.05$) at pre-puberty than post-puberty.

In conclusion, BC injection had an impact on number of observed estrous cases, vitamin A and estradiol-17β concentrations at puberty and post-puberty estrus, which might be applied in the animal farms to enhance estrus cases around puberty.

Keywords: beta-catrotene, ewe lambs, estradiol-17β, estrous activity, puberty, progesterone, vitamin A

INTRODUCTION

BC is essential for many of the biological processes at body level as the source of vitamin A (Schweigert, 1998). Vitamin A (Vit. A) is relatively low in blood plasma of sheep, due to the poor content of β-carotene (BC) in roughages as well as very low rate of absorption in intestine (Ross, 1999). This means that sheep should be supplemented with exogenous sources of BC to avoid deficiency of Vit. A. Inadequate nutrition in ruminants had negative effects on metabolic and hormonal profiles, puberty onset, regular estrous cycles and fertility (Scaramuzzi *et al.,* 2006 and Meza-Herrera *et al.* 2007)..

Oxidative stress (OS) occurs during breeding and pregnancy especially when animals are fed poor feed stuff. Thus, reactive oxygen species (ROS) are formed as a result of many stress factors (Mohebbi-Fani *et al.,* 2012**)**. OS induces lipid peroxidation, which promotes apoptosis, and causes some diseases via its effect on redox status, redox-sensitive signaling pathways and gene expression (Ames et al., 1993). Vitamin A and BC injections support the antioxidant system (Kamuloulu and Beytut , 2005) to avoid the harm effects of the free radicals (Little and Gladen, 1999). Up to the knowledge of the authors no data are available about the effect of BC treatment on age at puberty of Farafra ewe lambs.

The main purpose of this study is to study the role of exogenous BC treatment on puberty of Farafra ewe lambs with particular reference to post puberty estrous behavior, and blood concentrations of Vit.A, BC, progesterone and estradiol-17β.

MATERIAL AND METHODS

1. Animals, Management and Feeding

A total of 48 Farafra ewe lambs of four months of age and mean body weight of 13.25 ± 0.43 kg were divided randomly into two equal groups (n=24 each). Ewe lambs of the first group (G1) were injected intra-muscularly (IM) with 0.1 ml/kg body weight arachis oil (free from BC and Vit. A), while ewe lambs of the second group (G2) was injected IM just after weaning with 0.1 mg/kg BC loaded on arachis oil twice a week for four months (about 8 months of age). Lambs were housed in semi-open pens and were fed according to NRC (2007) on a ration contained 14.0% crude protein, 2.0% crude fat (2700 Kcal/Kg ration metabolizable energy, ME), 15% crude fiber, 9% ash and 12% moisture. No green forages were offered during the whole experiment. Water was available all the day time throughout the experimental period.

2. Estrous Detection

Prior to the expected time of puberty (The 8^{th} month of age) two fertile rams were allowed to run with the ewe lambs for 30 min (15 min for each ram) daily at 8:00 am to detect the ewes in heat. Behavioral signs of estrus (e.g. vaginal mucous discharge, vulva swelling, tail twitching) as well as observation of arborization (Fern-like pattern) in a smear made from vaginal secretion have been taken into consideration as a tool to estrous detection (Salem *et al.,* 2013). Estrous cycle length was classified as normal 14 -19, short < 14 and long cycle

>19 day. Silent estrus was determined by concentration of progesterone.

3. Blood Collection

Blood samples (10 ml/animal) were withdrawn via jugular vein (JV) at 8:00 a.m. before feeding from 6 animals/group chosen randomly. Blood samples were taken from each animal twice a week before the expected puberty by one month to post-puberty by 49 d. Samples were left in the refrigerator (5° C) overnight until clotting before centrifuging at 3000×g for 10 min for harvesting blood sera. Blood sera were stored at −20 °C until analyses.

4. Determination of Progesterone and Estradiol-17β Concentrations

Progesterone (P_4) and Estradiol 17β (E_2) concentrations were assessed using enzyme immunoassay kits (BioCheck, Inc, 323 Vintage Park drive, Foster City, CA 9404, USA). Standard curves were plotted as described by manufacturer. The minimum detectable concentrations of the progesterone and estradiol-17beta ELISA were 0.0625 ng/ml and 10 pg/ml. Cross reactivity with other steroids was reported by manufacturer to be < 1%**.**

5. BC and Vit.A assay

Both BC and Vit.A were assayed by Colormetric method (Suzuki and Katoh, 1990). Reagents used for BC (dye 0.2 mmol / L, buffer 1.0 mol/L and distilled water) and Vit. A (2, 6-dichlorophenol-indophenol, buffer and distilled water) analyses were purchased from ABC Diagnostic-Egypt.

6. *Statistical Analysis*

Data were analyzed using SPSS (2007). Data were presented as number, percentage, mean and standard error. Chi-square test was used to compare qualitative variables between treated and control groups. Mann-Whitney test was used to compare qualitative variables between treated and control groups. P-value was considered significant when reached ≤ P<0.05.

RESULTS

1. Concentrations of Beta-carotene (BC) and vitamin A around puberty

Based on the concentrations of P_4 concentration as well as the appearance of fern-like shape of vaginal mucous smears, day of the first estrus (puberty) was determined. Age of puberty in G1 and G2 was differed non-significantly being 269.2 ± 5.9 and 264.8 ± 8.4 day, respectively. Concentrations of BC and Vitamin A at puberty, estrus and met-estrus phases were higher (P<0.05) inG2 than G1 by more than 50% (Table 1).

2. Effect of BC on post – puberty estrous cycle aspects

Estrous cycles just follow puberty were short in length in both studied groups. Overall mean of estrous cycle cases differed non-significantly between G1 and G2. However, it is worth to underline that the detected number of estrous cycles 69 post puberty was almost double in G2 compared to G1 (Table 2).

3. BC and Vit A concentrations at puberty and pre-and post-puberty

Concentrations of BC and Vit. A around puberty had no consistent trend. However, BC at puberty was higher (P<0.05) in G2 than G1 and similar trend was observed 25 and 21 d prior to puberty and days 21, 25, 38 and 42 post –puberty, the difference in concentrations in other days was not significant, as the trend of Vit. during the three phases studied around puberty (Table 3).

4. Effect of Beta-carotene treatment on estradiol-17β concentrations

E_2 concentrations in G2 at puberty and estrous and met-estrous phases post-puberty was higher (P<0.05) compared to G1 (Fig.1).

Table 1. Concentrations (mean ±SE) of BC (mmol/L) and Vit.A (mg/L) during puberty, estrus and met-estrous phases of Beta-Carotene (G2) and Control (G1) treated groups

Trait	G2	G1
Beta-carotene concentrations		
At puberty	0.85 ± 0.04*	0.38 ± 0.04
At estrous phase post- puberty	0.91 ± 0.05*	0.42 ± 0.03
At met-estrus phase post- puberty	0.80 ± 0.11*	0.38 ± 0.06
Vitamin A concentrations		
At puberty	703.83 ± 129.83*	146.93 ± 19.02
At estrous phase post-puberty	635.50 ± 60.89*	237.62 ± 29.51
At met-estrous phase post-puberty	545.20 ± 130.18*	248.58 ± 31.97

* = P<0.5

Table 2. Effect (mean ± SE) of treatment of Beta-carotene (G2) on post-puberty estrous cycle aspects compared with the control group (G1)

Traits	G2	G1
Number of estruses during 69 d post- puberty	17 *	9
Length of First short estrous cycle (d)	6.20 ± 1.74	7.40 ± 1.97
Overall lengths of estrous cycles during 69 d	13.23 ± .81	15.67 ± 1.62
Length of regular estrous cycle (d)	16.83 ± .19	19.60 ± 5.68

* = P<0.5

Table 3. Concentrations (mean ±SE) of vitamin A and Beta-carotene as measured at different stages around puberty as affected by treatment with beta-carotene (G2) and control (G1)

Stage around puberty	Sampling time	Vit. A concentrations (mg/L)		BC concentrations (mmol/L)	
		G2	G1	G2	G1
Pre-puberty	25	512.5 ± 102.0*	201.6 ± 50.3	0.92 ± 0.05*	0.39 ± 0.06
	21	777.0 ± 2.0*	287.0 ± 82.9	1.03 ± 0.01*	0.52 ± 0.05
	17	574.0 ± 164.0	365.6 ± 45.9	0.70 ± 0.23	0.45 ± 0.08
	14	567.0 ± 177.0	240.9 ± 76.9	0.72 ± 0.19	0.36 ± 0.03
	7	789.3 ± 358.8	175.8 ± 8.8	0.75 ± 0.20	0.38 ± 0.03
	3	594.5 ± 287.0	179.4 ± 26.3	0.77 ± 0.15	0.41 ± 0.10
Puberty	0	703.8 ± 129.8*	146.9 ± 19.0	0.85 ± 0.04)*	0.38 ± 0.04
	3	471.5 ± 96.9	225.5 ± 51.3	0.79 ± 0.04*	0.42 ± 0.03
	11	230.5 ± 66.5	161.0 ± 16.0	0.94 ± 0.13*	0.38 ± 0.06
	14	563.8 ± 174.3	201.0 ± 54.0	0.94 ± 0.11*	0.39 ± 0.02
Post puberty	17	532.8 ± 307.3	210.5 ± 25.5	0.92 ± 0.06*	0.39 ± 0.04
	21	625.3 ± 51.3*	276.0 ± 12.0	0.78 ± 0.02*	0.38 ± 0.04
	25	492.3 ± 39.8*	178.8 ± 5.8	0.40 ± 0.03	0.49 ± 0.06
	28	478.5 ± 7.0*	167.4 ± 14.9	0.44 ± 0.03*	0.32 ± 0.01
	35	269.8 ± 3.3	129.8 ± 62.2	0.80 ± 0.01*	0.37 ± 0.07
	38	579.5 ± 35.5	157.2 ± 21.3	0.91 ± 0.03*	0.38 ± 0.02
	42	713.5 ± 65.5*	283.6 ± 12.3	0.95 ± 0.04*	0.41 ± 0.05
	45	425.3 ± 199.8	307.5 ± 0.0	1.05 ± 0.11*	0.41 ± 0.03
	49	378.5 ± 50.5	307.5 ± 20.5	0.69 ± 0.09	0.50 ± 0.01

* = P<0.5

DISCUSSION

The no effect of BC treatment on age at puberty is in harmony with the finding of Huffman *et al.* (1987) reporting no positive effect on onset of puberty in female goat treated with BC. Meanwhile, the present findings are in contrary with the results of Arellano-Rodriguez *et al.* (2007) and Kawashima *et al.* (2009) who reported that Vit.A deficiency in farm animals delayed puberty. This difference is most probably attributed to the genotype and to the ability of animal in converting beta-carotene to vitamin A.

The obtained results revealed that experimental animals exhibited short estrous cycles at puberty agrees with findings of Parish (2010). The present of the effect of BC on E_2 concentrations during estrous cycle phases are in harmony with findings of Haliloglu *et al.* (2002); Arellano-Rodriguez *et al.* (2009) and Kawashima *et al.* (2010) where they reported that BC had a major role in the ovarian steroidgenesis and reproduction of many species.
Hence, level of estradiol-17β observed in this study may be due to the influence of BC, which positively affected ovarian activity in goats (Arellano-Rodriguez *et al.*, 2007 and 2009).

Inconsistent trends of BC and Vit. A; obtained in this study; around puberty could not be explained. The most probably interpretation is the cessation of treatment before puberty, hence the observed difference is referred to the individual physiological condition.

It could be concluded from this study that BC injection may improve the estrous cyclicity of Frafra lamb ewes.

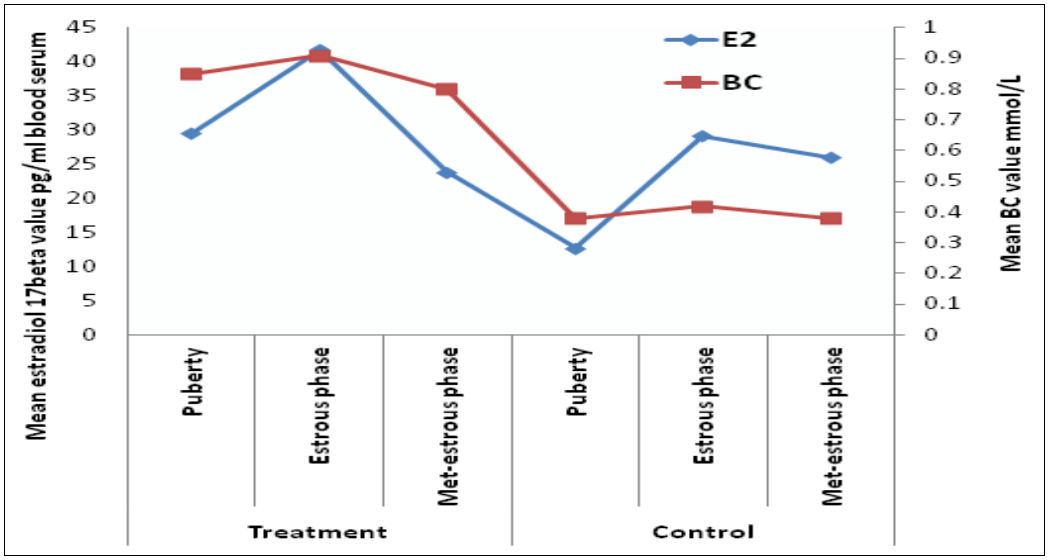

Fig. 1. Representation of BC and estradiol 17β concentrations at puberty, estrus and met-estrous phases after onset of puberty in the ewe lambs injected by BC compared with the controls

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حقن البيتا-كاروتين له تأثير قوى على شدة الشبق ، فيتامين أ ، هورمون الإستراديول ١٧ بيتا فى حملان الإناث البالغة

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يرجع سبب انخفاض مستوى فيتامين أ قى يلازما دم الأغنام إلى انخفاض البيتا كاروتين فى العلائق الغذائية الخشنة وإلى انخفاض معدل امتصاص البيتا-كاروتين فى الأمعاء الدقيقة . لذا يجب أن يؤخذ البيتاكاروتين (نواة فيتامين أ) من مصادر خارجية وذلك لملء مخازن فيتامين أ النسيجية المسئولة عن الحفاظ على الأنسجة الحيوية داخل الجهاز التناسلى. الهدف من هذه الدراسة كان لتحرى حقن البيتاكاروتين هلى بداية البلوغ الجنسى ، قوة الشبق بعد البلوغ الجنسى ، على تركيزات البيتاكاروتين ، فيتامين أ ، البروجيستيرون ، والإستراديول فى الحملان البالغة. اشتملت التجربة على ٤٨ حمل قسمت إلى مجموعتين متساويتين . حقنت مجموهة الكنترول بـ ١.٠ مجم زيت سودانى/كجم وزن/حيوان قى العضل بينما حقنت مجموعة الحيوانات الثانية بـ ١.٠ مجم بيتاكاروتين + زيت سودانى/كجم وزن/حيوان مرتين فى الأسبوع بدءاً من الفطام وحتى البلوغ الجنسى. قدرت جميع الجزيئات البيوكيميائية فى أمصال الدم المأخودة من ٦ حيوانات / مجموعة . تبين نتائج هذه الدراسة ان البيتاكاروتين يثير تنبيهيات وظائف الأستراديوا وفيتامين أ عند البلوغ الجنسى وعلى زيادة عدد الشبق بعد البلوغ الجنسى. حيث أن البيتاكاروتين أظهر قوة الشبق بعد البلوغ الجنسى فى الحيوانات المحقونة به أكثر من مجموعة الكنترول. جميع الحيوانات أظهرت دورات قصيرة عند بداية البلوغ الجنسى بينما ظهرت دورات الشبق المنتظمة مبكرة فى المجموعة المهاملة عن الكنترول. تركيز البروجيستيرون عند البلوغ الجنسى كان ≥ 1.0 نانوجرام/مل مصل دم. هناك ارتباط موجبى معنوى بين تركيزات البيتاكاروتين وفيتامين أ بعد البلوغ الجنسى. تركيز البيتاكاروتين كان أعلى معنوياً قبيل البلوغ الجنسى عن ما بعده. الخلاصة – حقن البيتاكاروتين كان له أثر قوى فى إظهار عدد شبق أكثر بعد البلوغ الجنسى ، وزيادة معنوية فى تركيزات الإستراديول ، فيتامين أ أثناء البلوغ الجنسى وهذا يمكن تطبيقه مستقبلاً فى المزارع الحيوانية.