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Effect of Evening Primrose Oil as Food Supplement on Reproduction in the Blue Fox

By *Anne-Helene Tauson* and *Mats Forsberg*

Department of Animal Nutrition and Management, Funbo-Lövsta Research Station, and
Department of Clinical Chemistry, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Tauson, A.-H. and M. Forsberg: Effect of evening primrose oil as food supplement on reproduction in the blue fox. Acta vet. scand. 1991, 32, 345–351. – Addition of evening primrose oil (EPO) to a blue fox diet in the reproduction period was evaluated in an experiment with 2 groups, each of 12 male and 25 female blue foxes, regarding the effects on reproductive performance. The experiment was carried out as a field trial and the experimental period lasted from March 10 until the end of the mating season (males) or early July (females). During this period the control group was fed the standard diet of the farm and the experimental group was fed the same diet supplemented with 4.5 g EPO and 2.5 mg zinc sulphate per animal and day. An addition of 10 mg vitamin E per 500 mg EPO was made. The results were evaluated regarding male and female treatment effects. There was an increased rate of abortions in the EPO-group, but simultaneously a non-significant decrease in the frequency of barren females, resulting in a similar level of females without litters in both groups. A tendency for increased litter size in the EPO group was found, mainly as an effect of male treatment, which might indicate an effect on semen quality.

reproductive performance; kit performance; lactation period; gamma linolenic acid.

Introduction

The blue fox is a seasonal breeder with a monoestrus breeding extending from late February until early May. The length of oestrus is 3–5 days and spontaneous ovulation occurs about 2–4 days after the LH peak (*Møller & Smith* 1982). Despite a high reproductive capacity, the reproductive results in practical blue fox production may be poor. About 15–20 % of the mated blue fox females are recorded as barren (*Einarsson* 1985), but the majority of these have implantation sites in the uteri, indicating that they have been pregnant for more than 15–17 days. Hence, *Einarsson* (1982) found implantation sites in the uteri of 55 % of the barren females and *Fougner* (1972) reported that 66 % of the empty females had implantation sites. Moreover, foetal mortality from day 17 until parturition is about 25 % (*Einarsson* 1982) to 27 % (*Fougner* 1972) in yearlings and 15 % (*Einarsson* 1982) to 20 % (*Fougner* 1972) in adults. Also the kit losses from parturition to weaning are high and may reach 30 % (*Einarsson* 1985). Thus, an improved reproductive performance in blue fox production may be possible.

Essential fatty acids (EFA) exert a profound effect on the reproductive capacity of many animal species of both sexes. Abnormal spermatogenesis, lack of oestrus, abortions and uterine inertia during parturition have been observed in experimental animals during EFA deprivation studies (*Horrobin* 1981).

EFA are important for 2 quite different rea-

sons. Firstly, they are constituents of membranes in all tissues of the body. Secondly, they are precursors of the prostaglandins.

Three of the EFA can act as precursors for prostaglandins, dihomogamma-linoleic acid (DGLA), arachidonic acid (AA) and eicosapentaenoic acid (EPA) (*Horrobin* 1981).

Evening primrose oil (EPO) is high in gamma-linoleic acid (GLA) which can be converted to DGLA, the precursor of the 1-series of prostaglandins or alternatively DGLA can undergo desaturation to give AA, the precursor of the 2 series (*Horrobin* 1981).

Conventional blue fox diets based on slaughter-house offal may be low in EFA, and therefore the dietary content of GLA may limit the synthesis of the 1-series of prostaglandins.

Supplementation with EPO has reduced foetal mortality in moderately zinc deficient rats (*Cunnane* 1982). This may be explained by an important effect of zinc deficiency being reduced prostaglandin production, which thus can be counteracted by dietary addition of EFA (*Cunnane et al.* 1979). Further, EPO treatment has shown a positive effect on the premenstrual syndrome in women and possibly also cyclicity was improved (*Brush* 1983, Horrobin 1983).

Due to these possible positive effects on reproduction, a field experiment was carried out in 1986 into the effects on reproductive performance of dietary supplementation with EPO during the reproductive season of male and female blue foxes.

Materials and methods

The experiment was carried out on a private fox farm situated in the vicinity of Uppsala, Sweden. The breeding stock of the farm consisted of some 250 blue fox vixens and some 25 silver fox vixens.

The experiment was carried out with 2 groups of blue foxes, each of 12 males and 25 females. The animals were grouped in early March with respect to age and previous reproductive results. The males used were aged 1–4 years, the majority being yearlings. Per female group, 16 animals were yearlings and the rest aged 2–5 years.

Matings were performed according to the ordinary routines of the farm. Attempts were made to mate the experimental males to a similar number of females from each experimental group, but they were also used for mating of non-experimental females. Similarly, the females were primarily exposed to experimental males, but some were mated to non-experimental males. The females were tried for remating on the day following the first mating. Rematings were in some cases performed with other males than the one which made the first mating.

The experimental feeding started in the 2nd week of March. The control group was fed the standard ration of the farm, consisting of rumen, cattle liver, muscular meat of cattle, cod offal, filleted scrap of Baltic herring, cattle skulls, cereals (wheat, barley, oats) and vitamin mixture. The experimental group was fed the same diet with added EPO (Efamol Ltd, Guildford, Surrey, UK; 5 ml equal to 4.5 g per animal and day) and zinc sulphate (2.5 mg per animal and day). The feed was prepared daily. For the males the experimental feeding was terminated by the end of the mating season. The females were fed the experimental diet until early July when all females had whelped.

An addition of 10 mg of vitamin E per 500 mg EPO oil was made. The oil was stored in plastic ampules containing the daily dose per animal. Until used the ampules were stored deep frozen. A solution of zinc sulphate was prepared, of which a dosage of 100 ml per 10 kg diet was supposed to supply the planned 2.5 mg per animal and day.

Samples of the diets were taken twice for chemical analyses. The samples were analysed regarding contents of dry matter (DM), ash, crude protein (CP) and fat. Carbohydrate (CH) was calculated by difference. Fat was determined according to the CEC method (*Anon.* 1971). The content of metabolizable energy (ME) and its percentage distribution on CP, fat and CH was calculated (Table 1).

Data were recorded as follows: at mating, date and mating partner were recorded. Date of parturition was recorded and as soon as possible after parturition the number of live and stillborn kits was recorded. The number of kits lost during the first part of lactation was also recorded.

Blood sampling was carried out at the start, during the course of the experiment and when the experiment was terminated. In the treatment group 4 males and 4 females were sampled and in the control group, similarly, 3 males and 3 females. Sampling was made to determine zinc status during the course of the experiment. Zinc was analysed according to methods described by *Sprague & Slavin* (1965), *Dawson et al.* (1968) and *Parker et al.* (1967). Health status of the animals was controlled by recording the following blood parameters: hematocrit, hemoglobin, leucocytes, S-ALT, and creatinine.

At pelting, the uteri of some experimental females were examined for implantation sites.

Statistical analyses were carried out according to the GLM procedure of SAS (*SAS Institute Inc.* 1982) The treatment effects were evaluated according to model (1):

$$Y_{ijkl} = \mu + a_i + b_j + c_k + (ab)_{ij} + (ac)_{ik} + (bc)_{jk} + \varepsilon_{ijkl} \quad (1)$$

Data where only one, or two mating partners with the same experimental treatment were used, were analysed according to model (2):

$$Y_{ijk} = \mu + a_i + b_j + (ab)_{ij} + \varepsilon_{ijk} \quad (2)$$

where

Table 1. Results of chemical analysis of the blue fox diets.

	Mating period		Lactation period	
	1 Control	2 EPO	1 Control	2 EPO
Dry matter %	32.3	31.2	24.7	36.9
Ash %	5.0	3.7	2.5	3.4
Crude protein %	16.6	16.4	11.0	16.1
Fat %	5.4	5.8	5.8	10.0
Carbohydrate %	5.3	5.2	5.4	7.3
Calculated content of ME				
MJ/kg[a]	5.23	5.34	4.49	7.05
MJ/kg DM	16.20	17.13	18.17	19.12
Percentage distribution of ME on				
Protein	51	49	40	37
Fat	36	38	45	50
Carbohydrate	13	13	15	13

[a] Calculated as described by *Tauson & Aldén* (1984).
MJ = Megajoule.
ME = Metabolizable energy.
DM = Dry matter.

$Y_{i...}$ = the i . . . th observation
μ = general mean
a_i = fixed effect of group
b_j = fixed effect of treatment of mating partner; for females treatment of male used for first mating; for males treatment of females (1; 2)
c_k = fixed effect of treatment of males used for 2nd mating (1)
$(ab)_{ij}$ = interaction effect between group and treatment of mating partner (1;2)
$(ac)_{ik}$ = interaction effect between group and treatment of males used for 2nd mating (1)
$(bc)_{jk}$ = interaction effect between mating partner and males used for 2nd mating (1)
$\varepsilon_{i...}$ = random error
The results reported are given as LS-means.

Results

The experimental diet was readily consumed by the animals, and no negative dietetic effects were recorded. Results from the chemical analyses (Table 1) showed that the protein content in the mating season diet was high and that the increase in fat content due to the addition of EPO was limited. In the lactation period the protein content was lower and the fat content higher than in the mating season diets. The EPO addition resulted in an increased fat level in the experimental diet in relation to the control diet.
The results of the blood samplings indicated that the health status of the animals was normal during the experiment and that the zinc status was adequate and not conclusively affected by the zinc supplementation in the EPO group.

Male treatment effects

Analysing the results according to model (1), showed few significant effects, but there was an increased frequency of aborting females and a tendency for increased litter sizes after mating to EPO-treated males. The analysis according to model (2) also indicated that the frequency of barren females was increased (Table 2). Also female treatment effects are recorded in Table 2. The increased rate of barren and aborting females after males in

Table 2. Reproductive results as affected by male treatment with dietary addition with evening primrose oil (EPO).

	Crude results		Male group			
			Control		EPO	
	Control	EPO	Control ♀	EPO ♀	Control ♀	EPO ♀
Independent of treatment of males used for 2nd matings						
Barren females %	11	13	17	4	13	12
Aborting females %	8	21[a]	3	12	7	35
Kits per mated female	6.2	6.1	6.8	5.7	7.1	5.0
Kits per litter	7.6	9.6	8.5	6.7	8.9	10.3
Males used for 2nd matings treated similar to the experimental males						
Barren females %	2	23	4	0	23	22
Aborting females %	2	9	4	0	0	18
Kits per mated female	8.1	6.1	8.1	8.2	6.5	5.8
Kits per litter	8.5	9.4	8.8	8.2	8.4	10.4

[a] Significant effect of group ($p = 0.02$*) and female treatment ($p < 0.001$***).

the EPO group was mainly recorded among EPO-treated females.

Female treatment effects

Four females in each group remained unmated and in the EPO group 1 female died before parturition.

Results analysed according to models (1) and (2) are reported in Table 3. There was a tendency for decreased rate of barren females, but an increased frequency of aborting females in the EPO group was indicated, resulting in a similar number of females not producing litters in both groups. Litter size at 1st control was not positively affected but there was a tendency for increased litter sizes in the treatment group at the 2nd control. Male treatment effects (Table 3) were somewhat contradictory, but in the treatment group there was a tendency for EPO-treated males to give an increased rate of aborting females and increased litter sizes as compared with control males.

The examination of the uteri of experimental females for implantation sites revealed that of 3 examined barren females in the EPO group, all had implanted foetuses.

Discussion

The main findings in the present investigation were the tendencies for increased litter size and the increased frequency of aborting females and reduced frequency of barrenness as effects of EPO treatment. Since the most pronounced effects were found when both male and female belonged to the treatment groups, physiological effects on both sexes are indicated. As reported in the literature (*Fougner* 1972, *Einarsson* 1982), about 55–65 % of barren blue fox females had

Table 3. Reproductive results as affected by female treatment with dietary addition with evening primrose oil (EPO).

	Crude results		Female group			
			Control		EPO	
	Control	EPO	Control ♂	EPO ♂	Control ♂	EPO ♂
Independent of treatment of males for 2nd matings						
Barren females %	14	0	28	0	0	1
Aborting females %	9	28	6	13	36	19
Kits per mated female						
At first control	7.1	5.6	5.0	9.3	4.8	6.4
At second control	4.9	4.8	3.9	5.9	4.8	4.9
Kits per litter						
At first control	9.0	8.0	7.3	10.8	7.8	8.3
At second control	6.3	7.9	5.6	7.0	9.2	6.5
Males used for 2nd matings treated similar to males used for first matings						
Barren females %	22	6	27	17	13	0
Aborting females %	0	13	0	0	0	25
Kits per mated female						
At first control	6.1	6.5	5.7	6.5	5.9	7.1
At second control	3.4	4.8	3.5	3.2	5.5	4.1
Kits per litter						
At first control	7.8	8.1	7.9	7.8	6.7	9.5
At second control	4.3	5.9	4.9	3.8	6.3	5.5

implanted foetuses and therefore it is likely that the major part of the females recorded as barren in the present investigation had resorbed their foetuses. This teory is supported by the results of the examination of the uteri of some barren females. The increased rate of recorded abortions in the EPO-treated animals may suggest that pregnancy was maintained for a longer period, which is likely, because the barren frequency simultaneously decreased.

The tendency for increased litter sizes was mainly found as a male treatment effect. This may indicate an effect on sperm metabolism or sperm penetration of the EPO treatment.

In the present investigation the results may have been affected by more than a single factor since the EPO-contained vitamin E and EPO-treated animals were also zinc sulphate-supplemented. The zinc supplementation was done to ensure an appropriate zinc status of the animals because a moderate zinc deficiency may result in foetal mortality (*Cunnane* 1982). From the analyses of zinc in blood samples from the experimental animals it was evident that zinc supply was not limiting and zinc status was not affected by supplementation. Thus, it is not likely that the zinc supplementation affected the reproductive results. Vitamin E was added mainly as a biological antioxidant of the EPO. The vitamin E requirement of the fox has not been established. The dose level, however, by far exceeded the requirement of the mink (0.16 mg/100 kJ ME; *NRC* 1982) and the diet was relatively low in poly-unsaturated fatty acids (PUFA). A vitamin E supplementation of 0.6 mg vitamin E per g PUFA is recommended (*Harris & Embree* 1963) but the levels in the EPO group are far above this. Moreover, the diet was supplemented with vitamins and therefore the supply of vitamin E was adequate also in the control group. Thus, it is not likely that the vitamin E supplementation of the EPO group affected the reproductive results.

The results of the present investigation indicate that dietary supplementation with EPO to blue foxes in the reproductive season may improve reproductive results, but the effects of the dose level and the length of the treatment period ought to be further evaluated in order to reduce the frequency of abortions and increase the number of successful pregnancies.

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Sammanfattning

Inverkan av jättenattljusolja som fodertillsats på reproduktionsresultat hos blåräv.

Inverkan av tillsats av jättenattljusolja till en foderblandning för blåräv på reproduktionsresultatet har utvärderats i en undersökning med 2 grupper om vardera 12 hanar och 25 honor. Försöket utfördes i fält, och försöksperioden sträckte sig från den 10. mars till parningssäsongens slut (hanarna) resp. början av juli (honorna). Kontrollgruppen utfodrades med farmens normalfoderblandning och försöksgruppen fick samma foderblandning men supplementerad med 4,5 g jättenattljusolja och 2,5 mg zinksulfat per djur och dag. Till jättenattljusoljan hade satts 10 mg vitamin E per 500 mg. Resultaten utvärderades avseende effekter av hanarnas och honornas försöksbehandling. I den supplementerade gruppen erhölls en ökad abortfrekvens, men samtidigt sänktes andelen gallhonor, vilket resulterade i en likartad frekvens honor utan kull i bägge grupperna. En tendens till ökad kullstorlek erhölls som huvudsaklig effekt av supplementering av hanarna, vilket kunde tyda på en inverkan på spermakvaliteten.

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Reprints may be requested from: Anne-Helene Tauson, Department of Animal Science and Animal Health, Royal Veterinary and Agricultural University, Bülowsvej 13, DK-1870 Frederiksberg C, Denmark.