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OVARIAN AND TESTICULAR FUNCTION IN THE BLUE FOX (ALOPEX LAGOPUS) AFTER ORAL ADMINISTRATION OF FENCHLORPHOS DURING THE BREEDING SEASON

By

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BERGE, G. N., M. MONDAIN-MONVAL, A. SMITH and O. M. MØLLER: *Ovarian and testicular function in the blue fox (Alopex lagopus) after oral administration of fenchlorphos during the breeding season.* Acta vet. scand. 1983, *24*, 200—210. — The possible effect of fenchlorphos, 0-0-dimethyl-0-(2.4.5-trichlorophenyl) phosphorothioate, upon the reproductive endocrinology in blue foxes (Alopex lagopus) was investigated. Five females were administered fenchlorphos orally at a dose of 100 mg/kg daily from 10 days before oestrus and up to the 21st day of gestation. This dose represents the therapeutic dose for the treatment of sarcoptic mange. Blood samples were collected for the analyses of progesterone, oestradiol-17β and luteinizing hormone (LH) in plasma. The vixens were ovario-hysterectomized on day 23, except 1 animal in the control group which was operated on day 17. Additionally, sperm quality and mating performance in 3 male blue foxes, which were administered 100 mg/kg fenchlorphos daily during the first 3 weeks of the mating season, were examined.

Pregnancy was recorded in 2 medicated and 4 control animals. No pathological changes were observed in the uterus and the ovaries. The plasma concentrations of the hormones were similar to those obtained from the control group. No evidence of any disturbances concerning spermatogenesis in the males was observed. However, their libido appeared to be reduced. None of the males achieved a mating during and after the period of medication.

reproduction; blue fox; fenchlorphos.

Sarcoptes canis infestation in the blue fox and silver fox represents a practical problem in Scandinavia with respect to treatment, because the animals are kept in outdoor cages which make dipping and spraying hazardous during the winter time.

Fenchlorphos, 0,0-dimethyl-0-(2.4.5-trichlorophenyl) phospho-

rothioate is an organophosphorus compound which has been shown to be highly effective as a systemic insecticide for the treatment of sarcoptic mange. Oral administration of this compound was found to be effective at a dose of 100 mg/kg for 3—5 weeks (*Berge* 1980). *Søli et al.* (1977) found that 100 mg/kg fenchlorphos was well tolerated by healthy foxes as measured by the level of acetylcholinesterase inhibition. Oral administration of 100 mg fenchlorphos/kg to pregnant foxes, however, at different stages of gestation revealed that the compound was potentially embryotoxic and teratogenic (*Berge & Nafstad* 1983). Of 19 medicated vixens the mean number of live whelps at term was 1.2 per vixens versus 9.5 in the control group. Different types of malformations like cleft palate, hydrocephalus internus and externus, cerebellar hypoplasia and congenital alopecia were observed. The mechanisms causing the embryotoxic and teratogenic effect could not be determined from the experimental data, but some preliminary analyses indicated that hormonal disturbances could be involved.

The purpose of the present study was to investigate the possible effect of fenchlorphos upon reproductive endocrinology. Congenital malformations have been reported in rats after ovariectomy and maintenance of pregnancy by low doses of progesterone (*Carpent* 1962). *Poulson et al.* (1965) observed abnormalities in foetal development in association with insufficient doses of progesterone when administered to ovariectomized mice during pregnancy.

In addition to the examination of female reproductive hormones, it was also considered to be of interest to examine whether fenchlorphos affected sperm quality and mating performance in males.

MATERIALS AND METHODS

Chemical

The organophosphorus insecticide fenchlorphos, 0.0-dimethyl-0-(2.4.5-trichlorophenyl) phosphorothioate, was administered as Ectoral® tablets (250 mg) produced by Pitman-Moore Inc., USA.

Animals and housing

The blue foxes (Alopex lagopus) used in the medicated groups consisted of 5 females and 3 males aged from 1—5 years. The non-medicated group was composed of 5 parous females

aged 2—5 years. All the animals were housed individually in outdoor cages before and during the investigation at the State Research Farm for Furbearing Animals, Heggedal. They were given standard Norwegian wet feed and water ad libitum.

The vixens were mated twice on the 2nd and the 4th day of sexual receptivity. The last day of mating was considered as day 0 of gestation.

Experimental design

Group I consisted of medicated females and Group II of medicated males. Each fox in the medicated groups was fed fenchlorphos at a dose of 100 mg/kg. The dose represents the therapeutic dose for the treatment of sarcoptic mange. The tablets were pulverized with an electric homogenizer, weighed and divided into daily portions which were then mixed with the individual wet feed ration before administraton. Indvidual food consumption was checked after 1 h. The animals' general condition was checked daily during gestation.

The administration of fenchlorphos in Group I started when the pro-oestrus vulval swelling was judged to correspond to the stage approximately 10 days before oestrus. Medication proceeded during oestrus and up to the 21st day of gestation.

The 3 male blue foxes in Group II were administered 100 mg/kg fenchlorphos daily during the first 3 weeks of the mating season, from 9th to 30th of March.

Blood samples

Blood samples were collected from the cephalic vein into heparinized tubes twice weekly from 3 of the medicated vixens and 3 animals from the control group. The samples were collected from about beginning of oestrus to the day of ovariohysterectomizing. They were taken at the same time of the day to attempt to eliminate the possible effect of diurnal variations. After centrifugation the plasma was frozen at —20°C until analysis. The complete series of plasma samples were collected before the assays were performed.

Analyses

The following hormones were analysed: Oestradiol-17 β, progesterone and luteinizing hormone (LH). The hormone levels were measured by radioimmunoassay.

Oestradiol-17 β and progesterone concentrations were determined from the same plasma sample using the methods of extraction and radioimmunoassay reported by *Mondain-Monval et al.* (1977) with the modification of *Møller et al.* (to be published). All the samples collected from a single animal were assayed within a single assay. High values were re-assayed in dilution with the intra-assay and inter-assay coefficients of variation listed in Table 1.

Table 1. Specification of hormonal analytical methods.

	Coefficients of variation		Limits of
	intra-assay	inter-assay	sensitivity
Oestradiol-17-β	7.8 % (n = 10)	11.8 % (n = 10)	9 pg/ml
Progesterone	9.9 % (n = 8)	13.4 % (n = 8)	300 pg/ml

The plasma samples were analysed for LH content using a modification of the double-antibody radioimmunoassay of *Nett et al.* (1975) described and validated for the red fox by *Mondain-Monval et al.* (1981) and for the blue fox by *Møllller et al.* (to be published). The inter-assay and intra-assay coefficients of variation were 10.3 % (n = 15) and 7.3 % (n = 60) at a level of 1.6 and 1.8 ng/ml respectively. Minimum assay sensitivity was 0.4 ng/ml.

Ovario-hysterectomy

All the vixens were ovario-hysterectomized on day 23 of gestation, except 1 animal in the control group which was operated on day 17. Ovario-hysterectomy was performed through a midline incision under xylazine (Rompun®) anaesthesia. The uterus and ovaries were removed for examination.

Clinical examination of the males

The reproductive performance of the males was evaluated by observing the mating performance when placed with several females in heat on repeated occasions during and after the medication period. Semen samples were examined by light microscopy 4 days after medication was terminated.

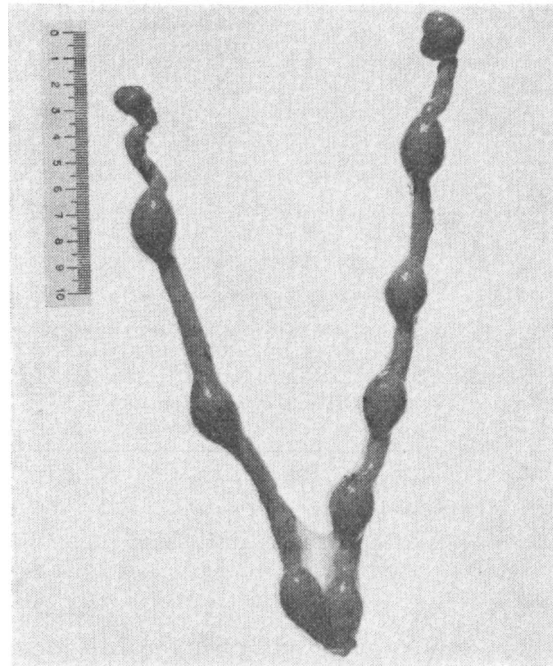

Figure 1. Pregnant uterus and ovaries in a blue fox vixen ovariohysterctomized on day 23 of gestation. The fox had been administered fenchlorphos (100 mg/kg) daily during the last 9 days before mating and up to the 21st day of gestation. No pathological changes were observed. The hormone profiles of this vixen are shown in Fig. 2b.

RESULTS

Females

Pregnancy was recorded in 2 medicated and 4 control animals. No pathological changes were observed in the uterus or the ovaries (Fig. 1). The embryos were alive at the time of operation. The number and average weight of embryos were about similar in the medicated group and the control animals. No implantation sites were recorded in the empty vixens.

The plasma concentrations of progesterone, oestradiol-17 β and luteinizing hormone in plasma are presented in Figs. 2 and 3. The hormone profiles obtained from the animals in the medicated group were similar to those obtained from the control group. The hormone profiles in 1 empty vixen were similar to those observed in the corresponding pregnant vixens.

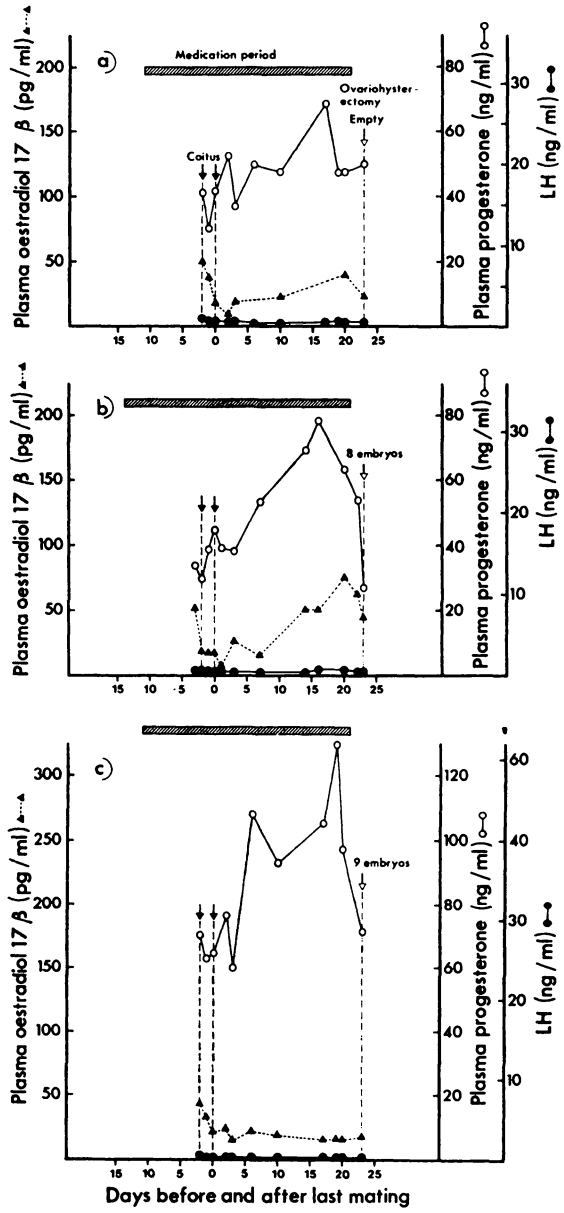

Figure 2. Plasma concentrations of progesterone, oestradiol-17β and luteinizing hormone (LH) in 3 blue foxes which were administered fenchlorphos (100 mg/kg) orally 9 or 12 days before mating and up to the 21st day of gestation.

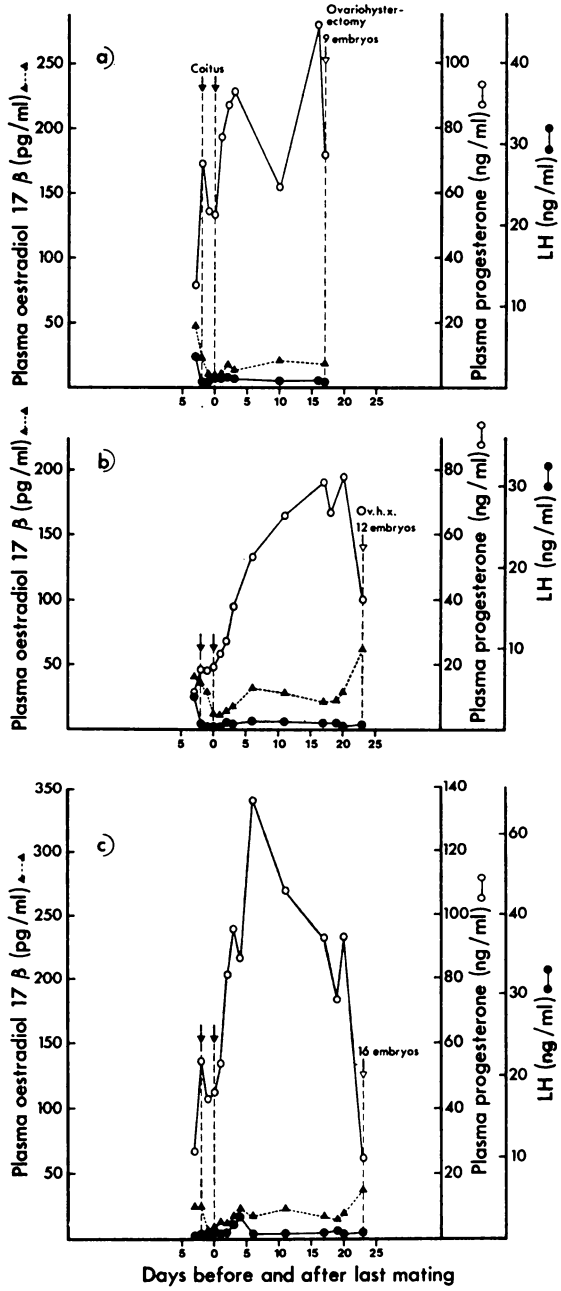

Figure 3. Plasma concentrations of progesterone, oestradiol-17β and luteinizing hormone (LH) in 3 non-medicated blue foxes from around oestrus and up to day 23 of gestation.

In all the vixens the concentration of plasma progesterone increased during oestrus. On the 2nd and 4th day of oestrus, i.e. when the vixens were mated, the plasma progesterone concentrations were about 50 ng/ml (range 41—64 ng/ml) in the experimental animals and about 40 ng/ml (range 19—53 ng/ml) in the controls. Plasma oestradiol-17β and luteinizing hormone levels remained relatively constant throughout the study period, basal levels of oestradiol-17β ranging between 9 and 75.9 pg/ml, and the basal levels of luteinizing hormone from 0.4 to 1.0 ng/ml in the medicated vixens. The corresponding levels in the controls were 7.0—63.0 pg/ml and 0.4—4.4 ng/ml.

Males

The medicated males were examined clinically 4 days after the period of medication. Palpation of the testicles, epididymis and other external genitalia failed to reveal any abnormalities. All the males gave semen samples after masturbation. The sperm fractions were examinated with the following results:

Motility (normal progressive motion)	> 70 %
Colourless sperm (eosin/nigrosin)	> 80 %
Morphology (normal sperm)	> 90 %

There was therefore no evidence of any disturbances concerning spermatogenesis in these animals. Their libido, however, appeared to be reduced. Despite repeated attempts with different vixens throughout the mating season, none of the males achieved a mating.

DISCUSSION

Of 5 ovario-hysterectomized foxes in the medicated group only 2 were pregnant compared to 4 in the control group. The low pregnancy rate may be due to the embryotoxic effect of fenchlorphos suggested by *Berge & Nafstad* (1983). When given prior to mating and during early pregnancy (i.e. about the same period as in the present study), these authors suggested that fenchlorphos might interfere with the process of fertilization. However it must be emphasized that poor breeding results may be caused by several factors.

The plasma progesterone levels in the medicated foxes were similar to the control levels and corresponded well with those

reported in earlier investigations (*Møller* 1973, *Møller et al.* to be published). No significant differences were observed in the mean peak level of progesterone between 1 none pregnant vixen and the pregnant vixens. This is in agreement with the suggestion by *Møller* (1973) that the factors controlling the release of progesterone during pregnancy do not originate from the conceptus.

There was no evidence that the profiles and plasma concentrations of oestradiol-17β or luteinizing hormone were influenced by medication. In the present study the values of luteinizing hormone in 2 of the control animals were higher than those recorded in the medicated vixens 3 days before oestrus. These values appear to be a part of the pre-ovulatory peak which normally occur 1—2 days before oestrus (*Møller et al.* to be published). The present results gave no evidence that the embryotoxic and teratogenic effects of fenchlorphos in the blue foxes are caused by hormonal disturbances.

The lack of libido registered in the medicated male foxes may have been due to the medication. The apparent loss of libido was not associated with abnormal spermatogenesis, since no indication of spermatogenetic disturbances was observed. Furthermore the number of experimental animals was too low to allow definite conclusions to be drawn. In an investigation on mating performance in blue foxes *Aamdal & Fougner* (1973) recorded that 269 of 1720 male foxes (15.6 %) were unwilling to mate.

Little information is available on the effect of organophosphorus compounds on male reproduction. *Dixon* (1980), however, listed organophosphorus compounds among chemicals causing disturbances in male reproduction. Further investigation into the possible effect of fenchlorphos upon male reproduction, including measurement of plasma androgen levels, is required.

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SAMMENDRAG

Ovarie- og testikkelfunksjon hos blårev (Alopex lagopus) etter oral administrering av fenklorfos i avlssesongen.

Fenklorfos, 0-0-dimetyl-0-(2.4.5-triklorofenyl) fosforotioat, 100 mg/kg/dag, ble administrert oralt til fem blårevtisper fra 10 dager før østrus til 21. drektighetsdag for å undersøke den eventuelle effekten av komponenten på det endokrine reproduksjonssystemet. Doseringen som ble valgt representerer den terapeutiske dose ved behandling av Sarcoptes skabb hos blårev. Blodprøver ble tatt til analyser av progesteron, østradiol-17β og luteiniserende hormon (LH) i plasma. Tispene ble ovariehysterektomert på dag 23 i drektigheten, unntatt ett dyr i kontrollgruppa som ble operert på dag 17. I tillegg ble sædkvaliteten og parringsvilligheten hos tre hanrever, som ble gitt fenklorfos 100 mg/kg daglig i de tre første ukene av parringssesongen, undersøkt.

Drektighet ble observert hos 2 forsøksdyr og 4 kontrolldyr. Ingen patologiske forandringer ble registrert i uterus og ovarier. Konsentrasjonen av hormoner i plasma viste et forholdsvis likt mønster mellom forsøksgruppe og kontrollgruppe. Det forelå ingen holdepunkter for å anta en forstyrrelse av spermatogenesen hos hanrevene. Imidlertid syntes revenes libido å være nedsatt. Ingen av hannene var i stand til å utføre parring under og etter medikamenteringsperioden.

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