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OVARIAN ACTIVITY DURING THE ANOESTRUS AND THE REPRODUCTIVE SEASON OF THE RED FOX (*VULPES VULPES* L.)

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SUMMARY

The ovarian activity of a species with seasonal monoestrous reproduction has been studied for two consecutive years. Several activity periods have been detected throughout the year: These are characterized by an increase in the peripheral plasma E_2 concentration ($\bar{x}$ = 254 pg/ml) and simultaneously by a thickening of the vaginal epithelium and an electrical activity of the myometrium, particularly during the rising phase of the E_2 peak. The increase in E_1 concentration was significant only during the reproductive season which occurs in March or April. Luteal activity was present during this period alone; the P concentration can reach 65 ng/ml. During the other oestrogen secretion periods which occur during anoestrus the P concentration remains below 3 ng/ml.

INTRODUCTION

The fox is a monoestrous species with only one reproductive season per year. According to several authors [1–4] its reproductive cycle is as follows: In mature females, the proestrus and oestrus take place between the beginning of January and mid-February. After a preovulatory period of 15 days, follows a sexual receptivity period of 2–4 days. Ovulation occurs spontaneously. Gestation lasts 51–52 days and ends in March. Lactation continues for 8–10 weeks. In females that have whelped, the corpus luteum is maintained during the five months following parturition; this maintenance is due to a slow, pituitary-controlled luteolysis [3, 4]. The anoestrus lasts 9–10 months until the following January. The young vixens born in March become adults in September and have their first oestrus the following January [2]. Their ovaries increase in weight from the end of June until the end of November; this also occurs in adults during the same period [1].

Little information is available on the changes occurring in the ovarian secretion in these monoestrous species, particularly during anoestrus. To our knowledge, nothing has been published on this in red foxes. The studies carried out on other wild monoestrous mammals, skunk [5], mink [6], and blue fox [7], are mainly concerned with pregnancy. However, in badgers, the histological examination of the vaginal epithelium and of the ovary [8], and the study of the uterine motility [9] have shown that the endocrine balance varied during the 10 months of luteal asthenia.

In order to investigate the ovarian activity in vixens, the pattern of oestrogen and progesterone concentrations was determined in peripheral plasma during two consecutive years at various intervals of time and was correlated to the myometrial activity and to the morphology of the vaginal epithelium.

MATERIALS AND METHODS

A. *Animals*

Vixens captured at the age of 3 months, were reared in the laboratory. They were 8–18 months old at the start of the experiment. Blood samples were always collected at the same time of the day for a given animal (09.00–11.00 or 14.00–16.00 h), every two weeks from September 1972 to October 1973 (Re 105, 109, 110, 118, 152, 163, 165, 237, 273), each week from November 1973 to October 1974 (Re 118, 123, 237, 311, 327, 328, 361), twice a week during the presumed reproductive season in March and April (Re 118, 123, 237, 327, 328, 361).

After ether anaesthesia, the blood samples were drawn from the radial vein into heparinized syringes and centrifuged immediately. The plasma was then frozen and stored at −20°C until analysed.

When an electrohysterography was performed, the animals were anaesthetized with intraperitoneal sodium pentobarbital. 67 Recordings were obtained from 14 vixens; and 117 biopsies were obtained from 15 vixens.

B. *Radioimmunoassays*

The estrone (E_1) and estradiol (E_2) concentrations were determined using a modification of the technique described by Castanier and Scholler[10].

Plasma samples (0.1 to 2 ml) were extracted twice with diethylether (5 and 4 ml). The pooled ether

extracts were evaporated to dryness and the residue dissolved in 120 μl of a benzene–ethanol mixture (85:15, v/v). The samples were transferred onto Sephadex LH 20 microcolumns and eluted with 3.0 ml of the same benzene–ethanol solution; E_1 was eluted in the 1.0–1.7 ml fraction and E_2 in the 1.9–3.0 ml fraction; 200 μl aliquots, in duplicate, of each of these fractions were then radioimmunoassayed.

The antiserum used was obtained by J. Adeline in this laboratory. It was raised in a rabbit immunized with estrone -17-(O-carboxymethyl)-oxime-BSA and was diluted in a sodium phosphate buffer containing 0.2% gelatin. The final dilution was 1:6000 for the E_1 assay and 1:8000 for the E_2 assay. It shows cross-reactions of 66% with E_2-17β, of 11% with E_2-17α, of 8% with estriol and below 0.1% with corticosterone, progesterone, testosterone, 5α-dihydrotestosterone and 16α-hydroxyestrone. When this antiserum was used against E_2-17β, the cross-reaction with E_2-17α was 28%. For this reason, the data are expressed as estradiol without designation of the orientation of the 17-hydroxyl group.

Without correction for losses which occur during the different phases of the assay the overall recovery was 89.3 ± 4.0 (S.D.) %, $n = 20$ for E_1 and 82.3 ± 3.2 (S.D.) %, $n = 20$ for E_2. The reproducibility of the standard curves is shown in Fig. 1. The detection limit was 6 pg/ml for E_1 and 5 pg/ml for E_2. The intra-assay coefficient of variation estimated for an E_1 concentration of 39.8 pg/ml and an E_2 concentration of 21.4 pg/ml was 9.4% and 8.2% respectively. The inter-assay precision was 10.7% and 10.0% at E_1 and E_2 level below 80 pg/ml.

Progesterone (P) was measured according to Tea *et al.*[11]. The antiserum, prepared by J. Adeline in this laboratory, was raised in rabbits immunized with progesterone -11α-hemisuccinate-BSA. The specificity of the method is ensured by chromatography on Sephadex LH 20. Among several steroids, 5α-dihydroprogesterone can alone interfere with progesterone since this steroid behaves in the same way as progesterone in the chromatographic system used and has a cross-reaction percentage of 13% with this antiserum. Reproducibility of the standard curve is shown in Fig. 1. Recovery is of 89.4 ± 5.9%, if no correction for losses is introduced. The detection limit was 0.10 ng/ml. The interassay precision was 11.2%. The variability of the values in the present study is given as a standard deviation (S.D.); n is the number of values.

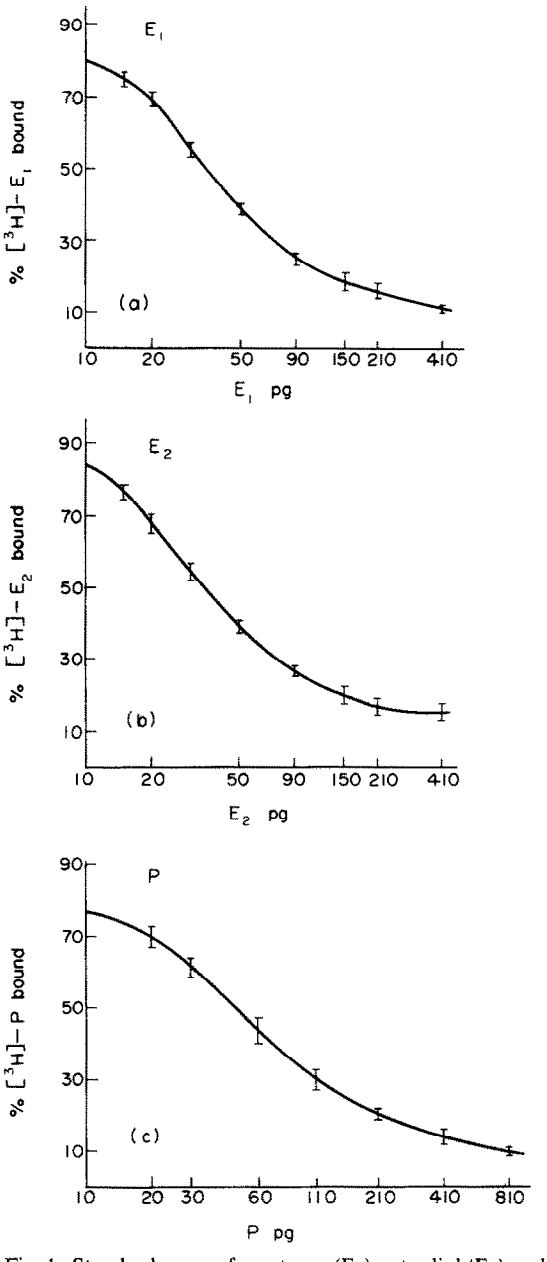

Fig. 1. Standard curves for estrone (E_1), estradiol (E_2) and progesterone (P) measurements. The first point corresponds to the mass of tritium-labelled steroid alone; the other points correspond to the total mass present in each assay tube. Each point of the curve is a mean value obtained from ten routine standard curves ($\bar{x}$ ± S.D.).

C. *Electrohysterography and histology of the vaginal epithelium*

The uterus and vagina undergo variations of structure and function depending on the cyclic activity of the ovary [12–13]. The changes in uterine motility were determined by electrohysterography. The electrical activity of the myometrium was picked up by bipolar macro-electrodes constituted by enameled copper wires of 0.1 mm diameter, stipped at their extremity. These electrodes were implanted in the smooth uterine muscle after laparatomy and connected to an amplifier with resistance-capacity link incorporated to an Alvar ink-jet recorder of EEG type. The potentials thus measured with a 0.1 s time constant correspond to the synchronous activity of several neighbouring cells. To study the modifications of the vaginal epithelium, biopsies were carried out periodically. The slides were stained by Masson's trichrome process.

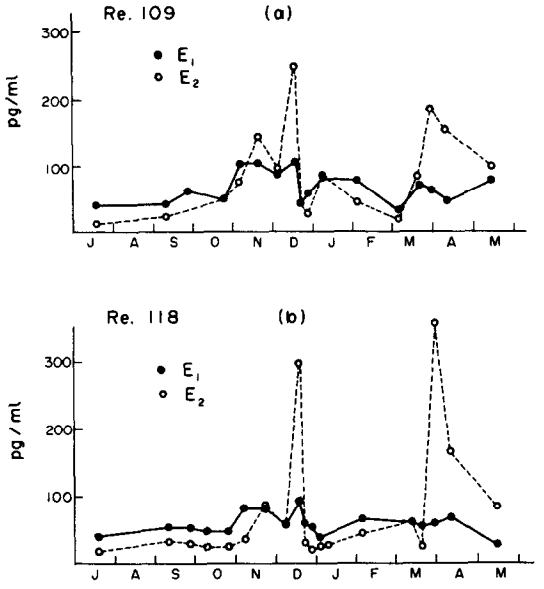

Fig. 2. Plasma E_1 and E_2 concentrations in two red fox vixens from July 1972 to May 1973.

RESULTS

Variations in the plasma oestrogen concentrations

A. *Year 1972–1973.* A preliminary study [14] was carried out between September 1972 and June 1973; it was shown that the E_2 concentration increased in December and in April (Fig. 2).

This study was continued until November 1973. The E_1 and E_2 profiles have been established from the results obtained every two weeks for a whole year: four of these profiles are shown in Figs. 3 and 4. Significant increases in E_2 concentrations were noted four or five times in the year. They occurred at comparable periods from one animal to the other: In November–December, March–April, June, August and October. The individual E_2 peak values ranged from 110 to 462 pg/ml, whereas the lowest values between the peaks were around the detection limit of the method (5 pg/ml). The E_1 concentrations at the time of the E_2 peaks ranged from 20 to 194 pg/ml.

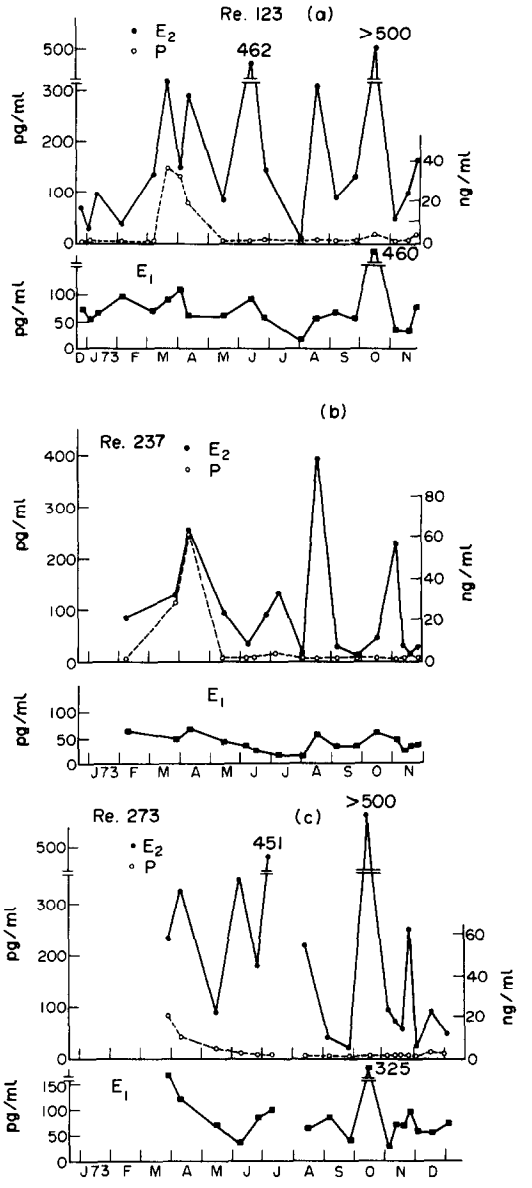

Fig. 4. Concentration of E_1, E_2 and P in peripheral plasma of three red fox vixens: (a) Re. 123, (b) Re. 237, (c) Re. 273. Blood samples were collected every two weeks between December 1972 and December 1973.

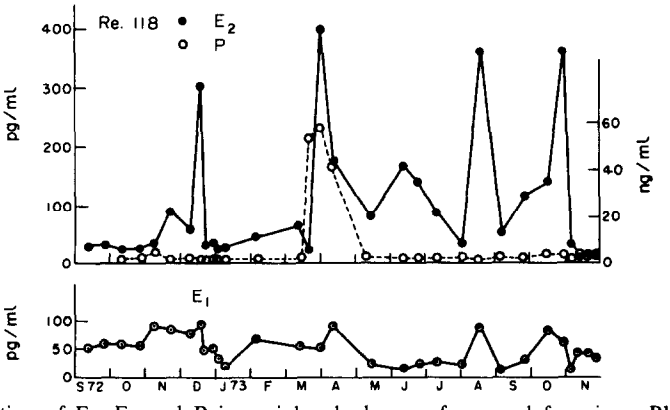

Fig. 3. Concentration of E_1, E_2 and P in peripheral plasma of one red fox vixen. Blood samples were collected every two weeks from September 1972 to December 1973.

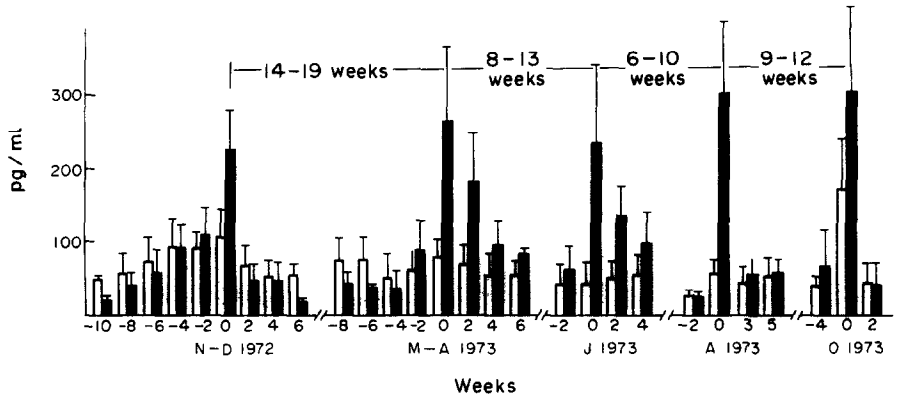

Fig. 5. Composite of mean E_1 and E_2 concentrations ($\bar{x} \pm$ S.D.) normalized to the day of the E_2 peak in peripheral plasma of the red fox vixen throughout the year. E_1: white bar, E_2: black bar. November–December 1972 (N–D), $n = 7$; March–April 1973 (M–A), $n = 8$; June 1973 (J), $n = 8$; August 1973 (A), $n = 6$; October 1973 (O), $n = 4$.

The lowest values were also around the detection limit (6 pg/ml).

In vixens 123 and 279 (Fig. 4), abnormally high values were measured in October 1973, namely 5212 ± 117 and 4121 ± 460 pg/ml ($n = 4$) for E_2 and, 735 ± 99 and 880 ± 109 pg/ml ($n = 4$) for E_1 respectively. These values were not taken into consideration in Fig. 5.

The overall data obtained during 1972–1973 has been summarized in Fig. 5 which shows up clearly this repeated oestrogen release. In this figure, the results obtained for each vixen were normalized to the day of the E_2 peak at each period of the year. The time zero corresponds to the day of the peak. The mean values determined at two week intervals before or after the peak were taken into consideration.

The mean E_2 peak value was 257 ± 116 pg/ml ($n = 33$); there were no significant differences from one peak to another. The dispersion of the results (*C.V.* = 42%) can be partially explained by the low frequency of sampling; under these conditions, the measured values do not always correspond to the real maxima. Nevertheless, these values are significantly different ($P < 0.001$) from the mean E_2 concentration obtained between the peaks from September 1972 to March 1973, namely 44.2 ± 25.3 pg/ml ($n = 77$).

The rhythm of this ovarian release is not constant: the time interval between two maxima varies from 6 to 19 weeks, the longest time interval being between January and March.

From Fig. 5, it can be seen that the same elements are not so evident with E_1. The variations of its concentration are less marked. Its increase does not always coincide with an increase in E_2 concentration and furthermore it was not detected in each of the animals. The mean concentration estimated at the time of the E_2 peak is 93 ± 101 pg/ml ($n = 34$). This value does not differ significantly from those recorded between the E_2 peaks from September 1972 to April 1973, namely 55.6 ± 26.6 pg/ml ($n = 83$).

B. *Year 1973–1974.* The experiment was repeated from November 1973 to October 1974 to see whether the pattern of oestrogen secretion already noted would occur again or whether it was an artefact due to captivity. Blood samples were collected every week from 7 vixens, three of which had taken part in the previous experiment.

Analysis of the results shows that the concentration of E_2 rose significantly at different time intervals but with a greater frequency between September and March. Three examples are given in Fig. 6. This same phenomenon can be noted by comparing the oestrogen pattern of the same animal from one year to the next, as in the case of vixens 118, 123 and 237 (Fig. 7).

A composite picture (Fig. 8) of the mean concentrations of E_2 and E_1, established in the same way as in Fig. 5, but at weekly intervals, shows this episodic secretion. The mean E_2 peak value (251 ± 149.0 pg/ml, $n = 28$) was significantly ($P < 0.001$) higher than the mean E_2 concentration between the peaks from November 1973 to March 1974 (36.1 ± 22.7 pg/ml, $n = 88$). During the same period, the mean concentration of E_1 was 35.2 ± 17.2 pg/ml ($n = 90$); at the time of the E_1 peak it was not significantly different (68.3 ± 45.3 pg/ml, $n = 28$).

As during the previous year, significant increases in E_2 concentrations were noted on several occasions. This finding in a monoestrous species has led us to determine whether a single oestrous existed or whether in captivity, vixens become polyoestrous. With this in view, progesterone levels were determined for the presence of corpus lutea.

Variations in the plasma progesterone concentration

The P concentration was determined every two weeks in six of the vixens included in the 1972–1973 study. Four profiles of P concentration are shown in Figs. 3 and 4. Determinations were also carried out weekly on six vixens of the 1973–1974 study. Five profiles are shown in Fig. 6 and 7. The P concentration reached a maximum ranging from 35 to 65 ng/ml in March or April. It then decreased slowly until May. This release coincides with the oestrogen

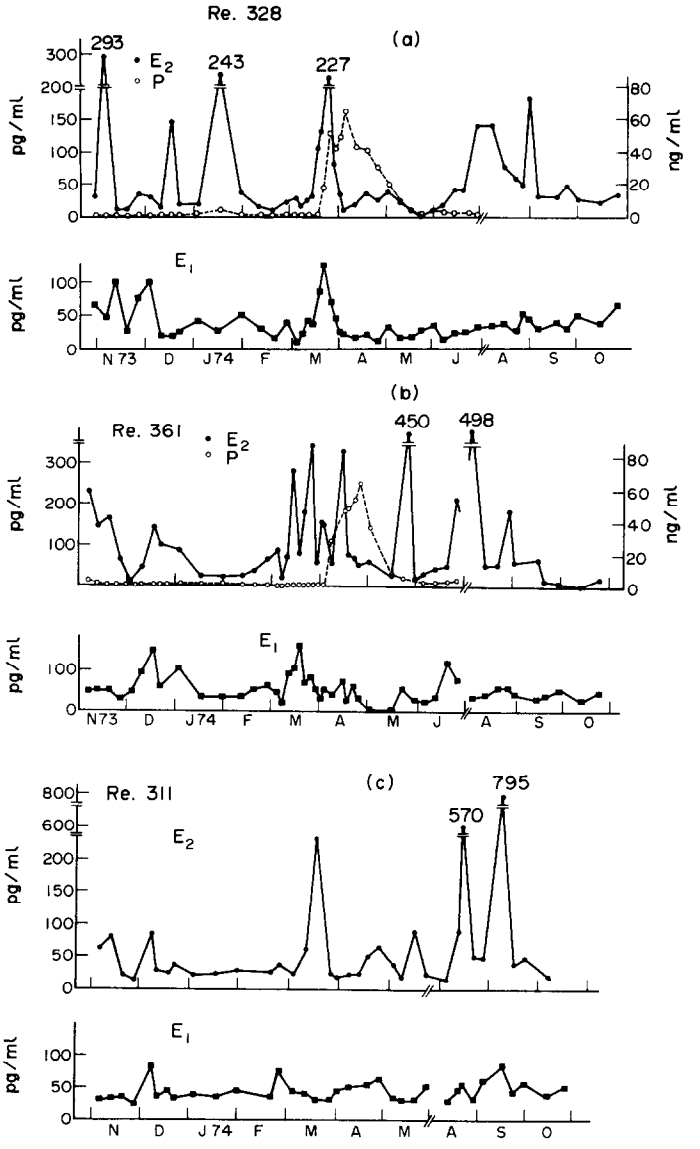

Fig. 6. Concentration of E_1, E_2 and P in peripheral plasma of three red fox vixens: (a) Re. 328, (b) Re. 361, (c) Re. 311. Blood samples were collected every week from November 1973 to November 1974 and twice a week in March and April 1974.

secretion noted during this same period. However, outside this period, the P level remains low, particularly during the other phases of estradiol secretion: It varies between 0.10 and 3 ng/ml; its mean value from August 1973 to March 1974 is 0.98 ± 0.82 ng/ml (n = 126).

These results confirm the existence of a single ovulation per year, but whereas ovulation in wild vixens occurs between the beginning of January and mid-February, in vixens in captivity it occurs between March 15th and April 15th.

Variations in E_2, E_1 and P concentrations during the reproductive season

From the results obtained during the first year, it was possible the second year to study with greater precision the plasma E_1, E_2 and P concentration patterns during the presumed reproductive period. Determinations were carried out twice a week in March and April 1974. Two facts were noted: (1) the E_2 level increased for 14–25 days before reaching its maximum and, (2) one or more successive E_2 peaks could occur (Fig. 6, Re 361).

The mean E_2 peak value was 216.3 ± 49.5 pg/ml, (n = 7); this was significantly different ($P < 0.02$) from the concentration measured three days before and four days later, *viz.* 96.2 ± 52.4 and 61.9 ± 25.2 pg/ml respectively. On the day of the E_2 peak, the E_1 concentration was 109.7 ± 69.6 pg/ml (n = 7). It was significantly different ($P < 0.05$) from those obtained two weeks before and two weeks later, *viz.* 29.0 ± 10.9 and 27.7 ± 8.5 pg/ml (n = 7). The E_1 increase was slower and smaller than that of E_2; it could only be witnessed during this period of the year.

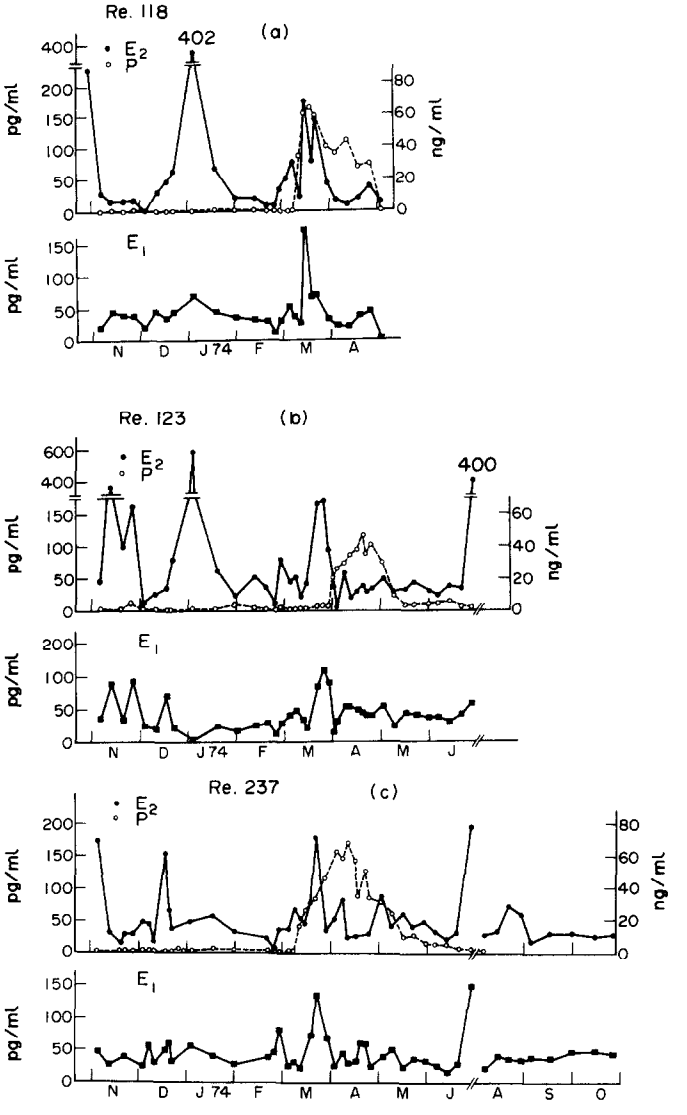

Fig. 7. Concentration of E_1, E_2 and P in peripheral plasma of three red fox vixens: (a) Re. 118, (b) Re. 123, (c) Re. 237. Blood samples were collected every week from November 1973 to November 1974 and twice a week in March and April 1974.

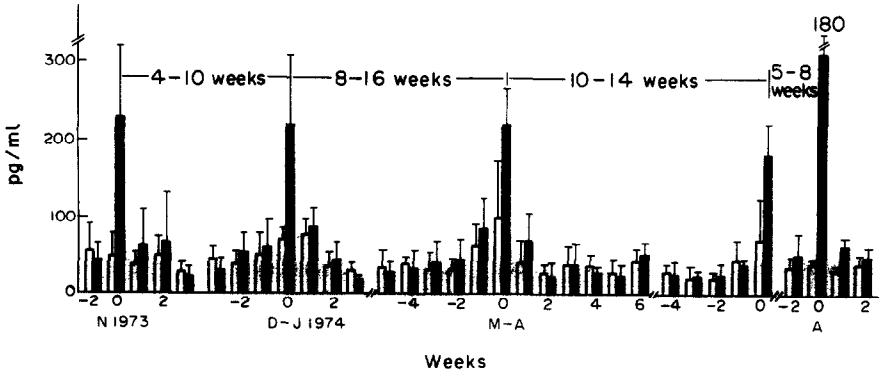

Fig. 8. Composite of mean E_1 and E_2 concentrations normalized to the day of the E_2 peak in peripheral plasma of the red fox vixen throughout the year ($\bar{x} \pm$ S.D.). E_1 = white bar, E_2 = black bar. November 1973 (N), $n = 6$; December–January 1974 (D–J), $n = 7$; March–April 1974 (M–A), $n = 7$; June 1974 (J), $n = 5$; August (A), $n = 3$.

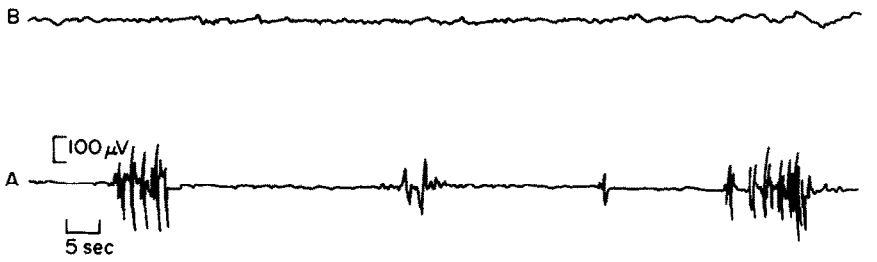

Fig. 9. Electrical activity of the myometrium in a vixen (Re. 118). Tracings were recorded at a paper speed of 5 mm/s. A—on 23-3-1973, intense activity during the rising phase of the E_2 peak; the amplitude of the potential discharges exceed 100 μV with a duration of 5–20 s. B—on 11-4-1973, no activity after the E_2 peak.

The P concentration began to rise before the estradiol level reached its maximum and varied widely (3 to 55 ng/ml) at the time of the E_2 peak; its mean value was 33 ng/ml. However, the E_2 peak concentration was reached 4–24 days before the progesterone peak.

Changes in vaginal mucosa and uterine motility

Period from March to April. When the plasma E_2 concentrations reached 100 pg/ml, and particularly during the rising phase of the E_2 peak, the uterine activity became intense and highly rhythmical; the amplitude of the potential discharges exceeded 200 μV with a duration of 5–20 s and with a frequency of 2–3 per min (Fig. 9A). The vaginal epithelium was very thick, many layered and very frequently keratinized on the surface (Fig. 10).

When the P release had risen to an average value of 30 ng/ml, the physiological criterion of the oestrogen activity disappeared: the vaginal epithelium was thin, two-layered (Fig. 11), the uterine activity was inexistent (Fig. 9B). However, Figs. 6, 7, 8 and 9 show that the E_2 concentrations are then above 100 pg/ml. The reversal of the response of the genital tract was caused by changing in the progesterone:oestrogen ratio [13].

Period from June to September. With the onset of uterine activity, E_2 concentrations were above 100 pg/ml. The vaginal epithelium was generally much thinner than during the preceding period (Fig. 12).

Period from September to March. The electrical activity of the myometrium and the thickening of the vaginal epithelium correspond to an increase in the circulating oestrogen as before. Epithelial proliferation and keratinization were stimulated. In conjunction with the increase in plasma estradiol concentration, the uterine muscle and the vaginal mucosa can show signs of activity during anoestrus as during the reproductive season.

DISCUSSION

In the red fox vixen, several phases of ovarian activity have been detected throughout the year. They

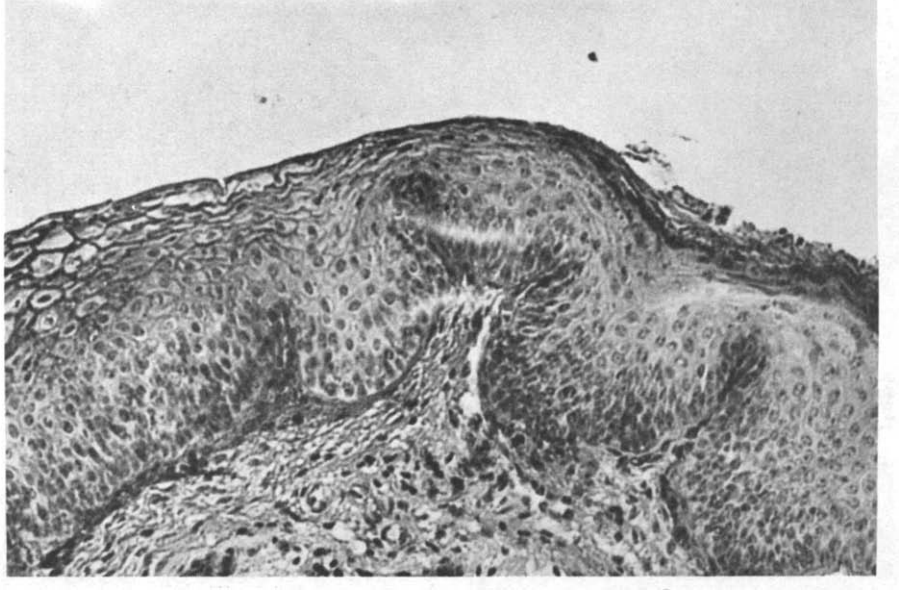

Fig. 10. Vaginal mucosa (Re. 118): on 23-3-1973, thick and keratinized epithelium (×250).

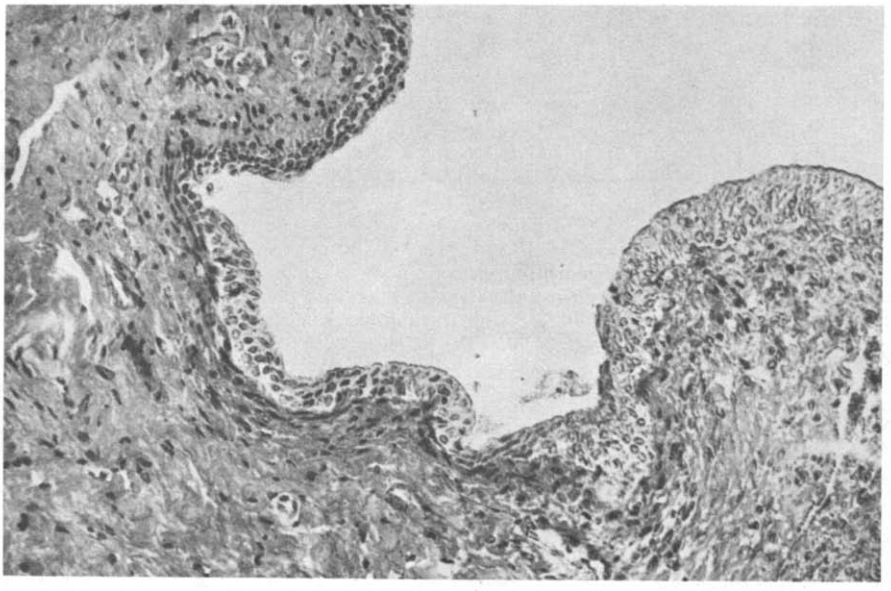

Fig. 11. Vaginal mucosa (Re. 118): on 11-4-73, thin epithelium (×250).

are characterized by an increased concentration of peripheral plasma E_2, by an electrical activity of the myometrium and by a thickening of the vaginal epithelium.

Increase in P concentration which occurs solely in March or April confirms the fact that ovulation takes place only once during the year. Vixens in captivity however, have a delayed oestrus of about two months compared to wild vixens; this concurs with Setton's observations as quoted by Rowlands and Parkes[2]. Ovulation is spontaneous since under our experimental conditions it occurred despite the absence of mating. With the results obtained, it was not possible to determine precisely the length of the proestrus and of the oestrus and the exact date of ovulation. The E_2 peak is not a sufficient criterion and furthermore, the P release is initiated before the level of E_2 has reached its maximum, thus very probably before ovulation. This fact has been reported in the blue fox by Moller[7]: The mean P concentration is about 30 ng/ml the 4th day of oestrus determined by the mating date; this corresponds to the mean level of P measured on the day of the E_2 peak (33 ng/ml) in the present study. Pearson and Enders[15] have shown from histological observations, that luteinization of the granulosa cells starts in the preovulatory follicles before oestrus and ovulation.

From these results, it is difficult at the present time

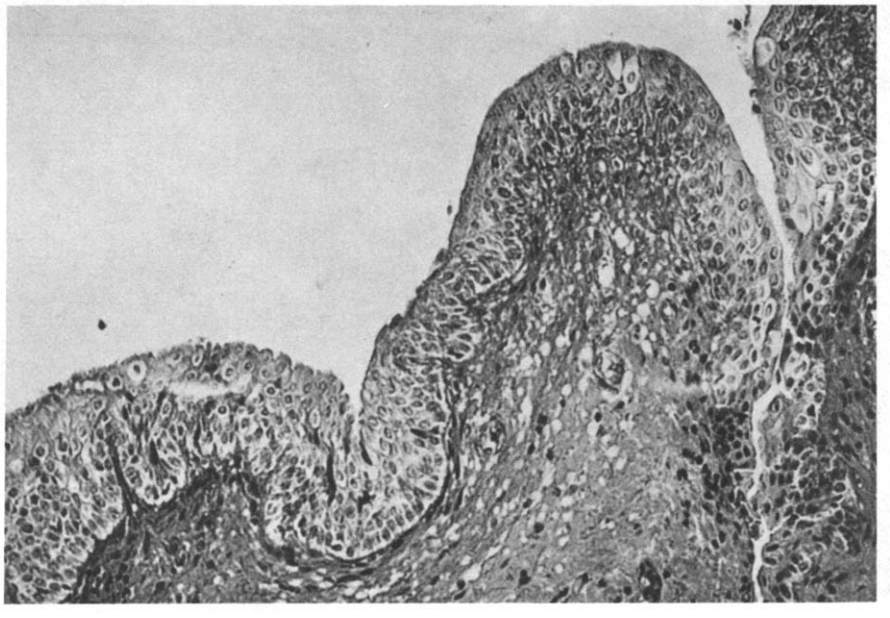

Fig. 12. Vaginal mucosa (Re. 118): on 25-6-73, thick epithelium (×250).

to describe precisely the sequence and development of the events occurring during the proestrus and the oestrus. Further study is necessary, in particular determination of FSH and LH levels.

Moreover, it is difficult to determine the duration of the corpus luteum. It is generally established from the day of ovulation or from the day of the LH peak in the other species. However, the plasma P level reflects the luteal activity, then the duration of this activity can be estimated at between 60 and 85 days, which is longer than the vixen's pregnancy (51–54 days). According to Bonnin *et al.*[3, 16], this cyclic corpus luteum is comparable to the corpus luteum of pregnancy which persists after parturition. In the blue fox, the progesterone profile is the same in pregnant and non-pregnant vixens [7], even in two non-receptive vixens in which typical profiles were obtained during 70 days.

In the dog, which is a fairly close relative of the fox, Smith and McDonald[17] noted a prolonged luteal phase in the case of non-pregnant bitches, whether or not these had been mated with vasectomized males. Similar observations were reported by Hadley[18].

During anoestrus, an ovarian activity exists and can be detected by changes in the plasma oestrogen concentrations. The E_2 level is as high as during oestrus and the genital tract response of the same order.

A constant and significant rise in E_1 concentration could not be detected, except during the reproductive season. This fact could be partly explained by the higher frequency of sampling during this latter period. Furthermore, as it was shown in women [19], it is possible that the peripheral plasma E_1 did not originate only from the ovary but also from adrenal and from extraglandular conversion of androstenedione and of E_2; so the changes occurring throughout the year in the ovarian secretion of E_1 are less detectable than those of E_2. The lack of peripheral conversion of E_2 to E_1 may also be suggested, but this remains to be investigated.

The E_2 surges observed during anoestrus could originate either from growing follicles or from atretic follicles. These structures have been observed in the anoestrous ovary. The latter are particularly well developed in certain monoestrous species during delayed implantation period as noted by Canivenc and Bonnin[8]. They take part in the formation of the thecal gland and then of the interstitial gland. According to Guraya[20] this gland possesses the cytological, histochemical and biological characteristics of steroid secretory cells; it could produce, according to the species, androgens, oestrogens and progestogens.

The present study gave no information about the determinism of this episodic release of oestrogen during anoestrus and about the physiological significance of this ovarian activity. Further investigations will be carried out, particularly investigations concerning the influence of gonadotropic hormones.

REFERENCES

1. Fairley J. S.: *Proc. R. Irish Acad.* **69** section B no. 5 (1970) 103–137.
2. Rowlands I. W. and Parkes A. S.: *Proc. Zool. Soc. London* (1935) 823–841.
3. Bonnin-Laffargue M. and Canivenc R.: *C.r. hebd. Séanc. Acad. Sci., Paris* **271** Série D (1970) 1402–1405.
4. Bonnin-Laffargue M., Canivenc R. and Lajus-Boue M.: *C.r. hebd. Séanc. Acad. Sci., Paris* **274** Série D (1972) 85–88.
5. Mead R. A. and Eik-Nes K. B.: *J. Reprod. Fert.* **18** (1969) 351–353.
6. Möller O. M.: *J. Endocrinology* **56** (1973) 121–132.
7. Möller O. M.: *J. Endocrinology* **59** (1973) 429–438.
8. Canivenc R. and Bonnin-Laffargue M.: *Le corps jaune.* Masson, Paris (1973) pp. 135–193.
9. Dutourné B. and Canivenc R.: *C.r. hebd. Séanc. Acad. Sci., Paris* **273** Série D (1971) 2579–2582.
10. Castanier M. and Scholler R.: *C.r. hebd. Séanc. Acad. Sci., Paris* **271** Série D (1970) 1787–1789.
11. Tea N. T., Castanier M., Roger M. and Scholler R.: *J. steroid Biochem.* **6** (1975) 1503–1516.
12. Zerobin K.: *Zentbl. VetMed.* **15** (1968) 777–798.
13. Naatgeboren C., Van Der Weyden G. C., Klopper P. J., Kroon C. H., Schoof A. G. and Taverne M. A. M.: *J. Reprod. Fert.* **35** (1973) 511–518.
14. Mondain-Monval M., Dutourné B. and Scholler R.: In *Entretiens de Chizé* (1973) Unpublished.
15. Pearson O. D. and Enders R. K.: *Anat. Rec.* **85** (1943) 69–83.
16. Bonnin-Laffargue M., Mondain-Monval M. and Dutourné B.: *J. Reprod. Fert.* (in press).
17. Smith S. M. and McDonald L. E.: *Endocrinology* **94** (1974) 404–412.
18. Hadley J. C.: *J. Reprod. Fert.* **44** (1975) 453–460.
19. Baird D. T.: *Reprod. Biol.* **4/1** (1974) 31–39.
20. Guraya S. S.: *Acta endocr., Copenh.* **12** (1973) Suppl. 171.