PLASMA PROGESTERONE LEVELS IN THE STOAT

A. P. GULAMHUSEIN* AND A. R. THAWLEY†

*Wellcome Institute of Comparative Physiology, Zoological Society of London,
Regent's Park, London NW1 4RY*

(Received 26th June 1973)

Stoats (*Mustela erminea*), in the wild state (Deanesly, 1943) and in captivity (Gulamhusein, 1973), produce only one litter annually due to the occurrence of delayed implantation which extends from the summer of one year to the spring of the next. Implantation occurs in early March and parturition takes place some 4 weeks later. The females come into heat during early summer and, in the wild, virtually all of them are impregnated presumably before the family bond is broken.

Unlike the roe-deer (Short & Hay, 1966) and the nine-banded armadillo (Talmage, Buchanan, Krintz, Lazowasem & Zarrow, 1954), the stoat (Gulamhusein & Thawley, 1972) and other mustelids have 'relatively inactive' CL during the delay period. Low concentrations of progesterone in the CL of the European badger (Canivenc, Short & Bonnin-Laffargue, 1966) and in the plasma of the spotted skunk (Mead & Eik-Nes, 1969) support the belief that nidation in mustelids may be delayed due to inadequate secretion of this hormone.

Blood samples (5 ml) were obtained by cardiac puncture under Fluothane (I.C.I.) anaesthesia from twelve pregnant females (eight laboratory bred and four wild mated) during different times of the year. Samples from another eight unmated females were used as controls. The animals were usually allowed to recover and were used again for the same purpose 4 or more weeks later. A radioactive tracer ($[4-^{14}\text{C}]$ progesterone, 1.35 ng, 250×10^{-6} μCi) and a synthetic steroid (oestr-4-en-3,17-dione, 1 or 5 ng) were added to each extraction vial as internal standards. Progesterone extraction was carried out by a method described by Nambara, Bae & Nokubo (1971).

The progesterone-containing fractions were allowed to react with heptafluorobutyric (HFB) anhydride (100 μl) and toluene (100 μl) at 60°C for 1 hr (Wilson & Fraser, 1971), evaporated to dryness under nitrogen, dissolved in toluene (100 μl) and an aliquot was applied to glass Pye Auto solids Injector ampoules. Known amounts of HFB derivatives of progesterone and estr-4-en-3,17-dione were prepared at the same time, and n-alkyl fluoro-esters (A. R. Thawley, in preparation) were placed on the first and last ampoules of each batch. Automatic injection of the whole batch onto a Pye 104 gas chromatograph fitted with a ^{63}Ni electron capture (EC) detector was carried out under the

* Present address: Department of Anatomy, University of Leicester, University Road, Leicester LE1 7RH.

† Present address: Beecham Research Laboratories, The Pinnacles, Harlow, Essex.

following conditions: a 9 ft x 0.25 in. (o.d.) glass column containing 1% OV-17 on Gas Chrom Q was used at 200°C with a nitrogen flow rate of 40 ml/min. The detector oven temperature was 300°C, the pulse space 500 μ sec and the attenuation factor 1000. No purge gas was used. Peak areas were measured by triangulation and the amount of progesterone in the plasma was given by:

$$\frac{\text{Area of progesterone}}{\text{Area of oestrendione}} \times \text{Amount of added oestrendione} - \text{Amount of added [4-}^{14}\text{C]progesterone}$$

Text-figure 1 illustrates the progesterone concentrations in the plasma of non-pregnant (unmated) and pregnant females. From May to March, progesterone levels in the non-pregnant females were fairly constant. Progesterone

TEXT-FIG. 1. Plasma progesterone concentrations in non-pregnant (-----) and pregnant female stoats. Mean concentrations are indicated and the vertical lines represent the S.E. The number above the vertical line represents the number of samples assayed.

levels in the pregnant females, whose uteri contain unimplanted blastocysts during most of the delay period (July to January), were not significantly different from those of the non-pregnant females but the levels gradually increased as implantation time neared.

Plasma progesterone levels seem to be correlated with the state of the CL. During most of the delay period, the secretory activity of the CL was reduced. It is possible, however, that low progesterone levels observed during this period may be due to an extraovarian source of this hormone and thus further work is essential to investigate which other endocrine organs contribute to plasma progesterone during the period of delayed implantation (Mead & Eik-Nes, 1969). When the CL begin to enlarge before implantation (Gulamhusein & Thawley, 1972), it is at this time that a rise in progesterone level is seen. In a more detailed hormonal study in the mink, Møller (1973) has

reported that progesterone levels increase about 10 days before the estimated time of implantation. Our study has indicated that the stoat progesterone profile during the delay period appears to be similar to that observed in the badger, the spotted skunk and the mink.

All attempts to induce implantation in mustelids with exogenous progesterone alone or in combination with other steroids have been unsuccessful (see Sheldon, 1972). On the other hand, ovarian hormones maintain the unimplanted blastocysts in a viable state in the long-tailed weasel (Wright, 1963) and the short-tailed weasel of North America (Sheldon, 1972), the latter being the same species as the European stoat. The blastocysts of both these weasels, unlike those of the badger (Canivenc & Laffargue, 1957; Neal & Harrison, 1958), degenerate 3 weeks after ovariectomy. Sheldon considers that degeneration of blastocysts in the short-tailed weasel is a secondary event brought about by uterine involution following ovariectomy.

It appears, therefore, that low levels of progesterone during most of the delay period are just sufficient to maintain the uterus and the blastocysts in a viable state and, with the approach of spring, increased progesterone secretion prepares the uterus for implantation. Progesterone may not be the only factor in the timing of implantation, however, since nidation has been induced prematurely by artificially increasing the photoperiod in many members of the family Mustelidae (see Mead, 1971), including the stoat (A. P. Gulamhusein, unpublished observation), and by decreasing the photoperiod in the European badger (Canivenc, Bonnin-Laffargue & Lajus-Boue, 1971). This has led many to suspect that light, acting through the hypothalamus, may be involved in the timing of implantation in this family.

One of us (A.P.G.) is grateful for a Ugandan Government Fellowship. The cost of research was defrayed by the O.D.A. (A.P.G.) and by the M.R.C. (A.R.T.). The authors express their thanks to the Z.S.L. for the hospitality of the Institute and to Dr I. W. Rowlands for his interest and help in this work.

REFERENCES

- CANIVENC, R., BONNIN-LAFFARGUE, M. & LAJUS-BOUE, M. (1971) Réalisation expérimentale précoce de l'ovoiimplantation chez le blaireau européen (*Meles meles* L) pendant la période de latence blastocystaire. *C. r. hebdomadaire. Acad. Sci., Paris*, **273**, 1855.
- CANIVENC, R. & LAFFARGUE, M. (1957) Relation des corps jaunes et des blastocystes au cours de la nidation différée du blaireau européen (*Meles meles*). *C. r. Séanc. Soc. Biol.* **151**, 561.
- CANIVENC, R., SHORT, R. V. & BONNIN-LAFFARGUE, M. (1966) Étude histologique et biochimique des corps jaunes du blaireau européen (*Meles meles* L). *Annls Endocr.* **27**, 401.
- DEANESLY, R. (1943) Delayed implantation in the stoat (*Mustela erminea*). *Nature, Lond.* **151**, 365.
- GULAMHUSEIN, A. P. (1973) *Reproductive studies in the Mustelidae*. Ph.D. thesis, University of London.
- GULAMHUSEIN, A. P. & THAWLEY, A. R. (1972) Ovarian cycle and plasma progesterone levels in the female stoat, *Mustela erminea*. *J. Reprod. Fert.* **31**, 492.
- MEAD, R. A. (1971) Effects of light and blinding upon delayed implantation in the spotted skunk. *Biol. Reprod.* **5**, 214.
- MEAD, R. A. & EIK-NES, K. B. (1969) Seasonal variation in plasma levels of progesterone in western forms of the spotted skunk. *J. Reprod. Fert., Suppl.* **6**, 397.
- MØLLER, O. D. (1973) The progesterone concentrations in the peripheral plasma of the mink (*Mustela vison*) during pregnancy. *J. Endocr.* **56**, 121.
- NAMBARA, T., BAE, Y. H. & NOKUBO, M. (1971) Analytical chemical studies on steroids. Part I. Determination of urinary metabolites of 16 α -chloroesterone methyl ether by gas chromatography. *J. Chromat.* **60**, 414.

- NEAL, E. G. & HARRISON, R. J. (1958) Reproduction in the European badger (*Meles meles* L). *Trans. zool. Soc. Lond.* **29**, 67.
- SHELDON, R. M. (1972) The fate of short-tailed weasel, *Mustela erminea*, blastocysts following ovariectomy during diapause. *J. Reprod. Fert.* **31**, 347.
- SHORT, R. V. & HAY, M. F. (1966) Delayed implantation in the roe-deer, *Capreolus capreolus*. *Symp. zool. Soc. Lond.* **15**, 173.
- TALMAGE, R. V., BUCHANAN, G. D., KRAINTZ, F. W., LAZOWASEM, E. A. & ZARROW, M. X. (1954) The presence of a functional corpus luteum during delayed implantation in the armadillo. *J. Endocr.* **11**, 44.
- WILSON, A. & FRASER, R. (1971) The estimation of plasma 11-deoxycorticosterone in man using gas-liquid chromatography with electron-capture detection. *J. Endocr.* **51**, 557.
- WRIGHT, P. L. (1963) *Variations in reproductive cycles in North American mustelids*. In: Delayed Implantation, p. 77. Ed. A. C. Enders. University of Chicago Press, Chicago.