LUTEAL FUNCTION IN MINK: THE EFFECTS OF HYPOPHYSECTOMY AFTER THE PREIMPLANTATION RISE IN PROGESTERONE

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ABSTRACT


Pearl variety mink bred twice between March 4 and 20 were hypophysectomized or sham hypophysectomized between April 4 and 10, after the preimplantation rise in progesterone. Four groups of hypophysectomized females each received one of the following treatments in the form of minipump (Alza) infusion for 170 h of: prolactin (PRL) 1 µg/h, 2 µg/h, luteinizing hormone (LH) 1 µg/h or LH 0.5 µg/h with PRL 1 µg/h. One group of hypophysectomized mink and the sham hypophysectomized mink received no further treatment. Blood samples were taken from all animals at the time of surgery (day 0) and day 3, 6, and 9. Hypophysectomized mink were killed by exsanguination and blood samples were taken from sham treated animals. Plasma progesterone was quantitated by radioimmunoassay. The mean level of progesterone increased in sham treated mink at day 6 and remained high through day 12. Mean progesterone declined significantly by day 3 in all hypophysectomized mink. At day 3, two subgroups were present in terms of luteal response: in animals receiving PRL 1 µg/h, 2 µg/h or PRL + LH, progesterone levels were significantly greater than animals receiving no infusion or LH alone. Plasma progesterone levels declined and were statistically homogenous in all hypophysectomized mink by day 6. Sham treated mink produced normal litters. Embryos degenerated in all hypophysectomized mink. It was concluded that the pituitary is necessary for support of the postimplantation corpus luteum and for the completion of gestation. PRL but not LH temporally ameliorated the decline in progesterone induced by hypophysectomy. LH together with PRL was no more effective than PRL alone. The results suggest that PRL is an important luteotropin in mink.

INTRODUCTION

Pregnancy in the mink (Mustela vison) is characterized by a period of obligate embryonic diapause. The corpus luteum (CL) forms after ovulation, and
soon after, the luteal cells become reduced in size. This condition persists during the preimplantation delay period (Hansson, 1947). Previous reports from our laboratory (Murphy and Moger, 1977) and others (Möller, 1973; Allais and Martinet, 1978a; Pilbeam et al., 1979) have shown that peripheral progesterone levels rise rapidly 5–10 days prior to embryo implantation. Progesterone concentrations remain elevated above preimplantation levels for the greater part of postimplantation gestation.

Reports by Canivenc et al. (1966) and Möller (1973) suggest that uterine and fetal influences on the mink CL are minor or non-existent. We previously reported that the pituitary is required for both the preimplantation rise in progesterone and subsequent implantation to occur (Murphy and Moger, 1977). Studies by Donovan (1963, 1967) showed that prolactin (PRL) can prevent regression of the CL in the hypophysectomized ferret (*Mustela furo*), a closely related species that does not undergo a delay in implantation. A recent study has demonstrated that PRL can qualitatively maintain the progesterone levels, morphology of the CL, and can induce embryo implantation in the hypophysectomized ferret (Murphy, 1979).

In discussion of the luteotrophic role of PRL in the ferret, Donovan (1967) suggested that this hormone might be luteotropic in the mink as well. The ratio of progesterone to its metabolite 20α-hydroxypregn-4-ene-3-one in mink hypophysectomized during the delay phase of gestation further suggested a luteotropic role for PRL (Murphy and Moger, 1977). A recent study by Papke et al. (1980) has shown that administration of exogenous PRL will advance the time of implantation in mink. The present study was undertaken to determine the effects of hypophysectomy on CL function in mink following initiation of the preimplantation rise in progesterone and to investigate the role of luteinizing hormone (LH) and PRL in the maintenance of the postimplantation corpus luteum.

**MATERIALS AND METHODS**

*Animals, surgical procedures, experimental protocol*

Experiments were performed during the 1977 and 1978 breeding seasons. Ranch mink of the Pearl variety were purchased from Montgomery Fur Ranch, Wetaskiwin, Alberta and shipped to Saskatoon 2 months prior to the mating season. Females were maintained out of doors under conditions of natural photoperiod and fed National Northwood mink pellets ad libidum. All females were mated twice between March 4 and 20, mating was confirmed by the presence of sperm in vaginal smears taken on the day the female was placed with the male.

Hypophysectomies were performed between April 4 and 10 under halothane (Somnothane, Hoechst) anesthesia by the parapharyngeal method of Hill and Parkes (1932) as modified in our previous study (Murphy and Moger, 1977). Since outdoor conditions are cold and often inclement during April
in Saskatchewan, animals were maintained inside at 25°C under simulated natural photoperiod following hypophysectomy. A group of four females were sham hypophysectomized, a procedure which consisted of all of the steps of hypophysectomy except removal of the pituitary. The day of hypophysectomy or sham hypophysectomy was designated Day 0. At the time of hypophysectomy, laparotomies were performed to determine the status of each mink relative to embryo implantation. In every case either uterine swellings or uterine enlargement and coiling indicative of incipient implantation were present.

In pilot studies, daily handling and injection resulted in stress-induced mortality in hypophysectomized mink. We therefore chose to use subcutaneously implanted controlled delivery minipumps (Alza) which allow delivery of peptide hormones at the rate of 1 μl/h for 170 h (Bowers and Folkers, 1976). Infusions began on Day 0 and continued through Day 7. Minipumps were charged with one of four saline solutions: PRL (NIH-P-B4), 1 mg/ml; PRL 2 mg/ml; PRL 2 mg/ml; LH (NIH-LH-S18), 1 mg/ml; or PRL 1 mg/ml and LH 0.5 mg/ml. The devices containing these solutions were implanted subcutaneously in the abdominal region into four, five, five and four females respectively at the time of hypophysectomy. One group of four mink received no further treatment after hypophysectomy.

A 2 ml blood sample was taken by cardiac puncture into heparinized syringes on Day 0 at the time of hypophysectomy or sham hypophysectomy under halothane anesthesia. Similar samples were taken under light ether anesthesia on Days 3, 6 and 9. On Day 12 all hypophysectomized females were killed by exsanguination under ether anesthesia. Blood samples were centrifuged at 1200 × g and the plasma aspirated and frozen at −20°C for progesterone analysis. Blood samples only were taken from sham operated control mink on Day 12.

At autopsy, both ovaries, two or more uterine swellings and the hypothalamic region of the brain from each hypophysectomized female was collected, fixed in Bouin’s fluid and processed for histological scrutiny. Efficacy of hypophysectomy was evaluated by gross observation of the hypothalamic region, by the absence of antral follicles in ovarian sections and by viewing serially sectioned hypothalami from representative animals.

**Progesterone assay**

Radioimmunoassay (RIA) of progesterone was performed according to methods previously described for mink plasma (Murphy and Moger, 1977) except that all samples were assayed in a single assay.

To prevent stripping of antibody bound hormone during separation with charcoal-dextran suspension, a 10 min incubation replaced the 30 min period previously used. These incubations were carried out on 50 tube batches, and duplicate zero tubes (containing no cold progesterone) were run with each batch. Combined coefficient of variation for all zero tubes was 3.8%.
The antibody employed in this assay system was Niswender's anti-progesterone-11-BSA serum. The cross reactivity of this antiserum has been reported by Gibori et al. (1977). The intra-assay coefficient of variation, determined between duplicates ranged from 0.1 to 9%. Sensitivity of the assay, defined as the smallest amount of progesterone significantly different from zero was 10 pg/tube or 100 pg/ml.

**Statistical analysis**

Comparison of mean plasma progesterone levels among treatments at the time of hypophysectomy, at each subsequent bleeding and at autopsy was performed by analysis of variance. Where a significant $F$ value was obtained, individual means were compared using Duncan's New Multiple Range Test.

**RESULTS**

Gross observation of the hypothalamic region of hypophysectomized animals at the time of autopsy indicated that the pituitary was removed in every case. Examination of serial sections of the hypothalamus of one mink in each treatment revealed no fragments of pituitary tissue. No antral follicles were observed at any time in sections of the ovaries from the hypophysectomized mink.

Sham operated mink underwent pregnancy and parturition that could not be distinguished from untreated mink in the experimental colony. The litter size did not differ from those of untreated mink.

The embryonic contents of the uterine swellings in all hypophysectomized animals at Day 12 were degenerate. Trophoblast, which has previously invaded the endometrial tissue, was found to be necrotic as evidenced by cytoplasmic dissolution as well as nuclear pyknosis and fragmentation. Luminal embryonic structures appeared to be undergoing resorption. The endometrium, however, was intact and signs of degeneration were not observed.

The structure of CL after hypophysectomy was less consistent than the uterine observations. All CL observed in the ovaries of hypophysectomized mink were in the process of degeneration. The degree of dissolution varied from cytoplasmic vacuolation and condensation of chromatin to nuclear pyknosis. Some CL were similar to the CL of the preimplantation delay phase in mink. No pattern was obvious.

Plasma progesterone concentrations are presented in Fig. 1. Overall pretreatment mean was 37.3 ng/ml. No significant difference was present among

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Fig. 1. Plasma progesterone levels at the time of surgery and at 3 day intervals thereafter during postimplantation gestation in hypophysectomized and sham treated female mink. (a) Sham treated and hypophysectomized mink; (b) Hypophysectomized animals receiving minipump infusions of 1 or 2 µg/h bovine PRL; (c) Hypophysectomized mink receiving minipump infusions of ovine LH (1 µg/h) or ovine LH (0.5 µg/h) and bovine PRL (1 µg/h).
pretreatment means for all experimental groups. Mean progesterone levels in sham operated mink initially increased and remained elevated through the 12 days of the experiment (Fig.1a). Levels had declined precipitously at the first sampling (Day 3) in females that were hypophysectomized and received no further treatment. Thereafter, mean progesterone remained significantly lower than sham operated females (Fig.1a). A similar pattern was observed in LH infused animals. Duncan's test revealed that these levels were significantly lower than sham operated controls at each bleeding date ($P < 0.01$) and not different from animals that received no replacement.

Hypophysectomy followed by treatment with 1 or $2 \mu g$ of PRL per h resulted in a reduction in the peripheral concentrations of progesterone by Day 3 relative to sham operated animals ($P < 0.05$). Day 3 levels, however, were significantly greater than those observed in untreated or LH treated mink ($P < 0.05$). By Day 6, progesterone declined in both PRL treated groups so that it was not different from that seen in hypophysectomized, untreated females.

Females receiving $0.5 \mu g$ LH and $1 \mu g$ PRL per h had progesterone profiles which were not different from those observed in females receiving PRL alone (Fig.1b). These values were significantly lower than those found in sham operated females on the corresponding day ($P < 0.05$), but were significantly greater than hypophysectomized, untreated mink at Day 3 ($P < 0.05$). By Day 6, levels had declined so that they were not different from hypophysectomized untreated controls.

DISCUSSION

Progesterone levels in sham treated mink (Fig.1a) were similar to those reported in intact mink during postimplantation gestation (Murphy and Moger, 1977, Pilbeam et al., 1979). Progesterone values, together with the presence of normal parturition in this group, suggest that the stress of anesthesia, surgical procedures, and blood sampling did not affect gestation.

Progesterone fell precipitously following hypophysectomy in mink that received no replacement therapy (Fig.1a), indicating that the pituitary is required for maintenance of the postimplantation CL in mink. Histological examination of CL from all hypophysectomized mink on Day 12, five days after cessation of replacement therapy, suggested that morphological regression accompanied the reduction in progesterone output.

Embryos taken from all hypophysectomized animals at the time of autopsy had degenerated. From the observations of the decline in progesterone and embryo degeneration following hypophysectomy, it was concluded that the pituitary is necessary for postimplantation gestation in mink. This conclusion extends our previous finding (Murphy and Moger, 1977) that the pituitary is required during the preimplantation period.

The ability of PRL to ameliorate the reduction in progesterone at Day 3 following hypophysectomy (Fig.1b) suggests that this hormone has a luteo-
tropic role in the mink. In the ferret, PRL has been shown to maintain the CL from a morphological standpoint following hypophysectomy (Donovan, 1963, 1967). PRL will also maintain progesterone levels and induce embryo implantation in the hypophysectomized ferret (Murphy, 1979). In ferret experiments, doses of PRL were considerably higher; 250–300 μg/day/animal compared to 24 μg/day/animal in mink in the present study. It is possible that higher doses of PRL may prove to be more effective in maintaining the CL in hypophysectomized mink. Other workers have implicated PRL as a luteotropin before implantation in mink by administration of ergocryptine (CB154, Sandoz) (Papke et al., 1980; Allais and Martinet, 1978b). The latter report indicates that ergocryptine administered after implantation can accelerate the decrease in progesterone which occurs during the last half of postimplantation pregnancy.

LH alone, in the doses used, was not capable of maintaining the output of progesterone from the CL (Fig.1c). The combined effect of LH and PRL (Fig.1c) was not greater than that of PRL alone (Fig.1b). Donovan (1967) determined that LH alone could not support the structural integrity of the CL in the hypophysectomized ferret, and LH together with PRL was no more effective than PRL alone. Previous experiments indicate that LH does not induce progesterone secretion from the ferret CL in vitro (Dimond et al., 1977). From the present study it can be speculated that LH may not be necessary for luteal function in the mink, as appears true in the case of the ferret. Confirmation awaits further experimentation.

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REFERENCES


