Effects of medroxyprogesterone acetate on gestation in mink

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Summary. Mink ovariectomized 14 days after the first of two matings received injections of 2 mg MPA, the same MPA treatment + an oestradiol-17 β implant or no replacement therapy. Some mink were ovariectomized after implantation and given a single dose of 2 mg MPA or no replacement therapy. MPA persisted in the serum at detectable levels for 13 or more days in all mink treated. MPA and MPA + oestradiol induced uterine growth but neither treatment was capable of inducing embryo implantation. More embryos were retained in mink treated with MPA alone and these appeared to be viable. Implanted embryos persisted for a longer period in animals that were ovariectomized and treated with MPA. MPA neither supported pregnancy nor permitted parturition. Serum LH was elevated by 1 week after ovariectomy and elevations persisted for a further 20 or more days. While MPA alone had no apparent negative feedback effects on LH, animals that received MPA + oestradiol did not display any elevation of LH, suggesting that oestradiol or a combination of MPA and oestradiol has a potent negative feedback in mink.

Introduction

Mink gestation is characterized by a period of preimplantation delay during which embryo development is quiescent and corpora lutea are small and relatively inactive (Hansson, 1947). At this time circulating progesterone levels are low compared to those in postimplantation pregnancy (Møller, 1973; Murphy & Moger, 1977; Pilbeam, Concannon & Travis, 1979). The corpus luteum undergoes activation under the influence of lengthening photoperiod (Murphy & James, 1974) about the time of the vernal equinox. Luteal activation can be induced by prolactin (Papke, Concannon, Travis & Hansel, 1980; Martinet, Allais & Allain, 1981; Murphy, Concannon, Travis & Hansel, 1981), is characterized by a precipitous rise in circulating progesterone, and results in embryo implantation (Pilbeam *et al.*, 1979). Progesterone alone will not induce embryo implantation if administered to intact animals during embryonic diapause (Hansson, 1947; Cochrane & Shackelford, 1962), suggesting that luteal activation consists of more than simply progesterone secretion. A study of implantation in a related species, the ferret, has shown that luteal factor(s) other than progesterone are required to initiate embryo implantation following ovariectomy (Foresman & Mead, 1978).

Administration of the long acting progestagen, medroxyprogesterone acetate (MPA), results in sustained elevations of this synthetic hormone in dogs (Hansel, Concannon & McEntee, 1977)

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and will abbreviate the delay state in mink by inducing precocious implantation (Concannon, Pilbeam & Travis, 1980). The purposes of the present experiment were to determine the time course of MPA disappearance from the circulation of ovariectomized mink, to examine the effects of MPA on uterine size and embryo retention, and to determine the effects of ovariectomy and hormone replacement on luteinizing hormone (LH) in mink.

Materials and Methods

Animals and surgical procedures

Brown-eyed pastel mink, maintained in outdoor cages and fed standard mink ration, were mated twice, once on 7, 8 or 9 March and once 7 days after the first mating. In two groups of animals (Groups 1 and 2), 2 mg medroxyprogesterone acetate (MPA: Depo-Provera, Upjohn, Kalamazoo, Michigan) were administered intramuscularly during preimplantation delay on the eve of the day of ovariectomy, the 14th day after the first mating.

Animals in Groups 1 and 2, as well as those in Group 3, were ovariectomized via midventral incision under halothane (Somnothane, Hoechst) anaesthesia, and the day of ovariectomy was designated Day 0 of the trial. Mink in Groups 1 ($N = 5$) and 2 received 2 further injections of MPA, one each on the 1st and 3rd days after ovariectomy. Mink in Group 2 ($N = 8$) also received a 5-mm length of Silastic (Dow Corning) tubing (i.d. 2.0 mm, o.d. 3.2 mm) packed with crystalline oestradiol-17 β (Sigma Chemicals, St Louis, Missouri) at the time of ovariectomy. Group 3 mink ($N = 5$), ovariectomized during delay, served as controls and received no hormone replacement therapy. At the time of surgery, blood samples, taken from each animal by cardiac puncture, were allowed to clot at room temperature and centrifuged at 1200 g; the serum was aspirated and frozen at -20°C until analyses were performed. On Day 6 after ovariectomy, all mink were examined by laparotomy and an average width of the uterus was determined from measurements made with a metric ruler at 3 sites on each horn. A cardiac blood sample was taken at this time and prepared as above. On Day 13, animals were subjected to a third laparotomy at which the uteri were removed, and the width and length of the uterine horns were determined. Uteri were trimmed of fat and mesenteric tissue and weighed to the nearest mg before being dissected for observation of embryos. A cardiac blood sample was taken at this time and at weekly intervals thereafter until Day 44.

Mink in Groups 4 and 5 underwent ovariectomy, on 16 April (Day 38–39 of pregnancy) after embryo implantation, indicated by the presence of uterine swellings, had occurred. On this day, mink in Group 4 ($N = 5$) were given a single intramuscular injection of 2 mg MPA, while those in Group 5 ($N = 5$) received no replacement therapy. Blood samples were taken at the time of ovariectomy at weekly intervals for a further 3 weeks. Animals were observed daily for signs of parturition. A final blood sample was taken at the time of laparotomy on 27 May, 41 days after ovariectomy, and the uteri were removed and weighed.

Hormone analyses

Serum concentrations of MPA were determined by radioimmunoassay as previously described (Hansel *et al.*, 1977), using an antiserum provided by Dr K. Kirton, Upjohn Laboratories, Kalamazoo, U.S.A. The cross-reactivity of this antiserum has been reported (Cornette, Kirton & Duncan, 1971) and the assay has been validated for use in mink (Concannon *et al.*, 1980). The between-assay coefficient of variation was 4.0% and the within-assay coefficient of variation, determined between duplicates, ranged from 0.1 to 9%. The minimum sensitivity of the assay was 50 pg/tube or 100 pg/ml.

Serum LH concentrations in all samples were determined by double-antibody radioimmunoassay with GDN No. 15 antiovine LH serum. This assay and antiserum have been

validated for use in the mink (Murphy, 1979). Minimum sensitivity of the assay was 60 pg/tube or 0.3 ng/ml. The between-assay coefficient of variation was 8.4% and the within-assay coefficient, calculated between duplicates, ranged from 0.1 to 9%.

Statistical analysis

A one-way analysis of variance was performed for each uterine measurement studied, i.e. uterine width, uterine weight and number of embryos present. In the presence of a significant F value, a least significant difference test was used to compare group mean values for each characteristic. Two-way analysis of variance which treated bleeding dates as columns and treatments as rows was used to analyse blood hormone data. A least significant different test was used to compare treatment effects among each of the bleeding dates or with pretreatment control values.

Results

Serum MPA concentrations

The first injection of MPA preceded the first blood sample by approximately 18 h. The resultant mean ($\pm$ s.e.m.) MPA levels (Text-fig. 1a) were 19.2 ± 2.5 and 20.9 ± 2.2 ng/ml in mink in Groups 1 and 2 respectively. The highest mean concentrations of MPA in these two groups were on the 4th day following the third and final injection. Levels had declined by the time the next sample was taken (Day 13) and continued to decline but remained elevated

Text-fig. 1. Concentrations (mean $\pm$ s.e.m.) of medroxyprogesterone acetate (MPA) in serum samples taken from mink ovariectomized (a) during preimplantation delay (Day 0) and (b) after implantation.

($P < 0.05$) until Day 20 of the experiment. By Day 30 there was no difference in MPA concentrations between mink in Groups 1 and 2 and those in Group 3.

In Group 4 mink, MPA concentrations on the 6th day after treatment were 10 ± 2.4 ng/ml (Text-fig. 1b). This value was significantly elevated relative to pretreatment values ($P < 0.05$). The concentrations had declined to 4.4 ± 0.7 ng/ml by Day 12 and were not different from those of Group 5 animals by Day 20.

Uterine measurements and embryo counts

No implanted embryos were found in the uteri of ovariectomized mink that received no further treatment (Group 3). In addition, neither MPA nor MPA + oestradiol (Groups 1 and 2) were capable of inducing embryo implantation in animals ovariectomized during embryonic diapause under the conditions of this experiment (Table 1). Compared with values for Group-3 mink, uterine width and weight were increased in Groups 1 and 2 ($P < 0.05$). Uterine width at Day 7 was increased in Group 2 ($P < 0.05$).

Table 1. Uterine and embryonic characteristics of mink subjected to ovariectomy on Day 0 during embryonic diapause and receiving various treatments

Group	No. of mink	Uterine width at Day 7 (mm)	Uterine width at Day 14 (mm)	Uterine wet wt (mg)	Uterine horn length (mm)	Embryos	
						No.	Viable appearance
1	5	3.5 ± 0.3^b	3.4 ± 0.17^b	643 ± 41^b	42.0 ± 0.9^b	4.5 ± 1.9^b	Yes
2	8	4.1 ± 0.04^c	3.3 ± 0.15^b	716 ± 40^b	41.6 ± 1.4^b	2.7 ± 0.6^a	No
3	5	1.9 ± 0.11^a	2.1 ± 0.15^a	219 ± 37^a	30.8 ± 0.72^a	1.8 ± 0.8^a	Yes

Values are mean $\pm$ s.e.m.

^{a, b, c} Means in the same column bearing different superscripts are different ($P < 0.05$).

More embryos were retained in Group-1 mink than in the other 2 groups ($P < 0.05$). Blastocysts recovered from the uteri of mink in Groups 1 and 3 were judged viable because the zonae pellucidae and the trophoblast were intact and of normal appearance. These embryos were expanded when removed from the uterus and shrank in response to alteration in osmotic concentration of the medium. In Group 2 the texture of the embryos appeared gelatinous and there was no response to altered tonicity of the medium. The zonae pellucidae of embryos recovered from this group also appeared to have lost their elasticity as they did not regain their spherical shape after indentation with a sharp probe. It was concluded that these embryos were probably not viable.

No litters were produced by animals in Groups 4 and 5. One female in Group 4 aborted on 5 May, 19 days after ovariectomy and MPA treatment. At laparotomies on Day 41, uteri from Group 5 animals were typical of anoestrus, with no signs of embryonic tissue present. Mink in Group 4 had larger uteri than those in Group 5 and two contained dead embryos in the process of degeneration.

Serum LH concentrations

The mean concentrations of LH in serum were elevated from basal levels on approximately Day 13 in Group 3 or Group 1 animals (Text-fig. 2a). The elevation persisted in Group 1 mink until Day 30, but were not different from pretreatment values by Day 44. Levels did not increase in animals in Group 2 at the time of ovariectomy; there was an apparent, but not statistically

Text-fig. 2. Concentrations (mean $\pm$ s.e.m.) of serum LH in mink ovarietomized (on Day 0) (a) during the preimplantation delay phase of gestation and (b) after implantation.

defineable, decline in LH within the first 6 days following initiation of the experiment. LH levels remained low until Day 44.

LH concentrations were elevated by the 6th day after ovariectomy in animals in Groups 4 and 5 (Text-fig. 2b). In most of these animals, LH remained above basal levels until the 12th day. In a few animals, elevations in serum LH persisted to the end of the experiment (Day 41).

Discussion

MPA in mink serum was elevated following injection, and was present for 13 or more days in animals receiving a single injection of the hormone (Text-fig. 2). Concentrations of MPA may

have persisted longer in animals subjected to multiple injections because slight elevations relative to the control group were present on Day 20 after ovariectomy. These results confirm an earlier study in which MPA was found in the sera of pregnant mink 23 days after administration of 2.5 mg of this hormone in two injections (Concannon *et al.*, 1980).

The ability of MPA to induce and maintain uteri of the size found in pseudopregnant animals provides strong evidence for the capacity of this hormone to interact with progesterone receptors in the mink uterus. MPA alone appeared to maintain a greater number of embryos in the uteri of ovariectomized mink, further suggesting that it is an active progestagen in mink. While the ovarian influences on the process of embryo implantation in mink have not been entirely determined, a precipitous rise in progesterone precedes implantation, and MPA injection provided similar elevations in progestagen levels. MPA administered to intact mink during embryonic diapause can hasten implantation (Concannon *et al.*, 1980). However, MPA alone and MPA plus oestradiol, in the manner administered in the present study, were incapable of inducing implantation, suggesting that there is an ovarian requirement other than progestagens for embryo attachment. The ovarian complement required for implantation in the ferret consists of progesterone and luteal factor(s) (Foresman & Mead, 1978). The presence of aromatase activity in ferret corpus luteum, as well as the failure of implantation in the presence of a blockade to aromatization, suggests that the luteal factor required in the ferret may be an oestrogen (Mead & Swannack, 1980).

The oestradiol requirement for implantation in rats appears to be secreted over a brief interval on the 4th day of pregnancy (Watson, Anderson, Alam, O'Grady & Heald, 1975). The continuous release of oestradiol to which mink in the present experiments were subjected may have interfered with embryo attachment rather than induced it. Further, the quantity released by the implants may have been greater than that required, and if so, oestradiol in excess may have inhibited the implantation process.

Oestradiol plus MPA (Group 2) appeared to induce changes in the zonae pellucidae and cells of retained embryos in these experiments. Fewer embryos were retained in these animals. Adams (1981) suggested that the process of mating results in expulsion of some or all of the blastocysts from previous matings. While it is not obvious that this expulsion is an oestrogenic effect, it did not occur when ovulation was induced by gonadotrophin in that study. Together these observations suggest that oestrogens may induce embryo loss in the mink.

The present study demonstrates that single and multiple intramuscular injections of MPA result in MPA elevations in the blood of ovariectomized mink and that MPA does have a progestagenic and embryo protective effect on the uterus of ovariectomized animals. These two factors, as well as the capacity of MPA to advance implantation in intact mink, lead to the suggestion that MPA treatment could improve embryo survival in commercial mink herds.

The basal levels of LH in the present study were within the range previously described for mink bled during the mating season (Murphy, 1979). Ovariectomy in the present study appeared to result in elevation of LH within 6 days (Text-fig. 3), presumably due to the removal of an ovarian substance which normally acts on the hypothalamus, resulting in suppression of LH secretion. Ovariectomy in mink will result in hypertrophy of gonadotrophic cells of the anterior pituitary (Murphy & James, 1976), suggesting that the ovary has a negative feedback effect in this species. In the present study, the duration of the elevation of LH was brief and differed little whether animals were ovariectomized before or after embryo implantation (Text-figs 3 & 4), although it was more variable after implantation. MPA appeared to have no negative feedback effect to reduce LH secretion in ovariectomized mink since the LH patterns of MPA-treated and untreated ovariectomized mink were similar. The addition of oestradiol to the replacement therapy regimen not only prevented the normal elevation of LH in ovariectomized mink but also appeared to reduce circulating levels. This suggests that oestradiol may be an important regulator of LH concentrations in the mink, as it is in other species of carnivores, such as the cat (Johnson & Gay, 1981).

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