EFFECTS OF PROGESTERONE TREATMENTS ON DELAYED IMPLANTATION IN MINK[1]

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ABSTRACT

Ova nidation took place in female mink treated with progesterone from the 2nd through the 30th day after mating. When treatments were stopped, the females began to resorb fetuses. Perhaps the treatments partially inhibited natural corpora production of hormones and, when treatments ceased, the corpora could not maintain all the embryos. A lack of progesterone may also have prevented proper breast development, for all kits died within a day or two after birth. The females did not appear to be producing milk.

Female mink maintained on progesterone treatments until average gestation length had passed, resorbed or aborted all embryos. Perhaps the treatment was sufficient to inhibit natural production of hormones and was not enough to maintain pregnancy.

Treatments of females for one and two days with injected or oral progestins did not inhibit kit production. Some animals that were treated had blastulae implanting or adhering to the uterine endometrium at 18 days after mating. Most of these animals had active corpora and their endometria were moderately developed.

INTRODUCTION

Mink (*Mustela vison*) belong to the family Mustelidae, several members of which exhibit a natural delayed implantation of the blastocyst. Enders (1952), Hansson (1947), Wright and Rausch (1955), Eadie and Hamilton (1958), Hamilton and Eadie (1964), and Pearson and Enders (1944) are among those reporting this phenomena in different species of Mustelids.

Enders (1952), Hansson (1947), and Holcomb et al. (1962) reported that an artificial increase in the photoperiod caused a slight decrease in the length of the gestation period and, in some cases, more kits per litter in mink.

The experiments described in this paper were attempts to discover if exogenous progestin treatments would effect the gestation length and litter size in female mink.

METHODS

In the spring of 1962, twenty-three pastel females were subjected to varying oral dosages of progesterone from the 2nd through the 30th day after mating. The progesterone was given to the females in the commercial form, Provera (6-methyl-17-acetoxy progesterone), at the rate of 1.5 or 3.0 mg per lb body wt per day. Eight females were given 3.0 mg per lb body wt per day beginning two days after mating, and a second mating attempt was made after seven days. Only one of these females mated a second time. Eight other females were mated but once and were given 1.5 mg Provera per lb body wt per day beginning two days following mating. Seven other females were given Provera beginning two days following a second mating. The Provera was added to the mink daily ration, in a premix of soybean meal used to facilitate mixing, and was fed for 29 days to all three groups. A control group of 10 females was maintained.

Average numbers of kits per litter and average lengths of gestation period were recorded. A Student's two-sided t-test was used at the .05 level to determine if any significant differences existed between the various groups.

In the spring of 1963, 41 pastel females were subjected to different dosages of progesterone for varying intervals of time after mating (table 1). Eleven control females were maintained.

Several females received oral dosages of Provera; others received it as injections. Two matings were obtained, the second mating occurring on the day following the first mating. All females were checked for sperm after mating. Females were picked at random from the herd for the various groups; there was an even distribution of first-year breeders and older females in each group.

An attempt was made to have an equal distribution of early- and late-mated females in each group, because females mated early in the season have a longer delayed implantation resulting in fewer kits than those mated later. Mating

Table 1. Treatment of females with progesterone and interval during which the treatment was given—1963

No. of females	Treatment	Date when treatment given	No. autopsied at 18th day
11	Control		2
12	Light	7th-whelping	2
13**	*	7th-whelping	2
3	15 mg[1]	7th-9th	1
3	10 mg[1]	7th-9th	1
3	15 mg[1]	7th day	1
3	30 mg[1]	7th day	1
3	5 mg[2]	7th day	1
3	2.5 mg[2]	7th-8th day	1
2	*	4th-whelping	1
2	*	2nd-whelping	1
2	*	6th-whelping	1
2	*	7th-11th	1
2	*	13th-whelping	1

*3mg of Provera per day per lb. body wt.
**Four females were moved from the oral progesterone treatment on the 30th day after mating. One was autopsied the 30th day after mating; one was autopsied on the 40th day after mating. The remaining two were maintained to whelping time. The object was to determine whether embryos would be resorbed or maintained until whelping.
[1]Oral Provera.
[2]Injected Progesterone.

times of all 52 females were subdivided into three mating intervals and then the females were placed into the separate groups. The three mating intervals were March 10 to 13, March 14 to 17, and March 18 to 22. Only females mated between March 14 and 17 were included in those autopsied.

Injections of progesterone were made intramuscularly in the hind leg, as described by Franklin (1958). The progesterone was injected in a propylene glycol solution of 50 mg per cc.

Seventeen females were autopsied on the 18th day after mating. Four other females were autopsied, one each on the 30th, 40th, 50th, and 57th days after mating.

In autopsied mink, several factors were considered:
1. Appearance of the ovary; number of corpora lutea present, and activity of the corpora lutea.
2. Condition of the uterine endometrium and glandular development, and presence or absence of implanted embryos.
3. Number and appearance of blastulae and/or embryos present in the two uteri of each female.

Females to be autopsied were killed with an overdose of seven per cent nembutal injected into the thoracic cavity. Uteri, after removal of ovaries and fallopian

tubes, were flushed from both ends with a 0.85 per cent saline solution. Any free blastulae were then visible in a watch glass viewed under a binocular dissecting microscope. Uteri were checked for implantation sites by viewing them against a bright light to find concentrations of blood vessels and the characteristic dark areas which indicate embryonic sites. When such a site was noted, a small portion was removed for total sectioning to determine progression of implantation. If no blastulae were flushed out and no implantation sites were visible externally, one horn of the uterus was slit lengthwise and examined for blastulae or implantation sites by examining the endometrium thoroughly under the microscope. Ovaries and uteri were placed in 10 per cent formalin for preservation. Portions of the uteri and the ovaries were then sectioned to determine whether the females had ovulated and the physiological condition of their uteri. The organs were sectioned at five microns and were stained with Hematoxylin and Eosin. Methods for determination of ovarian and uterine stages of development were taken from Franklin (1958), Enders (1952), and Hansson (1947). Blastocysts and embryos were compared with those whose descriptions are given by Enders (1952), Hansson (1947), and Kissen and Price (1962).

Activity ratings were given to corpora lutea of the ovary, and endometrial and glandular development of the uteri in the autopsied females. Aids in determining various stages were those given in Hansson (1947) and Franklin (1958). Corpora lutea were rated by the following criteria:

1—*Inactive Phase:* Luteal cells small with dense, strongly basophilic cell nuclei.

2—*Incipient—secretion phase:* Luteal cells increased in volume.

3—*Secretion phase:* Great infiltration of blood vessels; volume of lutein cells increased greatly and many pear-shaped; cell nuclei situated in the cytoplasmic periphery.

Uteri were rated in the following manner:

1. High cuboidal-type endometrial lining with basally situated nuclei; glandular development slight with mainly connective tissue-filled lumina.

2. Columnar-type endometrial lining with nuclei displaced apically and light fringing of the luminal surface; increased glandular development, lumen free of connective tissue.

3. Full glandular development with extensive fringing of endometrium.

RESULTS—1962

Table 2 gives the results obtained in 1962 with the various progesterone treatments compared with results obtained from control groups. In only one instance was there remating of a female which had received progesterone beginning on the

TABLE 2. Effects of progesterone treatment on reproductive
performance—1962

Dose in mg	No. times mated	No. fem. mated	Per cent whelped	Av. gest. length	Av. no. kits/fem. mated
0	1	6	83	48.2	2.83
3 mg	1	8	75	46.0 A**	2.38
6 mg	1	7	43	51.3 A**	1.57
6 mg	1 & 2ˣ	1	100	45.0	1.0
0	2	4	75	45.3	3.75
3 mg	2	4	25	45.0	2.0
6 mg	2	3	0		

**Two sided t-test.
A—Significant at .05 level.
ˣMated a 2nd time after receiving treatment on 2nd day after mating.

second day after the first mating; remating was on the seventh day after the primary mating.

There is a noticeable trend of fewer kits from females in the treated groups than from those in control animals. In most instances, there is very little difference in the average length of the gestation period. However, there was one instance where a significant difference was found to exist (.05 level, 2-sided t-test) between the average length of gestation in once-mated females treated with 3 mg and 6 mg of progesterone, respectively.

A trend is also noticeable in the average weight of the kits at birth. In most instances, those receiving progesterone had smaller kits than comparable control animals. In every instance, those females that received extended progesterone treatments lost their kits within two days after whelping, probably through starvation, since there was no indication of the presence of milk in these females.

On several occasions, record was made of possible aborted material given off by treated females between five and fifteen days after the progesterone treatment was terminated (30 days after mating).

Table 3. Reproduction records for mink on progestin treatments—1963

Days when treated	No. fem. mated	No. fem. autop.	No. fem. whelped	Av. gest. length	Av. no. kits/fem. mated
7–30 day[2]	4	2	2	50.0	3.5[1]
2–whelping[2]	2	1	0		
4–whelping[2]	2	1	0		
6–whelping[2]	2	1	0		
13–whelping	2	1	0		
7–11th day[2]	2	1	0		
7–whelping[2]	8	2	0		
7th day[3] 15 mg	3	1	2	49.0	4.5
7th day[3] 30 mg	3	1	1	51.0	4.0
7, 8, 9th days[3] 10 mg	3	1	1	53.0	2.0
7, 8, 9th days[3] 15 mg	3	1	2	52.0	2.5
7, 8th days[4] 2.5 mg	3	1	1	52.0	3.0
7th day[4] 5.0 mg	3	1	2	56.0	5.5
Control	11	2	9	51.4	4.6

[1]All of these kits died.
[2]3 mg Provera per lb. body wt per day.
[3]Total amount of Provera given in mg.
[4]Total injection.

RESULTS—1963

Tables 3 and 4 give the results of the progestin studies during the 1963 breeding season. There were 14 groups of females, including the control group.

In one group, the females were given extended oral progesterone treatments until the time when they should have whelped (the 47th to 50th day after mating). When no kits were whelped, one of these females was sacrificed and autopsied. Surgical inspection was performed on another female. There were small uterine "bumps" to indicate where fetuses had been either aborted or resorbed. Corpora lutea were small and there was apparently very little secretive activity. The luteal cells were involuting.

Table 4. Activity rating of uteri and ovaries from females
autopsied—18 days after mating—1963

Treatment	Ovary Activ. Rating	Uterus Activ. Rating	Blastulae or implanted fetuses
Control	5	2	1 blas.
Control	3.5	1	11 blas.
13th–Autop[1]	4	1.5	3 blas.
7th–11th[1]	4	1	4 blas.
7th–Autop.[1]	3.5	1.5	8 blas.
7th–autop.[1]	4.0	1.0	1 blas. possible implan.
2nd–autop.[1]	4.0	2.0	5 blas. possible implan.
4th–autop.[1]	3.5	1.5	5 blas.
6th–autop.[1]	4	2	7 blas.
7th–day 5 mg inject.	4	2	5 blas.
7, 8th day 2.5 mg inject.	5	2	Implan. fetus
7th–day 15 mg oral	3.5	1	5 blas.
7th–day 30 mg oral	4	1	2 blas.
7, 8, 9th day 10 mg oral	5	2	Implan. blas. adhering
7, 8, 9th day 15 mg oral	4	2	3 blas.

[1]3 mg Provera per lb. body wt per day.

Four females were taken off the oral progesterone treatments 30 days after mating (table 5). One of these females was sacrified and autopsied on the 30th day. She had what seemed to be fairly normal development of embryos. The corpora lutea were small but active. The uterine endometrium was fringed and glandular activity was abundant.

One of the females removed from the progesterone treatment on the 30th day after mating was sacrificed on the 40th day. There were three embryos in the

TABLE 5. Embryological development in progesterone treated
mink after 30 or more days

Female	Days after mating when autopsied[1]	Status of embryos
JP–454	30	3 small normal embryos
HP–16	40	3 small embryos with heart-beat; 2 embryos degenerating; resorbing
HPS–40	43	5 aborted fetal sites on uterus. 5 days previously possible aborted material was noted.
JP–600	57	Only slightly noticeable re-sorbed fetal sites.
HPS–32	47 (surgery)	6 slightly noticeable resorbed fetal sites.

[1]Dosage was 3 mg Provera per lb. body wt per day from the 7th day after mating until the date of autopsy.

right uterine horn and two in the left. The hearts of three embryos were beating. In one embryonic site, the amnionic capsule was as large as the previous three, but the necrotizing embryo was tiny. The last uterine "bump" appeared to be resorbing. Corpora lutea were small but active. The uterine wall was fringed.

Both remaining females, removed from the treatment at 30 days after mating, whelped. The average gestation was 50 days. These two females had a total of seven small kits, three of which were stillborn. All of the remaining kits died within two days after whelping.

Females receiving one-day or three-day oral treatments of progesterone had normal to good whelping success. Also those receiving injections of progesterone on the seventh or eighth day after mating had average to good whelping success. There was no unusual mortality in these kits.

There were too few animals in the groups to note any definite increase in average size of litter or any decided decrease in the average gestation length of those animals treated with progesterone when compared to control animals.

Table 4 shows the number of blastulae flushed out of uteri from females subjected to various treatments. At 18 days after mating, blastulae from progesterone-treated females averaged 0.36 mm in diameter. The largest blastulae were found in the females subjected to oral progesterone treatments. There was only a single blastula in one control female; eleven were found in another. Most of them were extremely tiny and fragile and were lost before staining.

Possible implantation, adhering blastulae, and an implanted fetus were found in four animals of the progesterone-treated groups. None were found in the control group.

DISCUSSION

Cochran and Shackelford (1962) treated female mink with exogenous estrogen alone and in combination with progesterone for several days, beginning between four and nine days after a single mating. They found that the combination treatments prevented whelping of kits and that estrogen alone did not significantly decrease the number of kits but increased the gestation length. Furthermore, they had no evidence of corpora involution.

Franklin (1958) gave a single intramuscular injection of 5 mg progesterone in oil to several female mink, six to eight days after the second breeding. This hormone injection appeared to cause a decrease in the number of "misses" or whelping failures. The corpus luteum appeared to be more active in treated females than in control animals.

Enders and Enders (1963) reported on the morphology of the female mink reproductive tract during delayed implantation. They found that glycogen, alkaline phosphatase, and lipids varied considerably in the endometrium throughout the delayed period. Because of this observed variation in blastocyst environment, they doubted the usefulness of interpreting the hormonal basis of delayed implantation by administration of exogenous hormones. They cited Hammond (1951) and Cochrane and Shackelford (1962) as those attempting, but failing, to induce implantation in mink by these methods.

It appears, however, from results obtained by Franklin (1958) and from the investigations reported here, that administration of exogenous hormones may effect implantation in mink. Seventeen female mink autopsied 18 days after mating showed no evidence of implanted embryos except in four individuals that had received Provera. Two of these four females had embryos definitely implanted in the endometrium, and the other two had blastulae that appeared to be adhering to the endometrium. The two females that had implanted embryos showed the highest degree of corpora lutea capacity for secretion. The uterus activity rating was two for both females. Two females with possible implantations had corpora lutea with a rating of four and uterine ratings of one and two.

Results as shown in table 3 indicate that some of the females had kits after receiving Provera for one, two, or three consecutive days, beginning on the seventh day after mating. However, those treated for one day had more kits than those treated for two or three days. Females that received Provera for an extended time period whelped but few kits, and all kits died, as did those whelped from females in 1962.

The earlier implantations and high degree of corpora activity in females receiving Provera suggest that progestin administered at a proper time after mating may somehow stimulate luteal cells to become active, or cause the uterine endometrium to become ready for implantation at an earlier date. The action of the progestin can only be postulated when so few numbers of female mink are represented.

When Provera was given to a female for only one to three days, the duration of its direct effect on body tissues was short-lived. The progestin may play a role in a feedback system. I would suppose that luteotrophic hormone is present in milk and that it affects early luteal cell growth and secretion. If the LTH titer were low in the early period of delayed implantation, there would be little or no corpus luteum growth or secretion, and progestin titer would remain low. Then, if progestin titer were raised to a high level for a short time, LTH secretion might be inhibited. When the progestin titer dropped rapidly after exogenous progestin was degraded, LTH secretion might then increase. The increase in LTH might then cause enough growth and secretive activity of the corpus luteum to allow readiness for the blastocyst earlier than would have happened under normal circumstances.

The original hypothesis of these experiments was that exogenous progesterone would shorten the gestation period and produce more kits in mink. However, there was no indication of a shorter average gestation period in mink treated with progesterone, and there is no proof of more kits being produced per litter. In those receiving Provera for the extended time, the average number of kits was reduced when compared to control females.

From the evidence presented, it is possible to explain the reduced number of kits from animals treated with Provera for the extended time interval. The Provera probably caused uterine growth and increased glandular activity. This may have resulted in earlier implantation. The exogenous Provera appeared to inhibit corpus luteum growth, preventing it from reaching its fullest capacity. When females were autopsied, the sectioned corpora appeared small and involution was apparent. When animals no longer received Provera at 30 days after mating, implanted embryos were resorbed. This could have been due to low natural production of hormones from the underdeveloped corpus luteum that was then incapable of providing enough secretion to maintain pregnancy. When females received Provera for 50 days, embryos may have remained small and some were probably resorbed because of the inhibition of natural hormone production. The exogenous hormones received by the females were not sufficient to maintain a normal pregnancy. Further evidence of low progesterone secretion was apparent in the inadequate breast development in the females that whelped kits but were able to supply them with little or no milk.

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