REPRODUCTIVE PHYSIOLOGY IN MINK (Mustela vison)

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ABSTRACT

REPRODUCTIVE PHYSIOLOGY IN MINK (Mustela vison)

by Larry Claude Holcomb

Studies dealing with delayed implantation in females and spermatogenic activity in males were the chief aspects of this investigation concerning the reproductive physiology in mink.

Dark, pastel, sapphire, violet, Heinen, hope, and pearl mink were the color phases used in the experiments. Mink were housed individually in wire cages to which in most instances a nest box was attached.

Groups of female mink were subjected to extended artificial photoperiods:

1. Both before and after mating.

2. Immediately after mating.

3. Seven days after mating.

One group received less light than normal, in an attempt to delay the mating season. Males were included in some of the light experiments.

Extended light was given in the evening only. The lighting intensities varied from 3 to 40 foot candles. Duration of the added light varied between 95 and 240 minutes. Treatments were with white light in most instances with red light being used in one treatment.

Laparotomy was performed on three females from 18 to 29 days after mating. Right and left uteri and ovaries were removed alternately; the left being removed 7 days after the right. Recordings were made of the

numbers of implanted and unimplanted embryos.

Several females were subjected to various progestin treatments at different dosage levels and at different intervals of time after mating. Females received either oral treatment or injections. Oral treatments began between 2 and 13 days after mating, and terminated between 9 and 50 days after mating. Oral doses varied from 1.5 mg. of 6-methyl-17-acetoxy progesterone (Provera) per lb. body wt. per day to 30 mg. per lb. body wt. per day. Injections were given on the 7th or 7th to 8th days after mating, at dosages of 5 mg. and 2.5 mg. respectively.

Autopsies were made on 21 females receiving progestins, extra light, or maintained as controls. Implantations were recorded together with numbers and condition of blastulae and corpora lutea and uterine activity.

Testes were removed from 81 males during the months of November to April. Many of them were weighed and histological sections were made. Spermatogenic activity ratings were then given the testes.

Estrus was hastened in females subjected to extra light both before and after mating. In most instances reproductive performance was comparable to that of control animals.

In most instances, females lighted beginning immediately after mating had shorter gestations and more kits per female mated than control females. Females lighted 7 days after mating had on the average, fewer kits than control animals. Red and white light seemed to have no different

effects upon whelping performance when given in this experiment. Lighted females that could not escape light exposure averaged more kits than those that could enter their nest boxes.

Females receiving less than the normal amount of light were restricted in estrus by approximately a month. Four of 8 females were mated and 2 of them whelped.

Laparotomy on 3 control females demonstrated implantation taking place between 22 and 25 days after mating. There was a considerably higher average number of blastulae and/or embryos present in these females than kits whelped per female in the control group.

In females autopsied at 18 days after mating, only those that had received progesterone treatments had implanted embryos. Blastulae were larger and less fragile in the progesterone treated groups than those in the control and lighted groups.

Corpora lutea and uteri were more developed in females receiving short term oral or injected progestins a few days after mating than in control animals.

Females receiving progesterone treatments until 30 days after mating whelped a few small kits. They failed to live. The females did not appear to have milk production.

It is possible that additional light after mating, or post-mating progestin treatments for short intervals may trigger some hormonal mechanism, probably luteotrophic hormone, to stimulate corpus luteum development.

Those females maintained on progesterone until they should have whelped, resorbed or aborted their embryos. Corpora lutea were involuting at 50 days after mating and were very small.

There is no spermatogenic activity in males before late November. Sperm are present in the seminiferous tubules by late December or early January. However, interstitial cell development is slight until February.

Sperm production is active throughout February and March. Involution of seminiferous tubules takes place in mid-April. Noticeable changes in weight of testes coincides with spermatogenic activity.

REPRODUCTIVE PHYSIOLOGY IN MINK (*Mustela vison*)

by

Larry Claude Holcomb

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Introduction

The raising of mink (Mustela vison) for their valuable pelts on an extensive basis has stimulated considerable research on various aspects of reproduction in these animals. Many studies were concerned with delayed implantation, which occurs in mink, in an attempt to explain reasons for this phenomena. Investigations have included work with hormones, alteration of the photoperiod, effects of temperature, and a combination of these factors. Several theories have been advanced, but no conclusive evidence has been obtained to explain the major factor responsible for delayed implantation.

The relationship between the number of hours of light and dark per day controls the timing of the reproductive cycle in many animals. The perception of this daily relationship is probably through the eyes. Pearson and Enders (1944) and Bissonnette (1935) explain that light activates the optic nerve to stimulate the hypothalamus which, in turn, stimulates the anterior pituitary to secrete gonadotropic hormones. In mink and in some other members of the Mustelidae, the fertilized blastocyst does not implant in the uterine wall until an elapse of a varied amount of time. In marten (Martes americana), for example, blastocysts are not implantated for a period of several months. In other groups of mammals, delayed implantation

has been reported in the roe deer (Caproleus) by Bischoff (1854), in the European badger (Meles) by Fries (1880), and in five species of rodents by Lataste (1891). In describing the early embryonic development of the polyembryonic armadillo (Dasypus novemcinctus), Patterson (1913) reported a quiescent uterine period previous to implantation. Delayed implantation was found in the house mouse (Mus musculus) by Kirkham (1916), but it was due to the suckling of young. In no other family of mammals does the gestation period vary as much as it does in the Mustelidae. The period in mink, according to Hansson (1947), varies generally between 39 and 76 days with the average close to 49 days for once-mated females. Hansson (1947) also reported that delayed implantation causes discontinous embryonic development. However, Hansson (1947) and Pearson and Enders (1944) showed that in mink there is a definite relationship between the date of mating and the length of the gestation period. Animals that were mated early had a longer gestation period than those mated later in the breeding season.

According to Hansson (1947), if a mated polyestrous female comes into estrus, it is due either to the egg not being fertilized or to conditions not being favorable for implantation. Earlier investigators had not thought of the possibility of a resumed estrus cycle as due to failure of the zygote to implant. According to Hansson, a fertilized egg in the free vesicle state perishes if the female is stimulated to ovulate again as a result of mating. The fact that mink again come into estrus after mating does not mean that eggs from the preceding mating were not fertilized or were not in a

condition for implantation, but may be due to the uterine endometrium not being in an optimum condition for implantation. Ball (1940) reported that copulation in rats does not always elicit corpus luteum activity; although fertilized eggs are in the uterus, it is not "primed" for implantation.

ENDOCRINE EFFECTS ON IMPLANTATION:

Opinions are diverse as to the internal secretions effecting delayed implantation. Some report that there is lack of gonadotropic hormones from the anterior pituitary; others report there is a lack of estrogen and/or progesterone secretion from the corpora lutea. These two factors are closely related.

Although only two major contributions, Hansson (1947) and Enders (1952), have been made to the field of reproductive physiology in mink, a host of other workers have contributed to the general knowledge of endocrine interrelations in mammalian reproduction. Since the primary problem seems to be dependent on the implantation of more blastocysts and their persistance and development into embryos, it is expedient to determine the influence of modification of some factors known to be involved in implantation.

Corner (1928) showed that corpus luteum hormone was effective in maintaining pregnancy in ovariectomized female rabbits through injection of corpus luteum extracts. He also noted that uterine proliferation was necessary for the nutrition of the blastocyst prior to implantation.

Allen and Heckel (1939) reported that pregnancy could be maintained to term in rabbits castrated on the 11th day. Two mg. of progesterone were administered daily from the 11th to the 15th day, and 4 mg. daily from the 16th to the 28th day.

Westman (1929) reported in his experiments on the mode of formation, and the tissue responsible for, corpus luteum development. He reported that the granulosa layer of the follicle gives rise to the cell elements responsible for corpus luteum hormone. He removed the granulosa layer and observed no corpus luteum, and subsequently no uterine development. Partial removal of the granulosa layer caused formation of a partial corpus luteum.

Block (1939) stated that the corpus luteum hormone is functional for development of the uterine endometrium, and for the accomplishment of implantation and development of the implanted egg. He also noted that a mouse uterus ready for implantation has an intense secretion from the glandular epithelium. This secretion is found only when free blastocysts are present in the uterus or any time the corpus luteum persists or progesterone is given artificially. It is assumed that this secretion is absorbed by the blastocysts thus providing nutritive elements until the placenta is formed.

Mossman (1937), in his work with rabbits, reported that uterine proliferation was not only necessary for implantation, but also for nutrition and protection of free blastocysts during the time between their arrival and their implantation in the uterus.

Hisaw and Leonard (1930) thought that the follicular hormone estrogen primed the uterus prior to the production of corpus luteum hormone and suggested a synergistic action between the follicular hormone estrogen and the corpus luteum hormone (estrogen and progesterone).

According to Enders (1952) and Hansson (1947), a change occurs in the cytological appearance of the corpora lutea, accompanying growth from the "resting stage" to the active stage in mink. There is a resting stage during the time the ova are fertilized until just previous to implantation.

Makepeace et al. (1936) demonstrated that corpus luteum hormone inhibits the contractile action of pituitrin (oxytocin) on the uterine mucosa. This inhibitory effect on uterine motility was also demonstrated in vivo by Allen and Reynolds (1935). A quiescent uterine period has been reported essential for complete implantation of the blastocysts.

Astwood and Fevold (1939) reported that progesterone suppresses the release of luteinizing hormone (LH) from the pituitary and that this suppression is responsible for the absence of estrous cycles during periods of active luteal function. They thought that some other pituitary hormone was responsible for the continued function of the corpora lutea.

Long and Evans (1922) showed that the female rat does not undergo an estrus period when suckling her young. If she is suckling a large number of young, the corpora lutea do not develop fully until implantation occurs. Hamlett (1932) also showed this to be the case in the nine-banded armadillo.

Selye, Collip, and Thomson (1935) demonstrated maintainence of the uterus and mammary glands in the rat for 6 days, after removal of the ovaries and all embryos. This indicates that progestins and estrogens are being released by other tissues, possibly the placenta or adrenal.

Astwood and Greep (1938) indicated that the placenta in the rat secretes a substance responsible for maintaining functional corpora lutea during the latter half of pregnancy. The pituitary gonadotropins support the corpus luteum of early pregnancy until the 10th or 11th day. Then chorionic gonadotropin maintains and enlarges the corpora lutea whose secretions maintain pregnancy. A similar mechanism is probably active in other mammals. They also found that hypophysectomized pregnant rats maintained functional corpora lutea due to placenta hormones.

Deanesly and Newton (1941) stated that if placentae are maintained, the corpora lutea remain functional even though hypophysectomy is performed on mice on the 12th day of pregnancy. Foetuses were also maintained. However, elimination of the placentae caused corpora lutea to degenerate whether the pituitary was present or not.

Long and Evans (1922) reported the cessation of corpus luteum activity in the rat shortly before parturition.

Walton and Hammond (1928) reported that no corpora lutea were formed if a fully developed follicle was punctured prior to mating. This demonstrates that the formation of the corpora lutea is not an isolated phenomena which is elicited by the rupture of the follicle. It demonstrates

that at least in rabbits, the corpora lutea formation is conditioned by other factors, probably endocrine.

Astwood (1941) reported that corpora lutea are dependent on pituitary luteotrophin during early pregnancy.

Follicle stimulating hormone (FSH) is essential to follicular development beyond the stage of antrum formation. This hormone is probably secreted from the pituitary during the latter part of the luteal phase and follicular phase up to the time of ovulation.

Chow et al. (1939), Fevold (1939), and Shedlovsky et al. (1940), demonstrated that LH is essential to estrogen secretion from developing follicles. This indicates that LH is secreted from the pituitary during the follicular phase in amounts possibly too small for luteinization. More LH is produced as the follicular phase advances and may account for the increased output of estrogen from the ovary. LH secretion or the ratio of LH to FSH eventually reaches a sufficient level to bring about ovulation of mature follicles. Luteotrophic hormone then becomes essential to the secretion of corpus luteum hormone. The role of corpus luteum hormone and its repression of LH release from the pituitary accounts for the absence of ovulation or luteinization of follicles while an active corpus luteum is present.

Meyer and Hertz (1937) showed that estrogen depresses FSH production. This mechanism is probably sufficient to explain the limited but constant number of follicles maturing at a certain time. In addition to the

above requirements, some species require a nervous stimulus in order to complete the cycle. The added stimulus of copulation or "riding" is necessary in the cat, ferret, rabbit and mink to stimulate release of LH to cause ovulation. The copulation or "riding" is a necessary stimulus for production of luteotrophin in the hamster. The stimulus needs only to be of short duration in both of these instances. The ovary and the pituitary both show a tendency toward cyclic activity. They regulate each other through endocrine reactions thus controlling the reproductive cycle. Estrogen apparently governs the output of FSH. Corpus luteum hormone may inhibit the production of luteotrophin from the anterior pituitary. The importance of these factors probably varies from one species to another.

It was first suggested by Hammond (1951b) that the injection of progesterone after mating might result in an increase in the litter size of mink. Further work has been done with progesterone by Hansson (1947) and Franklin (1958). Hansson reported that injections of progesterone after mating had no effect on the delayed implantation, but that if the ovary was removed and then injections of progesterone were made each day, the uterus lost the turgidity typical of estrus. Blastulae present showed a tendency to shrivel as is the case before implantation. Hansson believed that delayed implantation in mink was due to a continued active follicular phase in the ovary during the free vesicle state, producing estrogen which suppressed the effect of progesterone. Franklin reported that the injection of

progesterone decreased the number of "misses" in females. This resulted in a higher average number of kits per female mated. Hamlett (1935) had negative results from the injection of synthetic progesterone into mated armadillos.

EFFECTS OF PHOTOPERIODICITY UPON THE REPRODUCTIVE CYCLE

Considerable research has been conducted on the effect of light on animal reproductive cycles.

Pearson and Enders (1944) shortened the gestation period in the marten (Martes americana) by steadily increasing the length of the day by artificial light beginning in September. They had been successful in mating three of eight females under increasing artificial light conditions They believed that an increase in the length of daylight shortened the time to implantation and reduced loss of blastocysts in some females.

Bissonnette (1935) reported that decreasing light from 19 to 6 hours per day caused ferrets (Mustela putorius) to go from an estrous state into an anestrous state. When light was then increased in duration, estrus was exhibited again.

Hammond (1951 a) treated mink with alternate 7 and 5 hour light and dark periods in winter and then returned them to natural light conditions at the end of February. The mink had shrunken gonads in mid-March, a condition comparable to mid-summer. When ferrets were subjected to a constant 14 or 24 hours of light per day, they came

into estrus sooner on the 14 hour day. This stresses the importance of light-dark intervals.

Pearson and Enders (1944) noted that female mink, when subjected to 1.5 hours of added light per day both before and after mating, had a mean gestation length of 49.9 days. Females illuminated under a similar lighting regime only after mating had a gestation length of 50.5 days. A control group of females had a mean gestation length of 54.7 days. Decreasing the light day by 1.5 hours did not effect the length of the gestation period

Hronopulo (1956) subjected female mink to various lighting regimes after mating. He found that increased light increased the average number of kits per litter and a decrease in light decreased the number of kits per litter.

Holcomb et al. (1962) found that increased light both before and after mating hastened the mating season in mink. These females had an increased litter average compared to the control group. Females taken from the artificial illumination after mating had a significant increase in the length of the gestation period. Females lighted only after mating had a decreased length of the mean gestation period and a corresponding increase in the mean number of kits per female mated. It was also noted that sapphire mutations with limited eye pigment responded faster to changes in light duration. Bowness (1957) subjected mink to increasing light in October and was successful in mating several animals within twelve weeks thereafter.

Kirk (1962) found that when mink were subjected to darkness from late December to mid-May, several females mated and many of them whelped. The average gestation length was considerably lengthened.

Hammond (1951a) reported that initiation of the spring breeding season may be a short day phenomenon. Spermatogenesis may start in December and some females show an estrous smear in late January.

Enders (1952) wrote that in general, year-old males are somewhat later in displaying breeding activity than older males. Bowness (1948) commented on the phenomena that young males if handled and shipped, were better breeders in their first year. These observations suggested that testes activity should be investigated at different times of the year.

Mink have but one breeding season each year extending from late February to late March. The length of day during this period increases at a constant rate (Holcomb et al. 1962). It is probably this constant rate of increase in light which governs release of gonadotropins from the anterior pituitary. These gonadotropins without a doubt regulate gonadal development, mating behavior, and delayed implantation.

Experiments herein were designed to determine what effect various lighting regimes would have on the reproductive cycle with regard to:

1. Increased duration of light both before and after mating.
2. Added duration of light only after mating.
3. Restriction of light to delay the mating season.
4. Verification of varied light effects on different mutations.

5. Responses to different light duration, intensity, and red and white light.

In trying to answer questions regarding delay of implantation it was decided to:

1. Perform laparotomy on several mated females to determine the number of blastocysts or embryos present and the approximate time of implantation.

2. Perform experiments with progesterone treatments, both oral and injected.

3. Alter progesterone treatments as to dosage and interval during which the treatment was given.

4. Perform autopsies on females from lighted, control, and progestin treated groups to ascertain especially the blastulae present, embryos implanted, and condition of the corpora lutea and uteri. Animals would be sacrificed at different intervals in order to determine the changes taking place in the reproductive tract.

By performing these experiments, it was hoped that some knowledge might be acquired about physiological mechanisms governing reproduction in mink.

METHODS AND PROCEDURES

This research will be described in terms of 5 experiments. They will be divided as follows:

I. Alteration of the photoperiod at the Michigan State University Fur Experimental Station.

II. Alteration of the photoperiod at a commercial mink ranch.

III. Alternate unilateral removal of ovaries and uteri.

IV. Effects of progestins on delayed implantation.

V. Spermatogenic activity in male mink.

Many different color mutations, dark, pastel, sapphire, violet, hope, Heinen, and pearl, were used in the experiments to be described. Mink subjected to different treatments were compared as to such matters as litter size and gestation length only if they were of the same color phase.

Some abbreviations will be used to indicate various lighting regimes and the number of times the females were mated. (N) will refer to no alteration in the photoperiod. (L) will refer to additional light per day by artificial means. (R) will refer to less light per day than normal. One asterisk (*) will refer to one mating. Two asterisks (**) will refer to two matings. Thus (NN)* would designate a female mated once; not lighted before mating and not lighted after mating. (NL)** would designate a female mated twice; not lighted before mating, but lighted after mating. (R)* will refer to females exposed to less than the natural photoperiod for purposes of delaying the reproductive season.

Mink at the Michigan State University Fur Experimental Station were picked randomly to fill the various groups for experimentation. Mink at the commercial ranch were placed into categories strictly as to the discretion of the mink rancher. The manager of the ranch had full control of these arbitrary decisions. All experiments had a nearly equal distribution of old and young females. Methods of statistical analysis included the use of Students t-distribution. Both one and two tailed t-tests were employed at the .05 level depending on the nature of the assumptions made previous to the experiment.

Mink at the commercial ranch received a nutritious commercial mink ration. Mink at the Michigan State University Fur Experimental Station received a balanced ration including horsemeat, fish, tripe, liver, poultry products, cereal, vitamins, and minerals.

If any female died after placing it within a certain group, it was not considered in calculating the mean number of kits per female mated.

Experiment I. Alteration of the Photoperiod at the M. S. U. Fur Experimental Station.

A. 1961-62 -- Artificial light both before and after mating (LL)*.

All mink used were in outdoor wire cages with attached nestboxes. Therefore, the mink could avoid the added light by remaining in their nest. There was a canvas barrier between the lighted and control groups.

This lighting experiment was started on December 21, 1961. The objectives were to hasten the mating season by increasing the light per

day by means of artificial illumination. Mink received a photoperiod comparable to March 1st by the 12th of January. Then a natural rate of increase was given until whelping. The extra light was given in the evening only. Artificial illumination was provided with a string of outdoor, white, incandescent, 7-watt bulbs. There was approximately one light to each cage providing a minimum of 2 and a maximum of 5 foot candles of light.

Ten males (5 sapphire and 5 dark) and 30 females (15 sapphire and 15 dark) were subjected to the extra light. Forty females (20 dapphire and 20 dark), together with several males of both color phases, were used in a control group.

Throughout the breeding season, vaginal smears were obtained from several females to determine the onset of estrus. When a female was in estrus, a male was introduced into her cage. All females were mated once and were checked for presence of sperm.

B. 1962 -- Restricted Lighting (R)*

This experiment was started on December 21, 1961. The object was to delay the mating season. Ten females and 3 males (all pastel) were housed in a shed where light could be controlled. They were in wire cages with a hanging sack for a nest. The artificial illumination was supplied by two 75-watt white bulbs, which provided 10 to 15 foot candles of light. When light was increased, it was during the early morning hours. The light was turned off at 4:00 P.M. every day and doors were closed. Light traps were present over the windows to provide ventilation. These mink received

daily light conditions approximating those present on December 21 (the day when the experiment began), until January 21st. On the latter date, light then was increased at the regular rate to simulate natural conditions in late December and January. Therefore, a delay in mating of approximately one month was expected.

Vaginal smears from a few females were obtained weekly to determine the onset of estrus. Males were taken to the females in mating attempts. Any matings were checked for presence of sperm.

C. - 1. -- 1962 -- Added light after mating (NL).

The object of this experiment was to determine if an increase in light would decrease the length of delayed implantation and also increase the number of kits whelped. The experiment began when females (20 dark and 20 sapphire) started mating in the normal March mating season. These females were moved into the lighted cages on the day of mating. Ninety-five minutes of extra light was given each day after sundown. The same controls were used as in part A.

Artificial illumination, nests, cages, and methods of mating were the same as in part A. The majority of these females were mated by (NN) males. However, a few were mated by (LL) males.

C. -- 2. - 1963 Added light after mating (NL)*.

Objectives of this experiment were the same as C-1, but differences in the effect of red and white light and increased intensity and duration of light were to be recorded. Females were moved to the lighted area on the

7th day after they had mated. The mink were subjected to 3 hours of extra light after sundown.

Four dark females were subjected to red light and 4 dark females were subjected to white light. Sixteen dark females were used as a control group. Twelve pastel females were subjected to white light. A control group consisted of 12 females. Two females from both the lighted and control groups were autopsied as described in Experiment IV.

Artificial illumination was given by means of 3 strings of 7-watt bulbs attached to the front of the cages. The mink received either all white or all red light. Barriers were placed between lighted and control groups and also between those mink lighted with red and white light. There were approximately 3 bulbs to each cage. The white light provided a minimum of 1 and a maximum of 7 foot candles. The minimum was estimated as that amount received when the female was near the front of the cage.

Experiment II. Alteration of the Photoperiod at a Commercial Mink Ranch -- (NL)* and (NL)**.

A. 1962.

This experiment began when mink started breeding in early March. The objectives were the same as in experiment I-C. The mink were in outdoor type wire cages with a nestbox provided. These cages were under a roofed shed with open sides. There were 102 sapphire, 46 violets, 20 hope, and 34 Heinen females subjected to artificial light. There were

10 sapphire, 10 violet, 19 hope, and 92 Heinen females in the control groups. Ninety-five minutes of extra light were given after sundown. The extra 95 minutes of light was increased gradually over a 5-day period from March 5 to 10. Artificial light above the cages was provided by 75-watt incandescent bulbs, which gave 3 to 10 foot candles of light. All the treated female mink were subjected to artificial illumination on the day of the first matings. They were all subjected to the light at the same time, because of inadequate facilities for moving them to the lighted area after mating. Many of the females received extra light per day for several days before mating. Control females and all males received no extra light.

In most instances, males were taken to the cages of the females to mate. Sperm checks were not made on the females. Two matings were obtained if possible; the second mating occurred 7 or more days after the initial mating. Mouths of obstinate females were tied shut at mating during the middle and late breeding season to prevent injury to the males. This action enhances breeding success in many instances providing that the females are in estrus.

B. 1963.

The objectives and methods were similar to those in part A-1962. One hundred-eighteen sapphire, 10 pearl, 42 hope, and 10 Heinen females were in the treated groups. Ten sapphire, 67 pearl, 10 hope, and 123 Heinen females were in the control groups. Treated females were not

subjected to extra light until 9 days after the mating season started. Therefore, most females had mated once before receiving extra light. Some had been mated twice. These females were subjected to 2 1/2 hours of extra light per day after sundown from the same type of lighting setup as in part A-1962.

Some lighted females were in a shelf-like, uncovered nest where they could not escape the artificial illumination. Separate records were maintained on these females.

Experiment III. - ALTERNATE UNILATERAL REMOVAL OF OVARIES AND UTERI (NN)*

The objectives of this experiment were to determine the length of the delayed implantation, numbers of unimplanted blastocysts, numbers of implanted embryos, and improvement of surgical techniques for further investigations. The average numbers of blastocysts and embryos present in the uteri of these females were compared statistically with a one-sided t-test at the .05 level with the average number of kits whelped by the control group.

Three dark females were subjected to laparotomy during late March and early April. They had all mated at nearly the same time in March. All were mated once and were checked for sperm when mated.

The three females were first subjected to laparotomy, one each day, on the 18th, 20th, and 22nd day after mating. Females were anaesthetized with 1cc. of 7 percent nembutol solution per kilogram body wt. placed in the axial region of the foreleg. An incision was then made in the posterior

mid-ventral region of the linea alba. Semi-antiseptic procedures were employed. The left ovary and uterus were removed after tying off the base of the uterine horn. Several stitches were then taken in the incision and a powder antiseptic was placed on the surgical site. A week later the right uterus was removed in a similar manner.

Uteri and ovaries were placed immediately in .85 per cent saline solution to await further observation. In addition, each uterus was slit lengthwise and pinned out on a dissecting board to view under a binocular dissecting microscope. Each specimen was kept moist by applying saline solution at regular intervals. A probe was then used to examine every part of the exposed endometrium. Free blastocysts and implanted embryos were counted and removed when possible with a micropipette and were placed on glass slides. Observations were noted as to uterine development.

IV. EFFECTS OF PROGESTERONE TREATMENT ON DELAYED IMPLANTATION

In the 1963 and 1963 breeding seasons several pastel females (23 in 1962, and 41 in 1963) were subjected to varying progestin treatments.

In 1962, twenty-three 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 a form called Provera (6-methyl 17-acetoxy progesterone) at the rate of 1.5 or 3.0 mg. per lb. body wt. per day. Some females were given Provera 2 days after mating and a second mating attempt was made after 7 days. Others were mated but once and given Provera 2 days following mating. Still other females were

given Provera 2 days following a 2nd mating. The Provera was mixed with the mink ration on a daily basis in a premix of soybean meal used to facilitate mixing. The Provera was fed for 30 days after the first dose. A control group of 10 females was maintained. Because of a lack of females and because of the many aspects of this experiment, there were only a few mink in each group.

Average numbers of kits per litter and the average length of the gestation period were recorded. A 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. Eleven control females were maintained. Some females received 3 hours of extra light per day as described in part II-B, to compare with those receiving the progesterone treatments.

Several females received oral doses of progesterone. Others received it as injections. Two matings were obtained; one following the first by a day. All were checked for sperm after mating. Mouths were tied in all females as described in Experiment II.

Females were picked at random for the various groups. There was a fairly even distribution of kit and older females in each group. An attempt was made to have an equal distribution of early and late mated females in each group. This is because females mated early in the mating

season have a longer delayed implantation resulting in less kits per female than females mated later. Mating times of all 64 females were subdivided into three mating intervals and then the females were placed into the separate groups. The 3 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. Table I indicates the number of females in the various groups and the treatments to which they were subjected.

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. Therefore, an injection of 5 mg. required .1 cc. of the glycol solution.

Seventeen females were autopsied on the 18th day after mating. Four other females were autopsied; one on the 30th and one on the 40th day after mating. The others were autopsied on the 50th and 57th day after mating.

In autopsied mink, several factors were to be 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 2 uteri of each female.

Females to be autopsied were killed with an overdose of 7 percent

nembutol injected into the thoracic cavity. Uteri, after removing the ovary and fallopian tubes, were flushed from both ends with .85 per cent saline solution by means of a syringe. Any free blastulae were then visible in a watch glass viewed under a binocular dissecting microscope. Uteri were checked for implantation sites by first placing them over a bright light and viewing them in search for concentration of blood vessels and the characteristic dark area which indicates embryonic sites. If such a site was noted, this small section was removed for total sectioning to determine progression of implantation. If no blastulae were flushed out, and no implantation sites were visible externally, one uterus was slit lengthwise and examined for blastulae or implantation sites by examining the endometrium thoroughly under the microscope. The uteri and the ovaries were then histologically sectioned to determine whether the female had ovulated and the condition of each uterus.

Ovaries and uteri were placed in 10 per cent formalin for preservation. After tissue fixation and imbedding, the organs were sectioned at 5 microns and were stained with Hematoxylin and Eosin. Blastulae and small embryos were fixed in 95 per cent alcohol immediately and then stained with Eosin or Hematoxylin and placed on a glass slide under a cover slip.

Determination of ovarian and uterine stages of development was taken from Franklin (1958), Enders (1952), and Hansson (1947). Blastocysts and embryos were compared with those descriptions given by the aforementioned works and Abbott and Price (1962).

TABLE I TREATMENT OF FEMALES WITH PROGESTERONE AND INTERVAL DURING WHICH THE TREATMENT WAS GIVEN.

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

*3 mg. of progesterone per day per lb. body wt.

** Four females were removed 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 Progesterone.

[2] Injected Progesterone.

V. Spermatogenic Activity in Male Mink

Testes were taken from a total of 81 males in October, November, December, January, February, March, and April of 1961, 1962, and 1963. Weights of testes were recorded and histological sections were made to determine reproductive activity. If possible, differences were to be noted between the condition in older males and that in juvenile males at various months throughout the year, and what effect this might have on differences in reproductive behavior.

RESULTS

For aid in clarity, the results of the 5 experiments will be given in the same order as described in methods and procedures.

Experiment I. Alteration of the Photoperiod at the Michigan State University Fur Experimental Station

Table II gives the results of Experiment I for both the 1962 and 1963 reproductive seasons.

In every instance there was a decrease in the average length of gestation and an increase in the average number of kits per female mated from those mink subjected to additional light immediately after mating.

As indicated by vaginal smears and confirmed by successful matings during the first and second week in February, Estrus was advanced by approximately a month, in the (LL) females subjected to additional light prior to mating. One female was mated on January 23rd but did not whelp. The first litter from this (LL) group was whelped on March 31st, approximately a month in advance of those females whelping under normal lighting conditions. Mating success was good and production from these early matings was comparable to that of the controls. All successful matings in February were secured with lighted males.

Vaginal smears and trial matings from the (R)* females, restricted in light, indicated they were restrained to proestrus by the restricted photoperiod until late in March. Four matings verified by checking for sperm were obtained; two each on April 3rd and 4th.

TABLE II EFFECTS OF VARIOUS LIGHTING REGIMES

ON THE REPRODUCTIVE PERFORMANCE OF MINK - IN EXP. I

No. of mink	Type of mink	Lighting[1] regime	No. fem. mated	Percent whelped	Average gest. (days)	Av. No. kits/fem. mated
20[2]	Dark	(NL)*	18	89	48.6 B	4.22
20[2]	Saph.	(NL)*	18	68	48.6 C	2.21
15[2]	Dark	(LL)*	11	75	A 48.7	3.67
15[2]	Saph.	(LL)*	11	47	52.4	1.40
8[2]	Pastel	(RN)*	4	50	49.0	2.25
20[2]	Dark	(NN)*	15	80	A 51.5 B	3.80
20[2]	Saph.	(NN)*	13	38	52.4 C	.85
11[4]	Pastel	(NN)**	11	100	51.4	4.60
11[4]	Pastel	(NL)**	11	89	49.6	3.90
15[4]	Dark	(NN)**	15	87	47.8	4.80
4[4]	Dark[3]	(NL)**	4	75	50.0	2.75
4[4]	Dark	(NL)**	4	50	49.5	2.75

[1] Meanings of abbreviations are described in procedures.

[2] These females were experimented with in 1961 - 1962.

[3] Refers to the use of red light.

[4] 1963-Mated twice if possible; light when added was started at 7 days after mating.

Capitol letters present in conjunction with average gestation periods designates significance at the .05 level using a 1-sided t-test.

However, none of the restricted males demonstrated any mating attempts, and control males were used to secure the four matings. Since this time was later than the normal mating season (matings were tried from March 26th to April 27th), the demonstration of unwillingness of the males to mate may have been the cause of low percentage of matings. Mating attempts were made on alternate days until April 27th when vaginal smears showed the onset of anestrus in the females. Two litters were whelped; one on May 21st, the other on May 24th. Neither litter lived beyond the first week.

Experiment II. Alteration of the Photoperiod at a Commercial Mink Ranch

Tables III and IV give the reproductive performance of mink during the 1962 and 1963 breeding season in Experiment II. As demonstrated in the table, there were few animals in some of the groups. However the results still give strong support of an effect of the light upon reproductive performance.

1962. -- The average gestation length was shorter for the treated groups than untreated controls in every instance. Significant differences between the (NL) and (NN) groups were found in several instances by using a t-test.

In most instances, there was an increase in the average litter size per female mated in the sapphire and violet groups that were treated when compared to controls. There were no significant differences noted

in reproductive performance between the lighted and the control groups of these two color phases.

A different phenomena was present in the hopes and Heinens. In every instance, the treated groups averaged fewer kits per female mated than comparable control groups. However, in every instance the average length of gestation was greater in the control groups; in one instance there was a statistical difference between them at the .05 level using a 2-sided t-test.

One-sided t-tests were used in drawing conclusions about sapphires and violets since previous investigation gave evidence that these two mutations would react favorably to increased light. Two-sided t-tests were used in drawing conclusions about hopes and Heinens.

1963. -- With the exception of the hope strain, the average gestation length was shorter for the treated groups than comparable control animals. Only once was there a significant difference found between the average length of the gestation in the lighted and control groups.

The average litter size per female mated was as great or greater in the treated females than in the controls. There was a significant difference between these two groups in only one instance.

The same statistical analyses were used in this phase of the experiment as in the case of the 1962 groups. Any analyses of the data associated with the pearl mutation was subjected to two-sided t-tests.

Several of the small groups of lighted females could not escape

TABLE III EFFECTS OF VARIOUS LIGHTING REGIMES ON THE REPRODUCTIVE PERFORMANCE OF MINK IN EXP. II - 1962

No. of mink	Lighting regime	No. fem. mated	Percent whelped	Average gest. length	Av. no. kits/fem. mated
Saph.	(NL)**	67	87	43.6 A[1]	4.18
Saph.	(NN)**	8	63	48.2 A[1]	3.13
Violet	(NL)**	24	63	44.9 B[1]	2.71
Violet	(NN)**	7	43	53.7 B[1]	2.29
Violet	(NL)*	22	32	46.6	1.32
Violet	(NN)*	3	0		
Saph.	(NL)*	35	69	46.4 C[1]	3.17
Saph.	(NN)*	2	100	49.5 C[1]	4.00
Hope	(NL)**	11	73	41.6	3.18
Hope	(NN)**	7	100	42.6	4.57
Hope	(NL)*	9	56	47.0	1.22 E[2]
Hope	(NN)*	12	83	50.1	4.75 E[2]
Heinen	(NL)**	7	71	41.0 D[2]	2.71
Heinen	(NN)**	46	85	45.6 D[2]	4.00
Heinen	(NL)*	27	63	46.3	3.22
Heinen	(NN)*	46	67	48.6	3.46

Capital letters present in conjunction with av. gestation period and kits/female in table III designates significance at the .05 level using a t-test.

[1] 1-sided t-test used.

[2] 2-sided t-test used

TABLE IV EFFECTS OF VARIOUS LIGHTING REGIME S ON THE REPRODUCTIVE PERFORMANCE OF MINK IN EXP. II - 1963

Type of mink	Lighting regime	No. of fem. mated	Percent whelped	Average gest. length	Av. no. kits/fem. mated
Saph.	(NN)**	10	90	48.4	3.30
Saph.	(NL)**	83	76	49.1	3.84
Saph.	(NL)**	35	97	49.9	3.20
Heinen[1]	(NL)**	10	90	50.2	4.80
Heinen	(NN)**	77	82	47.3	4.05
Heinen	(NN)*	46	72	49.1	3.89
Pearl[1]	(NL)**	7	100	47.1 A[2]	5.86
Pearl	(NN)**	51	84	52.5 A[2]	4.59
Pearl[1]	(NL)*	3	100	48.0	3.67
Pearl	(NN)*	16	63	53.1	3.31
Hope	(NN)**	3	67	41.5	3.00 B[2]
Hope	(NL)**	16	69	42.3	3.94
Hope	(NN)*	7	43	46.3	3.00
Hope[1]	(NL)**	7	100	42.0	6.43 B[2]
Hope[1]	(NL)*	5	80	51.0	3.00
Hope	(NL)*	14	71	47.0	3.71

Capitol letters present in conjunction with av. gestation period and kits/female designate significance at the .05 level using a t-test.

[1] Could not escape artificial light exposure. [2] 2-sided t-test used

total light exposure. These females had large litters with a good percentage of mated females whelping. In the hope strain, (NL)** females that could not escape light exposure, averaged 2.49 more kits per female mated than comparable treated females that were able to avoid the light exposure.

Experiment III. Alternate Unilateral Removal of Ovaries and Uteri

Results of this experiment are given in Table V. In female I there were 6 normal-appearing unimplanted blastulae at 18 days after mating. There was no indication of an implantation site.

Female II had the right ovary and right uterus removed 20 days after mating. There was one unimplanted blastula present. The uterine endometrium showed signs of readiness for implantation. At 27 days after mating female II underwent laparotomy for removal of the left ovary and left uterus at which time five implanted foetuses were found.

Female III had the right ovary and right uterus removed 22 days after mating. In the uterus were five unimplanted blastulae that appeared abnormal; possibly degenerating. The uterus appeared ready for implantation. At 29 days after mating, female III underwent laparotomy for removal of the left ovary and left uterus disclosing 4 implanted foetuses.

Fifteen dark females maintained as control animals averaged 3.8 kits per female mated as given in Table II. There was an average of 7 blastulae (unimplanted and implanted) present in those females upon

TABLE V UTERINE CONTENTS FROM FEMALES UPON WHICH LAPAROTOMY WAS PERFORMED

	Date of surgery	No. of days post-mating	Blastula Rt. uter.	Blastula Left uter.	Implanted foetuses left uter.
Fem. 1	3-28-62	18	4	2	
Fem. 2	3-31-62	20	1		
	4- 7-62	27			5
Fem. 3	4- 3-62	22	5		
	4-10-62	29			4

which laparotomy was performed. There were, on the average, significantly (.05 level) more blastulae present in the uteri of the three females upon whcih laparotomy was performed, than the average number of kits whelped by the control females.

Experiment IV. Effects of Progestin Treatments on Delayed Implantation 1962. Table VI gives the results obtained under the various progesterone treatments in addition to those obtained in control groups.

As can be noted, the size of all groups was small. Some difficulty was found in mating these pastel females because of inadequate numbers of proven males. As a last resort, many were mated to dark males.

In only one instance was a female remated on the 7th day after the primary mating, when receiving progesterone beginning on the 2nd day after mating.

TABLE VI EFFECTS OF PROGESTERONE TREATMENT ON REPRODUCTIVE PERFORMANCE - 1962

Dose in. mg.	No. times mated	No. fem. mated	Percent 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 - Signigicant at .05 level
x Mated a 2nd time after receiving treatment on 2nd day after 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

TABLE VII REPRODUCTION RECORDS FOR

PASTEL MINK IN PROGESTIN AND LIGHT EXPS. - 1963

Days when t reated	No. fem. mated	No. fem. autop.	No. fem. whelped	Av. gest. length	Av. no. kits/fem. mated
7-30 day*	4	2	2	50.0	3.5[x]
2-whelping*	2	1	0		
4-whelping*	2	1	0		
6-whelping*	2	1	0		
13-whelping *	2	1	0		
7-11th day*	2	1	0		
7-whelping*	8	2	0		
7th day** 15mg.	3	1	2	49.0	4.5
7th day** 30 mg.	3	1	1	51.0	4.0
7, 8, 9th days** 10 mg.	3	1	1	53.0	2.0
7, 8, 9th days** 15 mg.	3	1	2	52.0	2.5
7, 8th days[y] 2.5 mg.	3	1	1	52.0	3.0
7th day[y] 5.0 mg.	3	1	2	56.0	5.5
control	11	2	9	51.4	4.6
7-whelping	11	2	8	49.6	3.9

[x] All of these kits died.

[y] Total injection.

* 3 mg. Provera per lb. body wt. per day.

** Total amount of Provera given in mg.

TABLE VIII ACTIVITY RATING OF UTERI AND OVARIES FROM FEMALES AUTOPSIED - 18 DAYS AFTER MATING

Fem.	Treatment	Ovary activ. rating	Uterus activ. rating	Blastulae or implanted foetuses
1	control	5	2	1 blas.
2	control	3.5	1	11 blas.
3	light	3.5	1	nothing
4	light	3	1	8 blas.
5	13th autop.*	4	1.5	3 blas.
6	7th - 11th*	4	1	4 blas.
7	7th - autop.*	3.5	1.5	8 blas.
8	7th - autop.*	4.0	1.0	1 blas. possible implan.
9	2nd - autop.*	4.0	2.0	5 blas. possible implan.
10	4th - autop.*	3.5	1.5	5 blas.
11	6th - autop.*	4	2	7 blas.
12	7th - day 5 mg. inject.	4	2	5 blas.
13	7, 8th day 2.5 mg. inject.	5	2	implan. foetus
14	7th - day 15 mg. - oral	3.5	1	5 blas.
15	7th - day 30 mg. - oral	4	1	2 blas.
16	7, 8, 9th day 10 mg. - oral	5	2	implan. blas. adhering
17	7, 8, 9th day 15 mg. oral	4	2	3 blas.

* 3 mg. per lb. body wt. per day.

TABLE IX SIZE AND MAINTENANCE OF BLASTULAE AT 18 DAYS AFTER MATING IN FEMALES UNDER VARIED TREATMENTS

Fem.	No. of blas. flushed	No. of blas. stained	Av. size of blas. * in mm.	Days treated	Implantation
6	4	1	.30	7 - 11th[x]	
9	5	4	.35	2 - autop.[x]	Possible implantation
7	8	8	.39	7 - autop.[x]	
8	1			7 - autop.[x]	Blastula attaching
5	3	3	.27	13 - autop.[x]	
10	5	4	.38	4 - autop.[x]	
11	7	6	.34	6 - autop.[x]	
1	1	1	.30	control	
2	11	2	.34	control	
4	8	7	.32	7 - whelp. Light	
3	0			Light	
17	3	3	.45	7, 8, 9th days 15 mg. - oral	
16	0			7, 8, 9th days 10 mg. - oral	Blastula attaching
15	2			7th day 30 mg. - oral	
14	5	3	.39	7th day 15 mg. - oral	
12	5			7th day 5 mg. - inject.	
13	0			7, 8th day 2.5 inject.	Implanted foetus

* Size in diameter.

[x] Oral progesterone treatment - 3 mg. per lb. body wt. per day.

TABLE X EMBRYOLOGICAL DEVELOPMENT IN PROGESTERONE - TREATED MINK AFTER 30 OR MORE DAYS.

Female	Treatment	Days after mating when autop.	Status of embryos
JP-454	1	30	3 small normal embryos
HP-16	1	40	3 small embryos with hartbeat; 2 embryos degenerating, resorbing.
HPS-40	2	43	5 aborted foetal sites on uterus. 5 days previously we had noted possible aborted material.
JP-600	3	57	Only slightly noticeable resorbed foetal sites.
HPS-32	4	47 (surgery)	6 slightly noticeable resorbed foetal sites.

1 - 3 mg. oral proges. per lb. body wt. per day; 7th to 30th day.

2 - 3 mg. oral proges. per lb. body wt. per day; 7th to death.

3 - 3 mg. oral proges. per lb. body wt. per day; 7th to 50th day.

4 - 3 mg. oral proges. per lb. body wt. per day; 7th to 47th day.

most instances those receiving progesterone had smaller kits than comparable control animals. In every instance, those females that received progesterone treatments lost their kits within two days after whelping, probably through starvation because there was no indication of the presence of milk in these females.

On several occasions, record was made of possible aborted material from treated females between 5 and 15 days after the progesterone treatment was ceased at 30 days after mating.

1963. --- Tables VII, VIII, IX, and X give the results of the progestin studies during the 1963 breeding season. There were 15 groups of females including the lighted and control groups.

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. Surgery was performed on another female. There were small uterine "bumps" to indicate where foetuses had been either aborted or resorbed. Corpora lutea were small and there was apparently very little secretive activity. The luteal cells were involuting.

Four females were taken off the oral progesterone treatments at 30 days after mating. One female wassacrificed 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 and autopsied on the 40th day. There were 3 embryos in the right uterus and 2 in the left. The hearts of 3 of the small embryos were beating. In one embryonic site the amnionic capsule was as large as the previous 3 but the necrotizing embryo was tiny. The last uterine "bump" appeared to be resorbing. Corpora lutea were small but active. The uterine wall was tremendously fringed.

Both remaining females removed from the treatment at 30 days after mating whelped. The average gestation was 50 days. These 2 females had a total of 7 small kits, 3 of which were stillborn. All of the remaining kits died within 2 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 7th or 7th and 8th day after mating had normal 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 litter average or any decided decrease in the average gestation length of those animals treated with progesterone or light when compared to control animals.

Table IX shows the number of blastulae flushed out of uteri from females subjected to various treatments. At 18 days after mating, blastulae from control animals averaged .32 mm. in diameter. Blastulae from the lighted female, also averaged .32 mm. in diameter while those from the

progesterone-treated females averaged .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 and eleven in another. Most of them were extremely tiny and fragile and were lost before staining.

Possible implantation, adhering blastulae, and an implanted foetus were found in 4 animals of the progesterone treated groups. None were found in the lighted and control groups.

When notice was made of resorbed foetal sites within treated females receiving 3 mg. provera per lb. body wt. per day, one of the females from this same group was placed on 9 mg. per lb. body wt. per day, from the 43rd to the 57th day after mating. When she ceased receiving this treatment, she failed to have kits.

Activity ratings were given to corpora lutea of the ovary and endometrial and glandular development of the uteri in the autopsied females. Aid in determining various stages was found in Hansson (1947) and Franklin (1958). Corpora lutea were rated in the following manner.

3 - Inactive Phase: Luteal cells are small with dense, strongly basophilic cell nuclei.

4 - Incipient secretion Phase: Luteal cells increased in volume. The nucleus is larger and less basophilic; Blood capillaries are dilated.

5 - Secretion Phase: Great infiltration of blood vessels. Volume of lutein cells increase greatly and many become pear-shaped. The cell

are situated in the cytoplasmic periphery.

Plate I shows the various phases described.

Uteri were rated in the following manner:

1 - High cuboidal type endometrial lining with basally situate nuclei. Glandular development slight with mainly connective tissue-filled lumens.

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

3 - Full glandular development with extensive fringing of the endometrium. Plate II shows the phases described in uterine development.

Experiment V. Spermatogenic Activity in Male Mink

In at least two instances during our experimentation, males mated by January 23rd. When the mated females were sperm-checked either no sperm were present or sperm that were present appeared immobile. These matings proved to be infertile.

When lighted males mated during the early part of February, active sperm were present, and fertile matings resulted.

In Table XI weights of testes and spermatogenic activity ratings are given resulting from histological analysis. Testes activity was rated on the following basis:

0- No development, possibly a few spermatogonia.

1- Spermatogonium and primary spermatocytes present in few numbers. A predominance of Sertoli cells. Interstitial cells not turgid.

TABLE XI SPERMATOGENIC ACTIVITY AND WEIGHTS OF TESTES TAKEN IN DIFFERENT MONTHS

Month	No. of males given rating	Range	Av. rating	No. of males for testes wts.	Av. wt. pair of testes in mg.
October					
Juvenile	2	0	0		
November					
Juvenile	6	0-1	.333	17	1275
Adults	3		0	2	1715
December					
Juvenile	26	0-4	1.077	9	1275
Adults				1	656
January					
Juvenile	7	3-4	3.143	2	2222
Adults				1	3382
February					
Juvenile	1		4.0	2	3985
March					
Juvenile	3		4.0	3	4451
Adults	3		4.0	3	2893
April					
Juvenile	3	3-5			

2- Spermatagonium, primary and secondary spermatocytes present. Interstitial cell cytoplasm increasing and the nuclei not basophilic. Very few spermatids present. Very few sperm in the epididymis.

3- Spermatids present in small numbers. Interstitial cells quite turgid with light staining nuclei. Some sperm in the epididymis.

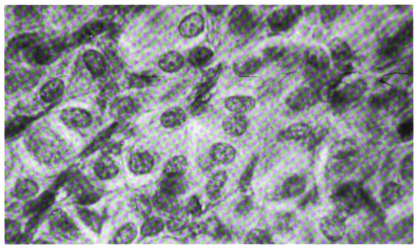
4- Sperm numerous; Interstitial cells have larger, basophilic nuclei. Sperm numerous in the epididymis.

5- Beginning of inactive period. Very few sperm present. Fat boies breaking from the walls of the seminiferous tubules. Few active interstitial cells. Plates III and IV. show the phases mentioned above.

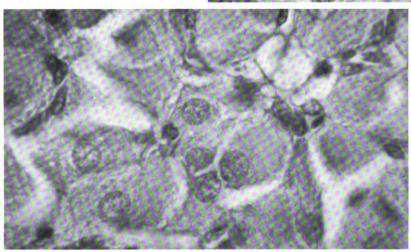
There is a definite increase in activity of the testes during the months of November through March. A coinciding increase in weight of the testes accompanies the increase in spermatogenic activity. Lack of adequate numbers prevented a good comparison of juvenile and adult mink with regard to either testes weight or spermatogenic activity.

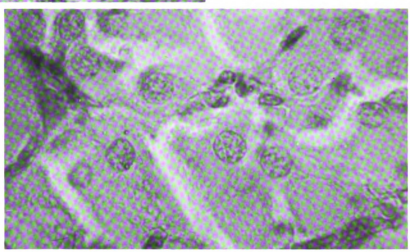
There is some range in activity of the testes until March. All of the males then exhibit fully active testes. In the middle to latter half of April, the testes begin involution, with degenerative processes taking place in both the seminiferous tubules and in the interstitial area.

A



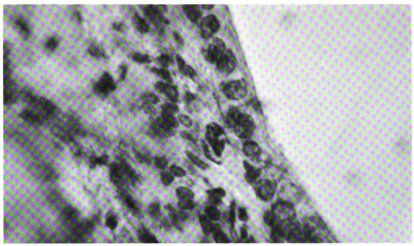
B



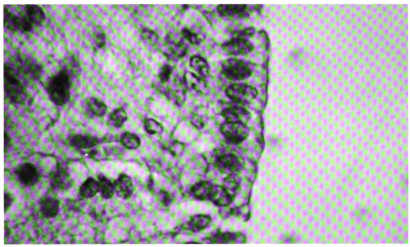


C

Plate I. Corpora lutea; A, B, and C have activity ratings of 3, 4, and 5 respectively with regard to secretion capacity; 970X.



A



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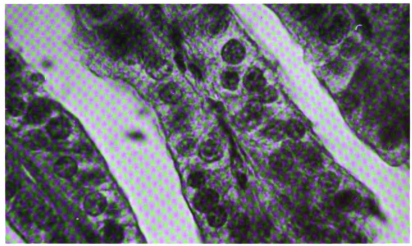


C

Plate II. Uterine endometrium; A, B, and C have activity ratings of 1, 2, and 3 respectively with regard to development; 970X.

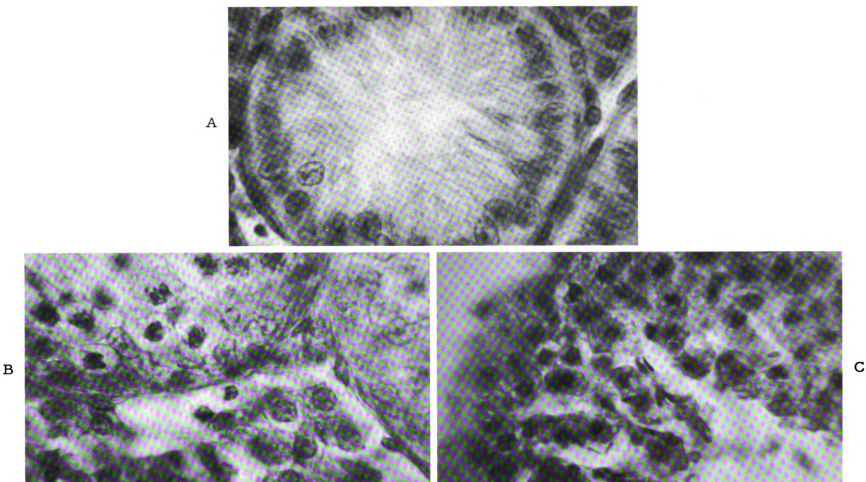
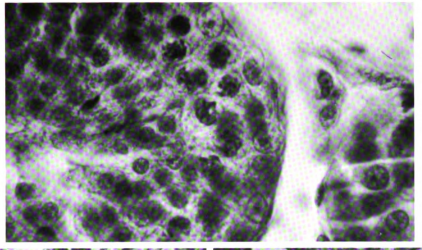
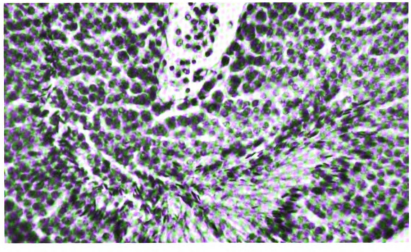


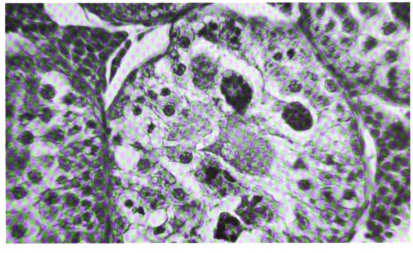
Plate III. Testes, seminiferous tubules; spermatogenic activity in A, B, and C with ratings of 0, 1, and 2 respectively, were taken in Nov., Dec., and Jan.; 970X.

A



B





C

Plate IV. Testes, seminiferous tubules; spermatogenic activity in A, B, and C with ratings of 3, 4, and 5 respectively, were taken in Jan., March, and April. (A 970X, B and C 430X.)

DISCUSSION

The role of different factors controlling the reproductive cycle in mink has puzzled scientists for years. Although no definite conclusions can be drawn from this experimentation, new evidence has been set forth for the role of both light and progesterone in influencing the reproductive cycle in mink.

Hansson (1947) investigated the effect of slight variations in temperature on reproductive activity from year to year. He found that mink mated a little earlier when the temperature averaged a little higher. The higher temperatures recorded may have been due to more sunlight.

Bowness (1957), Enders (1952), Hammond (1951a), Holcomb et al. (1962), Hronopulo (1956), and Kirk (1962) found that they could vary the length of gestation and the number of kits per litter by altering the photoperiod.

Franklin (1958), Hammond (1951b), and Hansson (1947), found some variation in reproductive performance when subjecting females to various amounts of such materials as estrogen, progesterone, and pregnant mare serum, throughout the mating season.

Hansson (1947) demonstrated that when female mink were subjected to an additional amount of light per day, both before and after mating, those receiving 90 minutes of light per day had an average gestation length almost 10 days shorter than those receiving 45 extra minutes of light per day.

Holcomb et al. (1962) found even when mink could enter their nest

boxes, the increased photoperiod of 82 minutes per day had an effect upon the mink. In this case between 3 and 5 foot candles of light were available to the mink.

In most instances investigators have failed to give the number of foot candles of light given in the artificial illumination. Certainly there must be some differences in reaction to both the duration and intensity of the added stimulus.

In the experiments described herein, variation in the lighting regimes were tried with regard to intensity, duration, light color, time with regard to mating when starting, and availability of the light.

Exp. I -- 1962:

The present study indicates an increase in the photoperiod prior to mating hastens estrus and supports the observations of Pearson and Enders (1944), and Holcomb et al. (1962).

Restricting natural light conditions caused a delay in matings but proved somewhat disappointing as only two litters were produced. Unwillingness of males to mate in this extended breeding season may have caused these disappointing results.

From the standpoint of efficiency in production, subjecting females to additional light only after mating was encouraging. The females on this treatment exhibited shorter gestation periods and whelped the largest average number of kits per female mated.

The experiments in 1962 may have yielded more kits had the females

been mated twice, as is customary procedure. However, a more accurate estimate of the length of gestation from once mated females was desired.

There were more kits whelped per female mated in the (NL)* and (LL)* groups, but partially due to the variance of kits per litter (0 to 8 kits) there were no significant differences noted.

Experiment I. -- 1963.

Lighted females in this experiment were subjected to 3 hours of extra light per day starting on the 7th day after mating. Hansson (1947) stated that blastulae did not complete their journey through the fallopian tubes for an average of 6 to 8 days. If light effected progesterone output, it was thought that possibly extra light immediately after mating might cause production of progesterone and partial constriction of the fallopian tubes. At present, it is not thought that the light has an effect this rapid on physiological changes.

Both the (NL)** dark and pastel females whelped fewer kits per female mated than comparable control females. The (NL)** pastel females had an average gestation length 2 days shorter than the control group, but both the (NL)** groups of dark females lighted with red and white light respectively had a longer average gestation length than control females.

There were no significant differences in average litter size or average gestation length in the treated and control females when subjected to a 2-sided Students t-distribution test. Why such a phenomenon took place cannot be explained. In previous experimentation extra light after mating

generally resulted in shorter gestations and more kits in the treated animals when compared to controls. Perhaps receiving the extra light 7 days after mating upset some hormonal balance.

There seemed to be no difference in gestation length of those females treated with white or red light. Those treated with red light had an average gestation length of 50 days while those treated with white light had an average gestation length of 49.5 days.

Experiment II. - 1962.

In the experiments at the commercial mink ranch, variables within and between various groups were more difficult to maintain constant. This was in most part due to the large numbers of mink involved and the lack of extra cages. If adequate cages had been available female mink could have been transported to the lighted area after mating. Instead, it was necessary to attempt to produce a lighting regime which would be optimum in obtaining a shorter gestation and more kits per litter. These treated animals received 95 extra minutes of light per day from white incandescent bulbs producing a maximum of 10 foot candles.

The additional light was given when the animals began breeding March 5th. Therefore, many of the females received extra light per day before they were mated. In every instance the average gestation length was shorter for females subjected to an increased photoperiod after mating.

The lighted sapphire and violet females reacted favorably to the extended light per day. They averaged more kits per female mated than did

comparable control animals. This may be due to the fact that when light strikes the less-pigmented eye of one of these color phases, the impulse passed on to the nervous and hormonal system is more intense than that in the dark and pastel strains.

The Heinens and hopes in this instance seemed to have an adverse reaction to the additional light. In every instance they averaged fewer kits than comparable control animals. This is a difficult phenomena to explain. The variation may have been due to chance alone.

Experiment II. -- 1963.

In most instances, (NL) females had a shorter average length of gestation and averaged more kits per litter than comparable control groups. A significant difference in the average length of the gestation period was found in but one instance.

The (NL) hope and Heinens all averaged more kits per female than comparable (NN) females. This was altogether different than what occurred in 1962. Possibly the phenomena observed in 1962 was due to chance, or possibly the extended length of the artificial photoperiod in 1963 altered the results. The treated females in 1962 had all been subjected to 95 minutes of extra light per day after sundown.

Those females in treated groups that could not escape light exposure all had large litter averages. There was one instance where females that were in such a group had an average of 2.49 more kits per female than comparable treated females that could enter their nest box. In this

instance, there was a significant difference between the (NL)** and (NN)** litter averages at the .05 level using a 2-sided t-test.

Females in Experiment II, 1963, when receiving 2 1/2 hours of extra light at approximately the date of mating (in a few cases before or after mating) had exceptional reproduction performance. This is in contrast to those in Experiment I-1963 where females were subjected to nearly the same treatment but at seven days after mating. The results in Experiment I- 1963 may be due to chance since small numbers of mink were used.

It is assumed that the light stimulus enters by way of the eye. The impulses then pass down the optic stalk to the optic chiasma of the hypothalmus. Since the pituitary is governed by the hypothalamus through nervous impulses, the light stimulus impulses when received at the optimal intensity or duration, may cause secretion of gonadotropic hormones. Follicle stimulating hormone (FSH) would be the first gonadotropin from the anterior pituitary. This would cause growth and maturation of follicles in the ovary of the female and probably initiate sperm production from the seminiferous tubules of the testes in the males.

Estrogens would be released from the developing follicles which would cause a decrease in production of FSH in the female. Release of luteinizing hormone in the male brings about testosterone production from interstitial cells of the testes aiding in sperm maturation. The female mink ovulates only upon copulation or rough physical contact with the

male (Hansson, 1947; Pearson and Enders, 1944). She then ovulates due to release of luteinizing hormone (LH) from the anterior pituitary. The LH causes the ova to ovulate and the corpora lutea to develop in the ovary. The fertilized ova then undergo cleavage while passing through the fallopian tubes and lie in the uterus as a blastula stage. This delayed implantation may last for an interval of 15 to 40 days. This may be the cause of failure to whelp, or decreased numbers of kits due to loss of blastulae of foetuses through resorption (Hansson, 1947).

Luteotrophic hormone (LTH) from the anterior pituitary is necessary for the secretion of estrogen and progesterone from the corpus luteum. These two hormones are necessary for conditioning the uterus for implantation. It may be possible that when the light stimulus becomes optimal during the spring months, LTH is secreted from the pituitary and enhances implantation through its effects on the corpora lutea. This may result in a shorter delayed implantation in those mink receiving additional light after mating. This could account for the shorter average length of gestation and higher average number of kits per female noted in the treated groups.

Experiment III.

Hansson (1947) found that there was an average of 8.73 ova shed from the ovaries. He found an average of 4.37 kits per litter for a large group of whelping females.

The experiment conducted in 1962 resulted in some interesting findings. Three females averaged seven blastulae and/or implanted foetuses between the 18th and 29th day after mating. Comparable females had an average of 3.8 kits per female mated; an average of 3.2 less kits whelped than blastulae and implanted foetuses present in the uteri of those females subjected to laparotomy. Several of the blastulae present seemed to be necrotizing.

Hansson (1947) believed that blastulae are resorbed during the free follicular period, and foetuses during pregnancy. This could be due to inadequate preparation of the uterus for implantation due to lack of progesterone and estrogen secretion from the corpora lutea. The blastulae could be resorbed before implantation due to lack of endometrial proliferation and failure of glandular development to secrete nutritive substances for the blastulae. Failure of adequate nutrition after implantation or implantation of already impaired blastulae may result in resorption. If the stimulus for activation of corpora lutea is not present in adequate amounts all of the blastulae may be resorbed before implanting. If the stimulus is present at an earlier date only a few of the blastulae may be resorbed before implantation.

Experiment IV. -- 1962.

As reported previously, a free follicle stage in mink is described by Hansson (1947). He reported that several successive waves of follicles ripen and are shed by a female mink if she is mated at intervals

throughout a breeding season. He believed that the persistence of a high estrogen level throughout this period may be one of the causes of delayed implantation. The comparative increase of progesterone after development of corpora lutea in the ovaries in comparison to estrogen increase at a later date after mating may enhance the readiness of the uterus for implantation. If a stimulus could provoke luteotropic hormone (LTH) production, the corpora lutea might be activated at an earlier date after mating, and thus ready the uterus for implantation. Supplementary sources of progesterone might also elicit the same end result.

In 1962, experiments were performed using oral doses of progesterone. It was thought that possibly an increase in progesterone (over the normal amount present) early after mating might hasten readiness of the uterus for implantation. This possibly could result in a shorter average gestation length and an average of more kits per female much the same as the added light per day had done in previous experiments.

The results were surprising. Since the progesterone treatment probably suppressed (at least partially) the normal corpora luteal development, when the treatment was ceased at 30 days after mating there was probably not enough progesterone secretion from the corpora lutea to maintain normal foetal development. The preceeding statement was verfied by further experimentation in 1963 and will be discussed later.

Experiment IV. -- 1963.

Many different progestin treatments were tried during the reproductive

season of 1963. The groups of females receiving the various treatments were small and after autopsies were made, some groups were very small.

Hansson (1947) and Franklin (1958) previously worked with some hormones with no definite findings. They both suspected that injections of progesterone facilitates quicker implantation, but there was no definite proof of this. Franklin believed that his single 5 mg. injection of progesterone at 7 days after mating activated the corpora to secrete progesterone. He was in doubt as to whether this injection triggered an endocrine mechanism within the ovary or within the pituitary.

It was thought that if both ovaries and uteri from various treated groups could be studied histologically, some further conclusions might be drawn about the physiological basis of delayed implantation in mink. Records were taken of implanted foetuses in the treated and control groups. Comparisons were also made between the progestin treated and lighted groups to discover any possible similarities or differences in development or corpora lutea and uteri. One group of females received oral doses of progesterone in the same manner as animals had received the previous year, but at varying times after mating. They were to receive the treatments until whelping. The results described previously, indicate that those females removed from progesterone treatments at the 30th day after mating had corpora lutea activity at the time the progesterone treatment was ceased. This was verified in 1963 by examination of ovaries taken from a treated female on the 30th day. At that time the corpora

lutea were small but were in the secretion phase. The uteri were fringed and glandular development was prominent. The foetuses were normal.

The female in this same type of progesterone treatment autopsied at 40 days after mating had three small normal foetuses and two resorbing foetuses. The corpora lutea were active but small, and the uterus was greatly fringed. The corpora must have remained psuedo-functional after the progesterone treatments were ceased. This enabled these mink to maintain a few foetuses, but not to the extent that they would normally be maintained. Thus, they whelped a few small kits incapable of surviving.

Those females maintained on progesterone treatments from the 45th to 50th days after mating, probably had normal to early implantation. The progesterone treatments were continued and the corpora lutea became either somewhat inactive or entirely non-functional. There was apparently no activity of the corpora lutea on the 57th day after mating, when an animal was sacrificed and autopsied. The progesterone treatment was probably too low to maintain the foetuses, thus the females resorbed or aborted the tiny unborn young.

The females treated similarly in 1962 probably had the same difficulties. All kits whelped were small and none survived.

There seemed to be no milk development in those females that ceased receiving treatments on the 30th day after mating. This was probably due to lack of progesterone for adequate mammary gland development.

These females might have raised their kits had they produced milk.

As noted in Tables VII, VIII, and IX, there is some correlation between the treatment given and the activity of corpora, development of the uterus, presence of implantation sites, and the size and status of the blastulae.

The four possible implantations were all noted in females receiving some progestin treatment. The blastulae present were, on the average, slightly larger in diameter when compared to the controls. The larger blastulae were not as delicate as those from the control group. It was easier to fix and stain them without damaging the external membrane.

In general, those females receiving progesterone treatments for several days had less active corpora, probably due to the supplementary role of the administered progesterone. In all instances the luteal cells were becoming active or were in the incipient secretion phase. The endometrium of the uteri varied from low cuboidal in some animals to high columnar cells in others, with some fringing. Uterine glandular development was also varied.

In those females receiving injections or large oral doses of progesterone for 1-3 days, the corpora lutea were more active than in the previously described animals. The uteri were correspondingly more developed with fringed endometrium and active glandular mucosae.

The lighted females had a lower average activity rating in the development of the corpora lutea and uteri than did any of the other groups. The

control group varied in ovarian activity from initial activity to the secretory phase, with corresponding uterine development.

Had the lighted females been given extra light on the first day mated, they might have had greater ovarian and uterine development.

In those females receiving injections or large doses of orally introduced progesterone for 1-3 days, the production was normal to good. This indicates that, as Franklin (1958) suggested, the progesterone may act as a triggering mechanism to activate either the pituitary or the corpora lutea.

Since light probably acts as a triggering mechanism for activation of the pituitary, it would seem more likely that the progesterone activates the anterior pituitary to produce LTH. It may be that the presence of progesterone stimulates the nervous system and that when the progesterone level is lowered through body metabolic activity, the hypothalamus stimulates the anterior pituitary to secrete LTH and allow subsequent corpora lutea development.

Another less likely possibility is that the short artificial span of progesterone in the body promotes uterine development to the extent that the glands of the uterus produce slight amounts of gonadotropins which, in turn, activate the corpora lutea.

The animals receiving large dosages of oral progesterone for one day or injections for one or two days, had the highest litter averages. If more animals had been present in the groups, more evidence might have been present for elucidating the mechanisms of delayed implantation.

If females receiving the extended progesterone treatments had received a higher progesterone dosage sometime between 20 and 30 days after mating, the kits might have been maintained. It is obvious that the 3 mg. of provera per lb. body wt. per day did not supplement the amount of progesterone normally produced by the corpora lutea.

One female was placed on a triple dosage of progesterone from the 43rd to the 57th day after mating. She whelped no kits when removed from the treatment. Presumably the kits may have been resorbed before the 43rd day after mating.

Experiment V. Success of reproduction in the mink hinges on both the reproductive behavior and competance of the male and the female. If a male is not in full spermatogenic activity, fecundity may be reduced. If the male exhibits no desire to mate, the reproductive process is stymied.

Untreated males that mated in January, had little desire to mate in most instances. The sperm were few and were immotile. Maturation of sperm requires the production of testosterone (androgen) from the interstitial cells of the testes. There was very little interstitial cell activity until January, although spermatids were present in many cases. Full interstitial cell development did not take place until February. When mating took place in February motile sperm were present.

Hammond (1951a) believed that the short days in December provided the initial stimulus for sperm production. It may be that the increasing

amount of light in January, February, and March stimulates the pituitary to produce FSH for interstitial cell activation. The continued increase in light in April after the mating season may then cause a shift in the hormonal balance with discontinued production of FSH and subsequent inactive interstitial cells. The decline in testosterone production along with a possible decreased thyroid activity during the warmer days may bring about the relatively inactive reproductive performance of males during the early part of April.

Although the testes seemed to be completely active by mid-February, the weight of the testes continued to increase until March. Although some activity in the testes was noted for the months of November and December, the rates of activity increase were much slower than in January and February.

SUMMARY

Mink (Mustela vison) was the subject of experimentation. Reproductive performance of females subjected to various lighting regimes and progestin treatments was of special interest with regard to delayed implantation. Reproductive activity of males with regard to performance in mating and spermatogenic activity was studied.

These studies on reproduction in mink were divided into 5 experiments.

Dark, sapphire, and pastel mink were used in Experiment I.

In 1962, 95 minutes of extra light per day was given after sundown to a group of dark and sapphire females lighted only after mating. Control groups were also maintained. Light intensity varied between 2-5 foot candles. Results showed a decrease in the length of the gestation period and an increase in the number of kits whelped when compared to control animals.

In 1962, a group of dark and sapphire females was hastened to estrous and mating by subjecting them to increased light beginning on Dec. 21. These females mated early in February and the first litter was whelped on March 31st. These females had litter averages comparable to those of the control groups.

In 1962, a group of pastel males and females were restricted in light by approximately a month. Four of these females mated on April 3rd and 4th. Two litters of kits were whelped. Males restricted on

light exhibited no mating interest and control males were used to secure matings.

In 1963, groups of pastel and dark females were subjected to 3 hours of extra light per day, 7 days after mating. The light intensity varied between 5 and 40 foot candles on white light and 1 to 7 foot candles with red light. Females in control groups averaged more kits per female than the females in treated groups. There was no evident difference in the effect of red or white light.

Sapphire, violet, hope, Heinen, and pearl mink were used in Experiment II.

In 1962, 95 minutes of added light after sundown was given to groups of sapphire, violet, hope, and Heinen females. Light intensity varied between 3 and 10 foot candles. Control females were also maintained in each strain. The average length of gestation was decreased in the treated females. The average number of kits per female mated was higher in the sapphire and violet treated females when compared to control females. The control females in the hope and Heinen strains averaged more kits per female mated than did lighted females. This latter phenomena may be due to chance.

In 1963, sapphire, Heinen, hope, and pearl strains of females were subjected to 2 1/2 hours of extra light per day after sundown with an intensity of 3 to 10 foot candles. Some of the lighted females were not allowed to escape the light exposure. Control groups of females were also maintained.

In almost every instance the average length of gestation period was shorter in the treated groups than in control females. In every instance the treated groups of females averaged the same or more kits per female than control females. Those females that could not escape light exposure had exceptionally large litter averages in some instances.

Statistical analyses using Students t-distribution of the data summarized for the photoperiodicity studies in both Experiments I and II, showed that significant differences existed in many instances between control groups and those lighted after mating.

Experiment III was concerned with determining the numbers of blastulae and/or embryos present in the uterus at a certain time interval after mating. It was also desired to obtain the approximate implantation time.

It was determined that implantation in three females would have taken place between 20 and 25 days after mating. There was a significant difference (.05) level between the average number of blastulae and/or embryos present in the uteri, and the average number of kits whelped by control females. This indicates a loss of blastulae before implantation or an abortion or resorption of embryos during pregnancy. This is probably due to lack of hormonal secretion by the corpus luteum which stimulates glandular and endometrial development of the uterus.

Experiment IV was concerned with progestin treatments. Both oral and injected treatments were used at varying dosages and at varying

intervals after mating

Extended progesterone treatments partially suppressed normal corpora lutea development. When animals were removed from these extended treatments at 30 days after mating, the corpora lutea remained sub-normally active as indicated by histological preparations. Small kits were whelped by these females but they all died. There appeared to be no milk production by the females, probably because of inadequate progesterone produced for mammary gland development.

Females subjected to the progesterone treatments a short time after mating to an approximated whelping time, had no kits. They were found to have aborted and/or resorbed their embryos.

Possibly the amounts of progesterone given after 30 days suppressed corpora lutea activity, but was not sufficient to maintain the embryos. It is obvious that inadequate doses of progesterone were administered.

Those females receiving larger oral doses or injections of progesterone between the 7th and 9th days after mating had normal to good reproductive performance. Possibly these doses triggered a mechanism for the release of luteotrophic hormone for stimulating corpora lutea activity.

Seventeen females were autopsied from the various groups (including control and light experiments) at 18 days after mating. Corpora lutea and uterine activities were varied although their developments coincided. Blastulae were less fragile and were on the average larger, in the progesterone treated groups than in the lighted and control groups. An implanted

foetus and adhering blastulae were found only in the treated females.

No correlation could be found between lighted and progesterone-treated females. Perhaps if the lighted females had received the extra light starting on the day of mating, they may have responded differently.

Males in Experiment V exhibited some unwillingness to mate in January. However, in February, males were generally agressive in mating behavior.

Weights of testes were found to increase steadily from the months of November through March. Greatest rate of gains in testes weights were found in February and March.

Spermatogenic activity increased from no activity in November to great activity in March. There is a decline in activity in April. Sperm are present in many instances by December or early January, but maturation has not taken place due partially to the lack of interstitial cell secretions (testosterone). Fully mature motile sperm were present in early February.

In brief, this research has furthered our knowledge of the physiology of mink in the following ways.

1. There was no beneficial effect of added light when given 7 days after mating.

2. The estrous period in females can be set forward by restricting the natural photoperiod in late winter.

3. Females in cages lacking nest boxes and unable to avoid added

light exposure had more kits than those receiving light but able to escape the exposure by entering nest boxes.

4. No differences in reproductive performance were found in mink when subjected to red instead of white light.

5. Significantly more blastulae and/or embryos were present in uteri of pregnant females at 18 to 29 days, than kits whelped by control females.

6. Oral treatments of progesterone at 7 or 7 to 9 days after mating trigger some mechanism which activates corpora luteal activity. Blastulae then implant more readily.

7. Corpora lutea and uteri were better developed in those receiving progesterone for a short time interval after mating than those structures in control and lighted groups.

8. Blastulae were larger and less fragile in progesterone treated groups than in control groups. Therefore, the uterine endometrium may have been secreting more nutritive substances.

9. Females receiving progesterone treatments until 30 days after mating whelped a few small kits. They died possibly because of no milk production by the females. Corpora lutea remained psuedo-functional.

10. Those females maintained on progesterone until they should have whelped, instead either resorbed or aborted their embryos. Corpora lutea were involuting at 50 days after mating and were very small.

11. Interstitial cell development in males is slight until February. Subsequent sperm maturation and motility is present. Involution of the seminiferous tubules takes place in April.

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