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Effect of Photoperiod on Time of Blastocyst Implantation in the Rat (37918)

NATHA LILIT AND VERNON L. YEAGER
(Introduced by Robert C. Holland)
Departments of Anatomy, Mahidol University, Bangkok, Thailand, and Saint Louis University, St. Louis, Missouri 63104

Mitchell and Yochim (1) recently showed that an increased photoperiod (22 hr light, 2 hr dark) caused a prolongation of pregnancy. They suggested that this may be due in part to a delay in implantation of the blastocyst, since they had previously shown that an increased photoperiod would delay the time of appearance of sensitivity to decidualization in pseudopregnant rats (2).

To determine whether or not increased photoperiods do in fact delay implantation, the size and number of implantation sites were determined on Days 6, 7, and 8 (Day 0 = day sperm found in vaginal smear) in rats which had been exposed to different photoperiods. The results reported here indicate that increased photoperiods delay the time of implantation of the blastocyst in rats, as suggested by Mitchell and Yochim (1).

Materials and Methods. Wistar strain virgin albino rats from animals bred in Thailand were fed an adequate diet of laboratory chow in pellets and tap water *ad lib.* They weighed from 180 to 258 g at the beginning of the experiment. The animals were housed three per cage. Daily vaginal smears revealed that estrous cycles ranged from 4 to 6 days in length. Animals with different length estrous cycles were distributed proportionately into three groups which were exposed to different conditions of environmental lighting for from two estrous cycles before being mated until time of sacrifice.

Control group. The animals in this group received approximately 12 hr of light and 12 hr of dark, these corresponding to the outdoor lighting periods. The entire south wall above desk level of this room was glass, allowing reflected (not direct) sunlight to enter. This light was supplemented by 40-W "daylight" fluorescent bulbs. The units had two bulbs each and were arranged in rows approximately 8 ft apart with 4 ft between units in a row. The cages were kept between rows, approximately 80 in. below the lights.

Vaginal smears were taken every morning between 8:30 and 9:30 AM. When the animals reached the proestrous stage of the second cycle, each animal was placed with two male rats. The following day the vaginal smear was examined for the presence of sperm and all animals were found to have mated. The day sperm were found was counted as Day 0 of pregnancy. Between 3:00 and 5:00 PM on Day 6 of pregnancy, ten animals were given sodium pentobarbital (30 mg/kg body wt intraperitoneally) and were injected with 0.5 ml Indian ink via the lingual vein to aid in the identification of implantation sites. One-half hour later the uteri were removed, weighed, and the diameters of the implantation sites were measured using a micrometer.

Dark group. The treatment of animals in this group was the same as for the control group, except that they were kept in a light-proof dark room in which the lights were on for 1 hr each morning from 9:00 to 10:00 AM when food and water was given and cages cleaned. Ten rats that mated in the second cycle after being placed in the dark room were killed on Day 6 of pregnancy.

Light group. The animals of this group were kept in constant light. The room was illuminated with 40-W "daylight" fluores-

cent bulbs, two bulbs per unit. The units were arranged in two rows 8 ft apart with 4 ft between units in a row. The cages were kept between rows approximately 80 in. from the lights. In addition, four 15-W "daylight" fluorescent bulbs were kept about 18 in. above the animals.

Daily vaginal smears revealed prolonged estrous cycles. On the day of pro-estrus of the second cycle in constant light, the females were placed with males as in the control group. Some of these animals had to be left with males for 2 or 3 days before sperm were found in the vaginal smear. Those which had not mated within 3 days were eliminated from the experiment. Fourteen animals were killed on Day 6 of pregnancy, 4 animals were killed on Day 7, and 4 animals were killed on Day 8.

All rooms in which the animals were kept were air conditioned and the temperature was kept at approximately 25°.

Results and Discussion. A summary of the results is shown in Table I. All of the animals in the control group which were exposed to males during pro-estrus had sperm in their vaginal smears on the following day. On the afternoon of Day 6 of pregnancy, the implantation sites were readily seen grossly in all control animals. Of the original 21 animals in the dark group, 11 failed to go through two normal estrous cycles. The vaginal smears indicated that they were in prolonged di-estrus. These animals were eliminated from the experiment. The remaining ten animals were successfully mated after two estrous cycles and, at autopsy on Day 6, had implantation sites similar in number and size to control animals. The uterine weights were also similar. Although short photoperiods altered the estrous cycles of about half the animals so that mating did not occur, short photoperiods did not cause a delay in implantation of blastocysts in rats which did mate. Mead (3) reported that blinding prolonged the preimplantation period in spotted skunks, but these animals normally have delayed implantation.

The rats exposed to constant light had prolonged estrous cycles. After reaching pro-estrus in the second cycle after being placed in continuous light, the animals were placed with males for 1, 2, or 3 days to mate. Of the animals in which sperm were found in the vaginal smear, implantation sites were found in only 6 of 14 animals killed on Day 6, 3 of 4 animals killed on Day 7, and 4 of 4 animals killed on Day 8. Whether the animals took 1, 2, or 3 days to mate had no relationship to whether or not there were implantation sites. Since Mitchell and Yochim (1) showed that rats could deliver live young after prolonged photoperiods, and since the percentage of animals with implantation sites increased from Day 6 to Day 8, it is concluded that implantation was delayed and not blocked.

The mean number of implantation sites on Days 7 and 8 was slightly increased, though not significantly. Since the number of implantation sites has been related to the level of progesterone present (4), the fact that they did not decrease in number is

TABLE I. Means of Data on Implantation Sites and Uterine Weights.

Group	No. of rats	No. with implantation sites	No. of implantation sites/animal	Diameter of implantation sites (mm)	Uterine weights (g)	Uterine weight body weight
Control	12	12	8.3 ± 0.5	3.38 ± 0.07	0.622 ± 0.023	$3.09 \times 10^{-3} \pm 0.13 \times 10^{-3}$
Dark	10	10	8.3 ± 0.9	3.39 ± 0.05	0.567 ± 0.036	$2.65 \times 10^{-3} \pm 0.22 \times 10^{-3}$
Light				2.78 ± 0.21[a]	0.515 ± 0.027	$2.53 \times 10^{-3} \pm 0.16 \times 10^{-3}$
Day 6	14	6	8.2 ± 1.2	99.5%*	99.5%*	99.0%*
				2.66 ± 0.18[a]	0.662 ± 0.06	$3.01 \times 10^{-3} \pm 0.18 \times 10^{-3}$
Day 7	4	3	10.7 ± 2.3	99.5%*	0.918 ± 0.157	$4.42 \times 10^{-3} \pm 0.61 \times 10^{-3}$
Day 8	4	4	10.3 ± 0.8	3.34 ± 0.28	99.5%*	97.5%*

[a] Mean from the animals which had at least 1 implantation site.

* Different from Day 6 control (% confidence).

surprising, because Piacsek, Hardy, and Martinson (5) found that exposure to continuous light caused decreased progesterone levels in adult pseudopregnant rats and in immature rats injected with PMS and hCG.

Animals kept in constant light and killed on Days 6 or 7 had implantation sites significantly smaller than those in the control group. By Day 8, they were equal in size to implantation sites in control animals killed on Day 6. Exposing the rats to constant light for two estrous cycles before mating appears to cause a greater delay than found by Mitchell and Yochim (1), who did not change the photoperiod until mating occurred.

Yochim and Mitchell (2) suggest that the delay in sensitization of the uterus in pseudopregnant rats after exposure to long photoperiods is due to an excess of endogenous estrogen. However, Hoffman (6) found that longer photoperiods were associated with 5-day cycles while shorter photoperiods were associated with 4-day cycles. She suggested that this might be due to a lower secretion rate of estrogen in the longer photoperiods. Piacsek, Hardy, and Martinson (5) have also suggested that constant light might cause reduced estrogen secretion. Our results show that the uterine weights were decreased from normal on Day 6 in the constant light group, but nearly doubled by Day 8. Since the uterus shows a marked increase in weight after an injection of estrogen in ovariectomized rats, this suggests that the estrogen surge, which normally occurs on Day 4 of pregnancy (7), was delayed.

Both decreasing and increasing the photoperiod altered the reproductive biology of the rats as evidenced by the fact that some animals of both groups failed to mate. However, only in the light group was there delayed implantation. Since the animals which did not mate were eliminated from the experiment, it is not known whether the endocrine alterations in the two groups differed qualitatively, quantitatively, or in timing of endocrine release.

Summary. Rats were exposed to 1, 12, or 24 hr of light per day for two estrous cycles, then were mated. On Days 6, 7, or 8 following the appearance of sperm in the vaginal smear, the animals were anesthetized, injected with Indian ink to mark implantation sites, and killed. The number and diameter of implantation sites were recorded and the uteri weighed. Although short exposure to light had no effect on day of implantation, 11 of 21 animals failed to complete two normal estrous cycles. Animals exposed to continuous light showed delayed implantation.

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