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SYNTHESIS OF THE RABBIT UTERINE PROTEIN, BLASTOKININ: INFLUENCE OF LIGHT DEPRIVATION

JOSEPH C. DANIEL, JR.
University of Tennessee
Knoxville, Tennessee 37916

ABSTRACT

Reproductive success is lowered when female rabbits are deprived of light during early pregnancy. This study concerns an attempt to correlate this phenomenon with changes in uterine protein.

Uterine flushings were analyzed by protein determinations of Sephadex gel filtration fractions from light-deprived animals in comparison with non-deprived controls. The two experimental groups (2 days or 4 days of deprivation) had twice the concentration of protein on day 5 *post coitum* as found in the controls and 10-17% more blastokinin. For the animals deprived of light from day three, blastocyst size and number were also reduced.

It is concluded that deprivation of light in rabbits during the first four days *post coitum* is related to coincidental changes in the protein of the uterine secretions.

INTRODUCTION

External stimuli received by the female mammal from her environment influence her reproductive success in various ways. This was particularly emphasized by a Ciba Study Group (Wolstenholme and O'Connor, 1967) which explored the influence of auditory, light, electrical and olfactory stimuli as well as climatic (e.g., temperature and rain-fall) and other environmental conditions (e.g., population density and food availability) on many aspects of reproduction. In mammals, light stimuli are particularly known to be important in relation to breeding behavior and ovulation, and the literature on this subject is extensive. Remarkably few studies, however, have been reported to relate light stimuli, or their absence, to successful *maintenance* of pregnancy, such as has been done for auditory and olfactory stimuli. Brown-Grant (1977) showed that rats exposed to constant light had a low incidence of pregnancy, but if pregnant either had small litters or stillborn pups. In total darkness, however rats apparently sustain their pregnancies and the pups will even demonstrate a rhythmic pattern of N-Acetyltranferase activity in their pineal glands (Deguchi 1975). Plaut et al (1970) and Mitchell and Yochim (1969, 1970) reported slight changes in gestation periods and times of parturition when rats were kept in continuous light or darkness or on shortened or prolonged photoperiods, and Arvay (1967) observed an overall reduction in reproductive success of rats kept under complex neural stimuli (including light). Blinding, or retention in complete darkness, has no effect on either the time or success of implantation. (Gilman and Fischer, 1971). Except for these, the main studies known to the author have been done with mammals having a delayed implantation (see review by Aitken 1977).

In some mustelids, increase of photoperiod after breeding shortens the gestation period, presumably by shortening the duration of the time during which the embryo is in the diapause state. For example, the eight and one-half to nine month gestation period of the marten was shortened by three to four months (Pearson and Enders, 1944), the 230± day period of the spotted skunk was shortened by more than 40 days (Mead, 1971) and the nine month period of the long-tailed weasel possibly by as much as five and one-half months (Wright, 1948). The gestation period of mink varies from 40 to 76 days with a mean of 51 days (Pearson and Enders, 1944), but it too has been reported shortened by three to fourteen days by different investigators (e.g. Aulerich et al., 1944; Pearson and Enders, 1944; Smith, 1970; Holcomb et al., 1962).

In some of these same animals, decreased photoperiod or deprivation of light (by blinding or by retention in a dark environment) after breeding may cause extensive prolongation or termination of the pregnancy (Mead, 1971) or reduction in the number

of young (Hronopulo, 1956; Holcomb, 1963). In the badger, however, gestation time is shortened by reducing the daylength (Canivenc et al. 1971).

Conversely, the reproductive success of some other mammals is apparently unaffected by light deprivation or changes in photoperiodicity during pregnancy. The gestation period of mice remains constant irrespective of the prevailing light conditions (Porter, 1972). Artificial manipulation of daylength has no effect on the gestation period of either the roe deer (Lincoln and Guiness 1972) or the northern fur seal (Daniel-unpublished) and female ferrets follow normal reproductive activity and bear normal litters even when bilaterally enucleated (blinded) before estrus (Thorpe, 1967; Rust and Schackelford, 1969). Kirchhoff et al. (1972) were unable to correlate seasonal photoperiodicity with the length of pregnancy in human females and of course many cases of successful pregnancies in blind humans are known.

In unreported studies from this laboratory we observed a reduction of 20%-50% in reproductive success (measured by numbers of young born, neonate viability or successful pregnancies/number of females mated) when rabbits were kept in complete darkness during part of their early pregnancy. We report here attempts to test the hypothesis that light deprivation during early pregnancy in rabbits alters the secretion of proteins in the uterus and specifically that proportion which is blastokinin (Krishnan and Daniel, 1967).

METHODS AND MATERIALS

In these experiments, New Zealand white rabbits were used from a herd which has had better than 90% fecundity over the last six years. Females were randomly bred at different times to a group of five fertility-proven males. On days 1 or 3 *post coitum*, these females were transferred from the colony room where they were exposed to a 12-hour photoperiod to a dark room where they were kept until day 5. At that time, the animals were killed, the uteri removed, the protein content of the uterine flushings determined, and the embryos counted and their diameters measured with an ocular micrometer. Protein was expressed as milligrams per uterine horn as measured by the procedure of Lowry et al., (1951) of 0.1 ml aliquots of the flushing fluid after 5 ml of physiological saline was forced through each horn. The remaining fluid was dialyzed 24 hours against distilled water and then lyophilized to a dry powder. Three mg. of this powder was used as a sample for Sephadex gel (G-200) filtration as described in earlier publications (Krishnan and Daniel, 1967; Daniel and Krishnan, 1969; Daniel and Booher, 1977). The column profiles were established by plotting the protein determinations of each 9-drop fraction collected on a LKB-7000 fraction collector and the relative proportion of the blastokinin fraction was estimated by measuring the area under that peak. Five rabbits were used for each experimental group and a control set of five animals was also taken on day five having been kept the entire time in the same room used for dark deprivation but in this case maintained under artificial light for a 12-hour photoperiod.

RESULTS AND DISCUSSION

Table 1 shows the uterine protein determinations and embryo number and size. The rabbits deprived of light

TABLE 1. *Effect of light deprivation on blastocyst size and uterine protein levels as measured on day five* post coitum.

Amt. of time deprived of light prior to sampling (days)	Animal Number	Number of embryos recovered	Diameter of blastocysts (mm) mean+SE	Mean diameter of blastocysts for group	Uterine protein mg/uterus	Mean uterine protein for group mg/uterus	Comments	Blastokinin composition of total protein (percent)
	1	13	1.09±.04		9.7			
	2	10	1.06±.09		10.0			
4	3	11	1.29±.06	1.15	>12.4	11.0+		41.8
	4	1	1.01		11.8			
	5	5	1.22±.07		11.4			
		40						
	1	3	.75±.07		10.6		3 d,m-cb*	
	2	5	.63±.05		>12.4		6 d,m-cb*	
2	3	5	.45±.04	.84	10.1	10.1+		48.2
	4	7	1.21±.04		10.9		1 d,m-cb*	
	5	5	.97±.05		6.7		1 d,m-cb*	
		25						
	1	7	.99±.04		4.9			
	2	7	1.01±.06		4.7			
0	3	6	1.44±.02	1.00	8.0	5.5		31.5
	4	8	.88±.06		4.9			
	5	9	.75±.02		5.0			
		37						

*d = degenerate; m-cb = mucin coated bodies

from day one produced eight blastocysts per animal with a mean diameter of 1.15 mm and their uteri contained an average of greater than eleven mg of total protein. The animals deprived of light from day 3 produced five blastocysts per animal of diameter .84 and greater than ten mg of protein per uterus. (Degenerate mucin-coated bodies, that may have been dead ova were found in four of these animals.) The control animals averaged 7.4 blastocysts of one mm diameter and 5.5 mg of protein per uterus. On the basis of molecular weight fractions, by molecular sieving (Figure 1), the protein from the three groups also varied qualitatively. The high molecular weight components in the void volume (fractions 35-50) were in significantly smaller proportion in the experimental animals and the relative concentration of blastokinin (fractions 75-94) was higher in both of the light-deprived groups than in the controls: The occurrence of the lowest molecular weight fraction (numbers 95-105) was highly variable in the four-day light deprived animals and in the controls, but was consistently absent in all of the two-day deprived animals.

The results of this experiment support the hypothesis that light deprivation influences the development of the embryo by causing an alteration in the production of uterine protein most notably that of blastokinin and of the heavier components. This is especially obvious for animals first exposed to the stress of continuous darkness on day 3, which is the same day that blastokinin first appears in the uterus as a measurable component in the secretions. The balance of these proteins is critical so that either too high or too low a concentration is presumably detrimental, as Beier et al. (1971) reported from studies of changing the concentration of these proteins by the injection of specific steroids and as noted with cultured embryos (Krishnan and Daniel, 1967).

The interaction between the endocrine and nervous systems of mammals in relation to reproduction has been concisely reviewed by Malven (1970). He noted the importance of light as an exteroceptive factor on the influence of the pituitary secretions and subsequently the production of specific sex steroids. He speculates that the external stimulus, after its reception by the higher sensory receptors of the brain, acts either directly or via the pineal gland to influence the hypothalmus: The hypophysiotropic regions of the hypothalmus, presumably through the secretion of special hormones which are carried directly to the pituitary via the portal system, influence the secretion of the pituitary hormones that in turn influence the secretion of the specific sex steroids by the ovary. It is already well established that the composition of the uterine secretions of various mammals is altered in a major way under the influence of estrogen and/or progesterone, and it has been demonstrated that these particular steroids in the right combination control both the quantity and the quality of the uterine proteins (Beier, 1970; Beier et al., 1971; Arthur and Daniel, 1972; Urzua et al., 1970; Rahman et al. 1975).

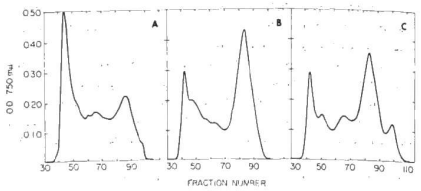

FIG. 1. *Typical sephadex gel filtration elution profile of rabbit uterine fluids collected on day five* post coitum, *after the animal had been deprived of light for A) zero days, B) two days, C) four days. The patterns are normalized to 1 mg of total protein in each case. Blastokin is represented by the peak from fractions 75-95.*

CONCLUSIONS

We conclude that the mode of action whereby light deprivation reduces reproductive success in rabbits may lie in part in the influence on the secretion of the uterine proteins.

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COLLOQUIUM ON BIOLOGY IN THE MID-SOUTH

A Colloquium on Biology in the Mid-South will be held at Memphis State University on Friday, February 22, 1980. Topics will include all areas of biology. Research papers (approx. 15 min.) are requested from faculty and students. An award will be presented for the best student paper.

Dr. Arthur Gentille, Executive Director of AIBS, will speak at the luncheon.

Titles of papers should be submitted by January 21, 1980. For further information, contact Dr. Melvin Beck, Department of Biology, Memphis State University, Memphis, TN. 38152. Telephone (901) 454-2955.