THE FATE OF SHORT-TAILED WEASEL, *MUSTELA ERMINEA*, BLASTOCYSTS FOLLOWING OVARIECTOMY DURING DIAPAUSE

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Summary. Blastocysts were obtained from intact or ovariectomized weasels, *Mustela erminea*, during embryonic diapause. Examination of thirty-five normal blastocysts revealed neither mitotic figures nor other evidence of trophoblast or embryonal knot development beyond the blastocyst stage during diapause. Uncleaved ova were found with normal blastocysts in three intact females, but no evidence of embryonic mortality during diapause was observed.

In spayed females, approximately 60% of the blastocysts recovered $1\frac{1}{2}$ to 3 weeks after ovariectomy showed evidence of deterioration, whereas all blastocysts recovered $3\frac{1}{2}$ to 9 weeks after ovariectomy were fragmented. Following ovariectomy, however, substantial proliferation of cells occurred in both embryonal knot and trophoblast tissues, cell numbers increasing by as much as five- to seven-fold as compared with normal blastocysts in diapause. These observations suggest that ovariectomy during the delay interval stimulated mitotic activity in the blastocyst, but, in the absence of ovarian support, the uterine environment was incompatible with continued embryonic differentiation and subsequent nidation.

INTRODUCTION

The general sequence of the reproductive cycle of several mustelids has been described (e.g. Hansson, 1947; Enders, 1952; Canivenc, 1960, 1966; Wright, 1963, 1966) but physiological parameters influencing embryonic diapause in these species are poorly understood. Earlier workers, relying on histological evaluation, have suggested that progesterone production is minimal during diapause but that a striking elevation of progesterone titre occurs in conjunction with reactivation of the blastocysts before nidation. This interpretation has been confirmed, in general, by biochemical investigations (Canivenc, Short, Bonnin-Laffargue, 1966; Mead & Eik-Nes, 1968), but exogenous progesterone is apparently incapable of substantially reducing the diapause interval (Hansson, 1947; Hammond, 1951; Wright, 1963; Canivenc, Bonnin-

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Laffargue & Lajus, 1967; R. M. Shelden, unpublished observations). Oestrone, either alone or in combination with progesterone, also failed to stimulate weasel embryos during diapause (R. M. Shelden, unpublished observation).

Although several investigators have attempted to stimulate nidation by administering exogenous hormones, there have been few attempts to determine the fate of mustelid blastocysts following ovariectomy during diapause. Canivenc & Laffargue (1957) and Neal & Harrison (1958) reported recovery of apparently normal European badger blastocysts 4 weeks and 10 weeks, respectively, after ovariectomy during diapause. Conversely, Wright (1963) found only fragmented blastocysts in the long-tailed weasel 2 weeks after spaying. In view of these contrasting observations, the fate of short-tailed weasel blastocysts was investigated following ovariectomy during the diapause interval.

MATERIALS AND METHODS

Short-tailed weasels, *Mustela erminea*, were trapped live during late summer and early winter months and maintained in the laboratory throughout the investigation. Female weasels so obtained were of undetermined age and reproductive history but most females were carrying blastocysts in diapause. Each weasel was maintained individually in a $\frac{1}{2}$ -in. wire mesh cage ($23 \times 19 \times 42$ cm) with attached nest box, at a room temperature of approximately 20° C. Although not precisely controlled, combined natural and artificial light provided a minimum light period of 10 hr per day. Water and food were freely available.

Bilateral ovariectomy was carried out under pentobarbitone sodium (40 mg/kg) anaesthesia by way of a single mid-abdominal incision. Each ovary was removed either alone through a tear in the ovarian bursa or together with the corresponding oviduct and proximal 5 mm of the uterine cornu. Uterine cornua were obtained subsequently by hemihysterectomy or autopsy.

Ovaries, oviducts and uteri with their blastocysts were submerged in Bouin's fixative immediately upon removal. After a minimum of 48 hr fixation, tissues were processed by routine histological methods and serially sectioned at $8 \mu\text{m}$. Sections were stained by Groat's modification of Weigert's haematoxylin procedure and examined under the light microscope. The complete serial section of each embryo was utilized for embryonic knot and trophoblast nuclei counts. Blastocyst diameters were estimated, where possible, from the size of gestation chambers occupied by the blastocyst since histological preparation frequently caused collapse of the trophoblast and rendered direct measurement of the blastocyst diameter impossible. All anatomical dimensions reported were obtained from fixed material with the aid of an ocular micrometer.

RESULTS

The gross morphology of normal short-tailed weasel blastocysts obtained during diapause was similar to that of unimplanted blastocysts of other carnivores. A complete zona pellucida 4 to $5 \mu\text{m}$ thick encompassed each embryo, which consisted of a small spherical inner cell mass at one pole of a unilaminar trophoblastic sphere (Table 1).

TABLE 1

CHARACTERISTICS OF *Mustela erminea* BLASTOCYSTS RECOVERED FROM NON-EXPERIMENTAL UTERI DURING DELAYED IMPLANTATION*

Month	No. of animals	No. of blastocysts	Approximate diameter (mm)† Mean (range)	No. of nuclei	
				ICM Mean $\pm$ S.E.	Trophoblast Mean $\pm$ S.E.
September	1	2	0.445 (0.43 to 0.45)	11.5 $\pm$ 0.50	184.5 $\pm$ 7.50
November	6	16	0.537 (0.45 to 0.66)	9.6 $\pm$ 0.61	140.6 $\pm$ 5.66
December	4	11	0.697 (0.48 to 0.88)	11.0 $\pm$ 1.36	167.4 $\pm$ 12.80
January	3	6	0.677 (0.50 to 0.76)	10.3 $\pm$ 1.58	149.3 $\pm$ 9.92

ICM = inner cell mass.

* In wild populations, diapause extends from May-June to February-March (Wright, 1963).

† Diameters estimated from size of gestation chamber.

The only noteworthy feature of the weasel blastocyst in diapause compared with the unimplanted mammalian blastocyst at other stages was the absence of mitotic figures in either the inner cell mass or the trophoblast of the quiescent weasel blastocyst. By contrast, as the weasel blastocyst approached nidation following termination of diapause, marked mitotic activity occurred in both tissues, the blastocyst diameter nearly doubled, and rapid endoderm differentiation transformed the unilaminar blastocyst into a bilaminar embryo before trophoblast attachment to the uterine epithelium.

The distribution of blastocysts within the uterine cornu during diapause varied between females, but most frequently the blastocysts occurred in a cluster near the utero-tubal junction, although in some cases, blastocysts were evenly spaced throughout the length of the uterine lumen. Transuterine migration of blastocysts had occurred in a few females, but this redistribution could not be correlated with the spaced or unspaced arrangement of embryos at the time of recovery. Spaced blastocysts were not more advanced in terms of development than were unspaced embryos.

The primary response by weasel blastocysts to ovariectomy during diapause

TABLE 2

FATE OF *Mustela erminea* BLASTOCYSTS FOLLOWING OVARIECTOMY DURING DIAPAUSE

	No. of animals	Days post-ovariectomy	Blastocysts recovered			No. of nuclei†	
			Normal	Abnormal	% normal	ICM	Trophoblast
Controls†	14	0	35	4	89.7	10.2 $\pm$ 0.68*	153.0 $\pm$ 5.44*
Bilaterally ovariectomized	7	9 to 12	10	13	43.5	69.8 $\pm$ 11.8*	701.3 $\pm$ 86.9*
	6	18 to 22	8	15	34.8	(200 to 500+)	(950 to 1500+)
	4	31	0	2	0	—	—
	4	65	0	10	0	—	—

ICM = inner cell mass.

† Data from Table 1. Abnormal embryos were uncleaved ova.

‡ Values expressed as mean $\pm$ S.E.; normal blastocysts only, or as range; seven blastocysts with multiple inner cell masses. See text.* $P < 0.001$.

was enhanced mitotic activity involving both the inner cell mass and the trophoblast (Table 2). This initial mitotic stimulation was followed by deterioration of the embryo, and by the 4th week after spaying only collapsed and fragmenting blastocysts with indistinguishable inner cell masses were recovered. This pattern of enhanced cellular activity followed by degeneration of the blastocyst was characteristic of all spayed females, irrespective of the time during diapause that the operation was performed, but the duration and degree of mitotic stimulation preceding death of the embryo varied considerably between embryos, even those within the same uterine cornu. Unequivocal endoderm differentiation did not occur in any of the stimulated blastocysts.

Seven blastocysts recovered from one female 18 days after ovariectomy were approximately the size of normal blastocysts at the time of nidation, and, although intact, their zonae pellucidae were much attenuated (less than 2 μm thick). Several inner cell mass-like protuberances were distributed about the trophoblastic sphere. The trophoblast was not folded in the vicinity of these protuberances, suggesting that their origin was not simply the result of general trophoblast expansion within the confines of an intact zona pellucida. Since these proliferations were morphologically indistinguishable, except on the basis of size, the largest in each blastocyst was assumed to represent the original inner cell mass (Table 2).

The uteri of spayed weasels followed an involutional pattern similar to that reported for spayed females of other species. The most striking changes occurred in the height of the luminal epithelium which declined by approximately 50% ($36.6 \mu\text{m} \pm 10.5 \text{ S.D.}$ to $19.5 \mu\text{m} \pm 2.2 \text{ S.D.}$) within the first 10 days after ovariectomy, and declined an additional 50% ($19.5 \mu\text{m} \pm 2.2 \text{ S.D.}$ to $8.2 \mu\text{m} \pm 0.6 \text{ S.D.}$) by the 9th post-operative week. The decrease in cell size appeared to involve primarily the loss of materials stored in the cytoplasm since nuclear volume remained essentially constant. By contrast, glandular epithelium was relatively insensitive to the loss of ovarian hormonal support, with cell height decreasing approximately 20% ($12.0 \mu\text{m} \pm 2.8 \text{ S.D.}$ to $9.4 \mu\text{m} \pm 0.5 \text{ S.D.}$) over a 9-week interval. A gradual but continuous decrease in the relative abundance of glandular elements occurred after spaying, but significant cellular necrosis did not occur in the uteri of spayed weasels.

DISCUSSION

These experiments establish that during diapause short-tailed weasel blastocysts, unlike blastocysts of the European badger (Canivenc & Laffargue, 1957; Neal & Harrison, 1958), were unable to survive in the uterus of the spayed animal for more than 3 weeks. Although the protocol employed does not demonstrate unequivocally that blastocyst mortality is a secondary event brought about by uterine involution rather than the result of a primary requirement for ovarian secretions by the blastocyst in diapause, the former possibility seems more likely in view of the temporal sequence of embryo loss following ovariectomy.

The absence of endoderm differentiation in stimulated weasel embryos recovered after ovariectomy suggests that the response reflected a general mitotic stimulation of the embryonic cells rather than an initiation of the nor-

mal developmental sequelae to embryonic diapause, but the stimulating effect was restricted to blastocysts and did not involve uterine epithelium or stroma except at the sites of surgical trauma. The possibility of an ovarian agent inhibiting blastocyst activity during diapause cannot be ruled out entirely, but preliminary observations following sham ovariectomy (which did not produce uterine involution) suggest that blastocyst stimulation may be more readily correlated with uterine traumatization than with loss of ovarian secretions.

Increased mitotic activity following ovariectomy has been reported for European badger blastocysts (Neal & Harrison, 1958, page 114) and for ferret blastocysts (Buchanan, 1969). However, since development of ferret embryos does not involve a period of obligatory embryonic diapause, this stimulation is observed only if ovariectomy is performed between Days 5 and 10 *post coitum*.

The data reported do not clearly establish the physiological significance of ovariectomy-induced mitotic activity in weasel blastocysts during diapause. While it is possible that the surgical manipulation induced elaboration of a specific blastokinin-like substance (Daniel & Krishman, 1969; Gulyas & Krishman, 1971), it is equally conceivable that the embryonic activity resulted from stimulation by non-specific growth factors synthesized in response to surgical trauma (Neuhaus, Balegno & Chandler, 1966; Shihabi, Balegno & Neuhaus, 1970). Additional experiments are necessary to elucidate the significance of this phenomenon.

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