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SYNTHESIS OF THE RABBIT UTERINE PROTEIN, BLASTOKININ: EFFECT OF A PROTEIN-FREE DIET

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ABSTRACT

Rabbits cannot sustain pregnancy when deprived of dietary protein. These studies were an attempt to determine if a failure in uterine protein synthesis, particularly blastokinin, might account for the abortions.

Only half of the does bred within two days after being put on a protein-free diet, had normal embryos and normal levels of total uterine protein and of both blastokinin and the high molecular weight fraction in their uterine fluids on day 5 *post-coitum*. The others had low levels and/or failed to ovulate. After three days without protein our rabbits would not breed.

Because of the reported relationship between starvation and low circulatory progesterone levels. ovariectomized rabbits were deprived of dietary protein for two weeks and then subjected to a series of four daily injections of progesterone at concentrations normally known to induce high levels of synthesis of blastokinin. These animals however were unable to synthesize significant amounts of blastokinin and only low levels of other uterine proteins.

It is concluded that protein-deprived rabbits *may* produce enough uterine protein to support preimplantation embryogenesis but prolonged deprivation results in an inability to synthesize essential uterine proteins in response to exogenous progesterone.

INTRODUCTION

The reproductive success of mammals under conditions of starvation or severe nutritional deficiency varies greatly from one species to another. For example, most laboratory rodents cannot sustain pregnancy on a diet deficient in protein (Leathem 1966, Rattner et. al., 1978), while pigs carry their litters to term on diets devoid of or low in protein content (DeGeeter et. al., 1972, Anderson et. al., 1979). In our experience, rabbits also are unable to maintain pregnancy when starved for protein and we have been interested in how starvation affects their uterine function.

Recently, Murray et. al. (1979) showed that the uterine capacity for protein secretion is maintained in gilts even after 122 days on either a low-protein or a protein-free diet. We were encouraged to examine this same problem using rabbits because of their reproductive failure on such a diet (as noted) and because they synthesize the uterine protein, blastokinin (Krishman and Daniel, 1967) which is believed to be critical for survival of preimplantation stage embryos. This paper reports experiments designed to appraise the effect of a protein-free diet on the synthesis of blastokinin. It relates to earlier papers in this series which report changes in rate of blastokinin synthesis (Daniel and Booher, 1977), age dependency (Booher and Daniel, 1977) and the effect of light deprivation (Daniel, 1979).

METHODS AND MATERIALS

The experimental protocol is shown diagramatically in Figure 1 Twenty-four young adult New Zealand White female rabbits, divided into groups of 6 each, were used in this study: Three of the groups were maintained on a protein-free diet (ICN-Nutritional Bichemicals 904666) and the fourth group was fed a normal diet (Purina Checkers) containing 16% protein.

Group 1 animals were started on the protein-free diet (PFD) and then bred within two days thereafter and maintained on PFD for the anticipated gestation period (32 days) to confirm rabbits' inability to sustain pregnancy under this nutritional deficiency. Three of these same does were then returned to the normal diet (ND) for one month, bred again and retained on ND and the success of those pregnancies recorded.

Group 2 animals were also started on PFD, then bred as before and continued on the PFD until day five *post coitum*. At that time they were killed by cervical dislocation (Daniel and Boyce, 1978) and the uteri flushed with physiological saline solution into a watch glass to facilitate collection of embryos and uterine fluids. The blastocyst stage embryos were counted, measured and their normalcy appraised. The uterine flushings were kept cold and analysed by molecular seiving as described earlier (Daniel and Booher, 1977). The essential steps involved centrifugation to remove cellular debris, dialysis in Spectrapore membrane tubing overnight against distilled water, concentration with Aquacide (3-4 hours) and then used as the sample for filtration in a Sephadex G-200 column using citrate buffer at pH 7.4. Nine-drop fractions (0.5 ml) were collected with a LKB ultrorac fraction collector and each fraction (and the original sample) analysed for protein content by the method of Lowry et. al. (1951). These data were displayed graphically by plotting the optical density on the vertical axis against fraction number on the horizontal axis. The relative proportions of the blastokinin fraction and the high molecular weight fraction (as a second indicator) were determined by measurement of the area under each graph peak.

Group 3 animals were ovariectomized, using standard surgical techniques, and permitted to recuperate for one week. They were then placed on the PFD for two weeks at which time they were given 3 mg of progesterone/kg body weight subcutaneously daily for four days (Booher and Daniel, 1977) and killed on the afternoon of the fourth day. The uteri were excised, flushed and the flushings analysed by Sephadex gel filtration as described for Group 2 above.

The control was provided by Group 4 which was treated identically to Group 3 except that these animals were kept on the normal diet throughout the experiment. Additional control comparisons were made with embryo and uterine protein data from normal pregnancies as reported in other publications from this laboratory (i.e. Arthur and Daniel, 1972, Murray and Daniel, 1973; Daniel and Booher, 1977; Booher and Daniel, 1977; Dunbar, 1977).

RESULTS

The results of these experiments are presented in Table 1 and representative uterine protein patterns shown in Figure 2.

None of the group 1 animals sustained pregnancy on the protein-free diet. When three of these six were

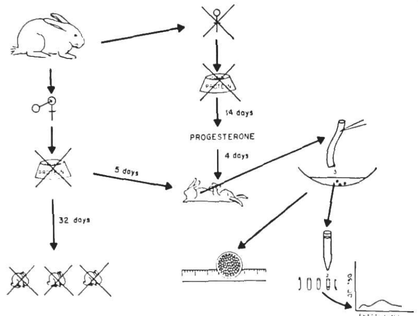

FIG. 1. *Experimental protocol. Bred does were put on a protein-free diet and either left to go to term or killed on day 5 post coitum and their uterine fluids and embryos collected. Another group of does were ovariectomized, deprived of dietary protein for 2 weeks, then treated with progesterone and their uterine fluids collected.*

TABLE 1. *Uterine protein and reproductive success in rabbits maintained on a protein-free diet.*

Experimental Group	Animal ID No.		Started on Protein-Free Diet	Breeding Date	Anticipated Parturition Date (32 days)	Actual Parturition Date	Number of Young				
1	1		2/9	2/10	3/14	—	0				
	2		2/12	2/12	3/16	—	0				
	3		3/13	3/14	4/15	—	0				
	4		3/14	3/14	4/15	—	0				
	5		4/2	4/2	5/	—	0				
	6		4/17	4/18	5/20	—	0				
					Uterine Flushing Date	Number of Corpora Lutea	Number Embryos Recovered	Blastocyst Diameter (mm)	Total Protein (mg/uterus)	% HMW	% BKN
2	7		2/14	2/16	2/21	9	4	0.9-1.1	5.2	25	40
	8		2/15	2/16	2/21	0	0	—	1.1	—	—
	9		3/15	3/16	3/21	7	1	0.5	1	15	12
	10		3/17	3/17	3/22	0	0	—	<.7	—	—
	11		4/12	4/12	4/17	10	10	0.8-1.1	6.0	21	36
	12		5/2	5/2	5/7	9	8	0.7-1.0	4.9	28	41
		Date of Ovariectomy		Dates of Progesterone Administration							
3	13	2/27	3/3	3/13-3/17	3/17				0.9	N.D.	N.D.
	14	2/27	3/3	3/13-3/17	3/17				5.3	9	N.D.
	15	3/28	4/3	4/13-4/17	4/17				2.5	15	4
	16	3/28	4/3	4/14-4/18	4/18				2.2	8	N.D.-3
	17	4/18	4/23	5/3 -5/7	5/7				(infected uterus)		
	18	5/9	5/14	5/24-5/28	5/28				2.8	10	N.D.
4	19	2/27		3/13-3/17	3/17				5.5	30	44
	20	3/28		4/13-4/17	4/17				4.8	27	50
	21	4/18		5/3 -5/7	5/7				2.5	19	32
	22	4/18		5/3 -5/7	5/7				5.3	20	38
	23	5/9		5/24-5/28	5/28				7.2	23	40
	24	5/11		5/26-5/30	5/30				5.0	21	41

returned to complete diet and then bred again, to determine if they suffered any permanent damage to their reproductive capacity as a result of the deficient diet, all three had successful full term pregnancies and gave birth to 6, 9 and 10 normal young respectively.

Four of the six (66%) does in Group 2 had embryos and corpora lutea on the fifth day p.c. but in one case (#9) only one tiny blastocyst was recovered. The uterine fluids in three of these were within the normal range for total protein content and for the percentages of high molecular weight components (HMW) or blastokinin (BKN). In the animal having only one blastocyst the total protein and the relative amounts of HMW and BKN were much lower than normal. For the two animals having neither C.L. nor embryos, the uterine protein was also very low and neither HMW nor BKN were measurable.

As noted in the literature and confirmed here by Group 4, ovariectomized rabbits provided with a complete diet for two weeks and then given progesterone for 4 days synthesize uterine proteins in predictable and significant amounts. Conversely, as demonstrated by Figure 2, if castrated does are denied dietary protein for two weeks before and also during the progesterone treatment, they fail to produce normal levels of total uterine protein including the HMW and BKN fractions (Group 3). Only those fractions which represent predominately serum proteins are retained at their typical levels.

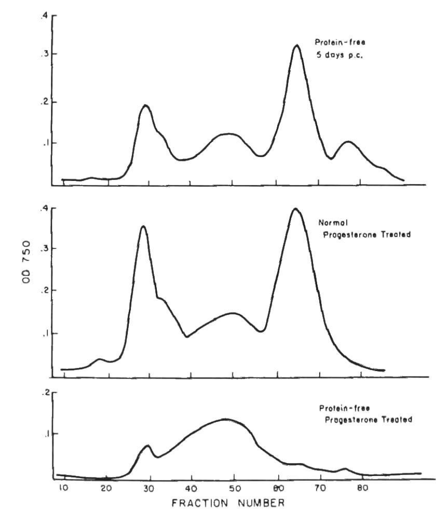

FIG. 2. *Typical protein patterns obtained after Sephadex gel fractionation of uterine proteins of rabbits under various experimental conditions. See text for details.*

DISCUSSION AND CONCLUSIONS

In mice restricted dietary intake interrupts blastocyst implantation and the levels of plasma progesterone are significantly reducd on the day of implantation in these animals (Rattner et. al., 1978). In rats kept on a protein-free diet the pregnancies are aborted on about day 11 which is coincidental with the time when Giannina and Leathem (1974) found a dramatic decrease in circulating progesterone levels. These same workers speculated that this decline in progesterone may reflect a defect in placental development because the rat placenta is becoming steroidogenic at this time. Pregnancies can be sustained in protein-starved rats by daily injects of estrone and progesterone, and to a lesser extent by prolactin (Callard and Leathem, 1970). Also, corticosterone improved the success of early pregnancy when given to adrenalectomized rats deprived of dietary protein (Morishige and Leathem, 1973). These, and similar observations have caused a number of workers to think that restricted food intake produces a condition of "dietary hypophysectomy" (e.g. Lamming, 1966; Leathem, 1966; Everitt and Porter, 1976).

Conversely, Anderson et al. (1979) found that progesterone levels were sustained in starved pigs throughout their successful pregnancies. Considering that uterine protein secretion in swine is progesterone regulated (Knight et. al., 1973) then the findings of Murray et. al. (1979) about adequate uterine protein levels in gilts on a protein-free diet are not surprising.

That female rabbits on a protein-free diet are unable to maintain pregnancies, is clearly shown here by the animals in Group 1 and that this is only a temporary condition is demonstrated by subsequent successful litters of three of these animals, when returned to an adequate protein intake. The question of whether the pregnancy fails prior to implantation and whether it can be associated with inadequate uterine protein was asked with the animals in Group 2. The results were ambiguous in that only half of these animals had normal embryos and normal levels of uterine protein on day 5 p.c. Protein levels were deficient in the one doe from which only one retarded embryo was recovered and in the two others without either embryos or corpora lutea. Obviously these latter two failed to ovulate. We had hoped to get more definitive results by prolonging the period of protein insufficiency before breeding, but when this was tried, we were unable to get females to breed after they had been on the protein free diet for 3 days or longer. (In reality the animals starve at the beginning of each experiment because they refuse to eat the protein-free pellets for approximately five days when first introduced to them.)

To try to clarify the relationship between dietary protein insufficiency, and progesterone regulation of uterine protein production, the experiment with exogenous progesterone was conducted using the animals in Groups 3 and 4. It is apparent that prolonged (14 days) dietary protein deficiency interferes with a rabbit's capacity to synthesize uterine protein in response to progesterone stimulation. Blastokinin and the high molecular weight proteins of the rabbit uterus are not synthesized in response to progesterone after an animal has been denied dietary protein. Whether this results from inadequate precursors, failure of progesterone transport or receptors, the effects of starvation stress, or other causes remains to be determined but it appears to contradict related findings in other species.

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SYNTHESIS OF THE RABBIT UTERINE PROTEIN, BLASTOKININ: IN AGING ANIMALS WITH REPRODUCTIVE FAILURE

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ASTRACT

One of the major causes of the decline in reproductive success which accompanies ageing in mammals has been identified as "uterine failure". In an attempt to clarify the meaning of this phenomenon the uterine flushings, taken on days 5, 6 or 7 *post coitum*, from ageing ($\geqq$ 2 years old) rabbits with a history of aborted pregnancies were analysed for embryo normalcy and for protein content and compared with those of younger does as reported in earlier publications from this laboratory. Blastokinin was specifically measured as an indicator of uterine protein adequacy.

No significant differences were found between the two age groups. Considering that implantation in the rabbit occurs on day 7 p.c., it was concluded that the "uterine failure" associated with ageing in this species probably relates to a post-implantation event.

INTRODUCTION

Reproductive success declines in ageing animals. For mammals, many reasons for this decline have been implicated including oocyte abnormalities, immunological response, reduced maternal steroid levels, chromosome deterioration, increased resorption and/or failure of ovulation, gamete transport, fertilization, implantation, placentation, hormonal interaction, "uterine support," sperm viability, etc. (see reviews by Biggers 1968, Adams 1970, Talbert 1977). For the rabbit, from his embryo transfer studies, Adams (1964) concluded that "the failure of aged does to support pregnancy is due to defects in the maternal environment, particularly the uterus," but that the nature of the defects is obscure. We find no record of any studies of changes in the uterine secretions which might be correlated with reproductive failure; important because these secretions are considered to be especially critical to embryogenesis prior to implantation. This paper reports studies of blastokinin (Krishnan and Daniel 1967) as an indicator of possible changes in proteins of the uterine fluids of "ageing" rabbits with a history of reproductive failure.

Rabbits have been reported to live as long as thirteen years (Altman and Dittmer 1972) and to continue to reproduce up to almost six years of age (Adams