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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ABSTRACT

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 (≥ 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

1970) but such animals are extremely rare. More reasonable expectations are reported by Weisbroth (1974) who notes that even though "the life span of normal healthy rabbits under domestication is approximately 7-8 years, the reproductive efficiency ordinarily declines rather quickly after the second year of life." Thus we are defining "ageing" rabbits as those 24 months old or older.

METHODS AND MATERIALS

Fourteen rabbits of mixed breeds were used in this study. All were over two years old (up to 3½) with a known breeding performance. Each had successfully produced at least 2 litters (and up to 5) followed by a history of 5-10 matings where pregnancy was not sustained. The animals were bred and half of them were killed (by cervical dislocation) on day 5 *post coitum*, the uteri isolated and flushed with PBS and the flushings dialysed, concentrated and fractionated on a Sephadex G-200 column as described earlier (Daniel and Booher 1977). Protein content of the concentrated flushings and of each fraction obtained by gel filtration was determined by the procedure of Lowry et. al. (1951). Total protein and the proportion that was blastokinin, determined by measuring the area under that peak in the profile obtained from the Sephadex fractionation, is reported here for each animal. The numbers and sizes of blastocyst-stage embryos are also reported.

Of the remaining seven does, 5 were killed on day 6 p.c. and 2 on day 7, for each of which total uterine protein and embryo size and number were recorded.

For comparisons, data from previous publications of work with younger does (9-18 months of age), were used.

RESULTS

Table 1 gives the pertinent information about the animals used and the data generated in these studies. As indicated by the corpora lutea, eleven of the fourteen animals ovulated and normal-sized embryos were recovered from nine of the eleven. Two undersized embryos were recovered from one more and the uterus was infected in two of the animals which failed to ovulate. Total uterine protein levels and that proportion which was blastokinin were within typical ranges for all of the animals with normal embryos for all 3 days tested (i.e. 5, 6 and 7 p.c.) One that failed to ovulate and the one with the two abnormally small embryos (Animals #7 and 10) had very low levels of uterine protein with blastokinin low to undetectable. The uterus from the doe which had ovulated but from which embryos were not recovered (#1) had a normal amount of protein and the blastokinin level was the largest of all the experimental animals. Figure 1 compares the fractionation pattern for uterine proteins between a young adult and an older animal on day 5 p.c.

TABLE 1. *Uterine protein and reproductive success in aged rabbits.*

Animal I.D. #	# of earlier litters	# of recent sterile matings	Age at this mating (mos)	Day of Sacrifice (p.c.)	# of corpora lutea	# of embryos recovered	Mean Size of embryos (m.m. diameter)	Range Size of embryos (m.m. diameter)	Total Protein mg/ uterus	% Blastokinin	Comments
1	2	5	26	5	9	0	—	—	3.8	54	Neither embryos no degenerate eggs recovered.
2	2	5	24	5	10	0	1.00	0.8-1.2	4.6	45	
3	3	5	30	5	8	9	0.95	0.7-1.2	4.1	48	Dark crystalline bodies in fluid.
4	2	7	25	5	6	5	1.11	0.9-1.3	3.5	38	
5	2	5	24	5	8	8	1.05	0.6-1.2	3.2	42	
6	2	10	42	5	0	—	—	—	—	—	Uterus full of pus.
7	5	8	32	5	0	—	—	—	0.95	0	
8	2	7	28	6	9	10	2.90	2.0-4.3	6.75	41	
9	2	6	27	6	0	—	—	—	—	—	Uterus full of pus.
10	3	6	36	6	6	2	0.50	0.4-0.6	0.7(?)*	15(?)*	One degenerate egg also recovered.
11	2	6	31	6	6	4	3.87	3.2-4.2	7.5	30	
12	2	6	32	6	10	7	2.92	2.5-3.2	8.1	26	One degenerate egg also recovered.
13	3	5	24	7	11	11	5.5	5.2-5.8	6.0	37	
14	2	7	30	7	8	9	4.9	4.0-5.5	7.2	28	
Young Animal Comparisons			6-18	5			1.02	0.5-1.4	3.6-5	40.43	These control data are taken from previously reported studies from this laboratory: Embryo size from Daniel 1964; Uterine protein ranges from Johnson 1972, Arthur and Daniel, 1972, Daniel and Booher, 1977 and Dunbar 1977.
			6-18	6			2.80	1.8-3.9	4.4-7.6	26-45	
			6-18	7			5.01	4.5-5.9	3.8-7	20.36	

*Accuracy questionable because of low concentrations.

DISCUSSION AND CONCLUSIONS

Even though all of the fourteen animals listed in Table 1 had failed to sustain pregnancies from five to ten previous matings, nine of them had normal embryos and normal levels of blastokinin and total uterine protein when examined on days 5, 6 or 7 *post coitum*. Since she had ovulated but no embryos were recovered on day 5, it may be assumed that doe #1 was pseudopregnant and would therefore be expected to have a typical uterine protein pattern. The other four animals had abnormally low proteins but three of them had failed to ovulate and therefore were not progestational. These data reinforce the hypothesis that normal, healthy rabbit blastocysts require high levels of uterine protein, specifically blastokinin. That doe #10 had low protein *and* abnormally small embryos on day 6 p.c. is further support of this hypothesis.

Although most investigators of the phenomenon of embryonic death in ageing mammals assign "uterine failure" as the primary cause, there has been little success in identifying the nature of that failure. Maurer and Foote (1971) found that the mortality of embryos transferred to aged does was 3-fold greater prior to implantation than it was post implantation, a finding which would suggest a preimplantation phenomenon. Larson et. al. 1972 found a reduction in uptake of both progesterone and estradiol by the uteri of old rabbits which, because uterine protein secretion prior to implantation is regulated by these steroids (Arthur and Daniel, 1972), led us to believe that we might demonstrate low levels of these proteins in ageing does. From our results, however, it is obvious that ageing female rabbits which are experiencing reproductive failure can still produce typical uterine protein levels and normal embryos prior to implantation. Thus the cause of the failure would seem to be associated with the period during or after implantation, as Finn (1970) also concluded from his work with mice. After implantation in the rabbit, the uterine secretions are less specific and contain mainly serum proteins.

Larson et. al. (1973) found that most embryonic wastage in aged rabbits occurred during the first twelve days *post coitum* and Adams (1970) claimed that prenatal loss mainly occurred after day 10. Collectively then, these observations would identify a critical period between days 10 and 12 *post coitum* which, in the rabbit, is coincidental with initial placental development and function, and not obviously related to early uterine protein synthesis.

Blastokinin production, specifically, is quite adequate in these older does and apparently not correlated with the termination of their pregnancies.

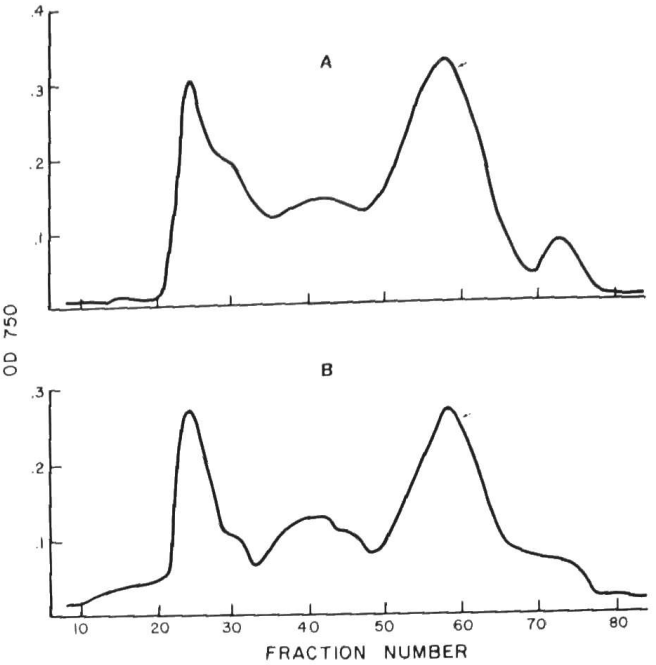

FIG. 1. *Comparison of proteins after Sephadex gel fractionation of uterine flushings collected on day 5 p.c. from young (A) and from ageing (B) rabbits. The arrows identify the blastokinin peak.*

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