LETTERS TO THE EDITORS

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An Attempt to cross the Domestic Rabbit (*Oryctolagus cuniculus*) and Hare (*Lepus europæus*)

PREVIOUS attempts¹ to obtain young from domestic rabbits by insemination with epididymal spermatozoa from hares (*Lepus timidus ainus* or *Lepus europæus*) have proved unsuccessful. The possibility, however, that fertilization and a limited amount of embryonic development may have occurred has not so far been investigated. Recently², it was found that in rabbits inseminated with epididymal spermatozoa from the eastern cottontail rabbit (*Sylvilagus floridanus*), 37 per cent of the eggs were fertilized but degeneration occurred at all stages before or at blastulation. The experiment described here was undertaken to determine whether fertilization occurred in rabbits inseminated with hare spermatozoa and, if so, at what stage development ceased.

During May and June, four male hares (Nos. 1-4) were shot locally and returned to the laboratory within one hour of death. Spermatozoa were obtained from the epididymides and suspended in Krebs's Ringer bicarbonate solution at 37° C., which induced a high degree of motility. Each female rabbit was inseminated with 1 ml. of sperm suspension. The ten adult female rabbits, A-I, used for this experiment were caged separately and not mated for a period of at least 30 days prior to the experiment. They were given an intravenous injection of 1 mgm. equivalent of pituitary lutealizing hormone (PLH, Armour Laboratories) to induce ovulation. Immediately afterwards, rabbits A and B were inseminated with 110 million spermatozoa (from hare No. 1), rabbits C-H received 160 million spermatozoa (mixed sample from hare Nos. 2 and 3) and does I and J received 692 million spermatozoa (from hare No. 4). One Fallopian tube was removed from rabbits A and B at 31 and 54 hr. after insemination. These rabbits were allowed to recover and were then autopsied at 122 and 145 hr. after insemination. The remaining eight rabbits were killed at intervals varying from 29 to 124 hr. after insemination. The technique of egg recovery has already been described³. The recovered eggs were examined microscopically to determine their general condition, stage of development and numbers of spermatozoa present.

The mean total number of ovulations per rabbit was 10.6 ± 0.6 (standard error of mean), range 8-15. Details of the number and condition of eggs recovered are given in Table 1. In rabbits examined at, or later than, 73 hr. after insemination, forty-nine eggs (equivalent to 100 per cent of the ovulations) were recovered from the Fallopian tubes; two of these eggs were uncleaved, four showed retarded development, whereas the remaining forty-three had reached a stage of development appropriate to their age. A total of thirty-eight eggs (66.7 per cent of the ovulations) were recovered from the uteri of the rabbits killed at 122-145 hr. after insemination. Although all thirty-eight eggs had undergone cleavage, no egg had developed beyond the early blastocyst stage, which is normally observed 84 hr. after mating;

Table 1. EGG DEVELOPMENT FOLLOWING INSEMINATION OF RABBITS WITH EPIDIDYMAL SPERM OF HARES

Rabbit	Interval between insemination and egg recovery (hr.)	No. of ovulations	No. of eggs recovered and stage of egg development
A	31*	4	2, 1-cell; 2, 2-cell
B	145	4	1 early blastocyst
	54*	4	3, 4-cell; 1, 16-cell
C	122	6	3 late morulae; 1 early blastocyst
D	29	10	10, 4-cell
E	67	11	11 late morulae
F	122	15	9 late morulae; 1 early blastocyst
G	123	11	10 late morulae
H	124	12	5 late morulae; 2 early blastocysts
I	124	9	6 late morulae
J	49	10	4, 8-cell; 3, 12-cell; 3, 16-cell
	73	10	1, 16-cell; 9 late morulae

* Tuballectomy performed

there were five early blastocysts and thirty-three late morulae in which development had ceased between 60 and 70 hr. after insemination. Small numbers of spermatozoa, varying from one to six per egg, were observed in the perivitelline space, zona pellucida and mucin; there appeared to be no relationship between the number present and the number used for insemination. The proportion of eggs 'fertilized', as determined by cleavage, was 97.7 per cent, which is not significantly different from the figure obtained from normally mated rabbits⁴.

Three questions which these observations raise are: (1) Were the eggs fertilized? (2) Did parthenogenetic development occur? (3) Why did development fail at the late morula or early blastocyst stages? The presence of hare spermatozoa in the perivitelline space and zona pellucida of rabbit eggs suggests that they provided the stimulus for development. Since no egg reached the five-day blastocyst stage, no attempt was made to determine the chromosome number⁵, and it is therefore not possible to state whether male hereditary material was transmitted to the eggs. For the time being, no explanation other than incompatibility can be advanced to account for the failure of the eggs to develop beyond the early blastocyst stage. Interspecific crosses are rarely possible, and this is often thought to be due to an immunological incompatibility, of a statistical nature, between combining groups on the surfaces of the heterologous eggs and spermatozoa⁶. These observations suggest that in certain cases this incompatibility may not exist, though the other barriers to successful interspecific crosses do.

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⁴ Adams, C. E., *J. Endocrin.*, **13**, 296 (1956).

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⁶ Rothschild, Lord, "Fertilisation" (Methuen, London, 1956).