J. Reprod. Fert. (1971) **25**, 329–341

MATERNAL AGEING AND EMBRYONIC MORTALITY IN THE RABBIT

I. REPEATED SUPEROVULATION, EMBRYO CULTURE AND TRANSFER

R. R. MAURER AND R. H. FOOTE

Department of Animal Science, Cornell University, Ithaca, New York 14850, *U.S.A.*

(*Received 9th July* 1970)

Summary. Embryos from ageing (20 to 148 weeks of age) and young (20 to 30 weeks of age) donors were transferred to ageing (52 to 221 weeks of age) and young (18 to 30 weeks of age) recipients to partition the effects of ageing oocytes and uterine environment on embryo mortality. More than 3300 two- to eight-cell embryos collected following superovulation at six 6-month intervals were transferred. The average number of ovulation points per ageing donor doe superovulated at the six intervals declined with age and repeated superovulation. The average number of ovulations for the young donors at the six intervals also differed slightly, the low number (forty-one) for the last group possibly being due to the crossbred strain used. Both embryo recovery and cleavage rates usually exceeded 80% and did not differ between young and ageing donors. The percentage of viable young developing from embryos transferred from ageing and young donors showed that the potential for embryo development had not been impaired during 3 years of ageing. The percentage of viable young developing from embryos transferred to ageing and young recipients indicated that conditions for maintaining pregnancy had been impaired in the ageing recipients. The average number of ovulations for a group of old does superovulated for the first time at 229 weeks of age was fourteen compared to sixty-two for young controls, and only 26% of the embryos transferred from the old does developed into neonates, whereas 45% of those from young donors developed normally. As the female ages, the uterine environment may become less conducive to prenatal development and the oocytes then show the effects of the ageing process directly or as a result of exposure of the oocyte or young embryo to the ageing oviduct. At laparotomy 12 days after transfer, the ageing recipients had 45·3% pre- and 14·8% postimplantation mortality. Corresponding values for young recipients were 33·5 and 16·0% respectively. Laparotomy increased embryo mortality in ageing females only. The percentage of embryos which developed to blastocysts *in vitro* paralleled the development *in vivo* of embryos in ageing and young donors. The overall sex ratio of 81·6

males/100 females resulting from transferred embryos was significantly different ($P<0{\cdot}05$) from the expected figure.

INTRODUCTION

The maternal reproductive system of mammals becomes less efficient with increasing age (Krohn, 1962, 1964; Talbert, 1968; Biggers, 1969). Embryo transfer studies by Boot & Mühlbock (1954) and Talbert & Krohn (1966), with mice, and Adams (1964), with rabbits, showed that the gametogenic function of the ovary outlasts the ability of the uterus to maintain the reproductive process. Blaha (1964) concluded that poor reproduction in old golden hamsters was due to an inadequate uterus, although fewer oocytes ovulated and there was more abnormal development of those that did. Krohn (1962) found that old ovaries grafted into young animals produced more pregnancies and neonates than young ovaries in old mice. He concluded that the uterus or its hormonal control becomes incompetent.

Corner (1923) classified the causes of embryonic mortality into defects of zygosis, defects of the zygote and faulty maternal milieu. The present study was undertaken to determine the significance of each of these categories in causing embryonic mortality in the rabbit.

MATERIALS AND METHODS

Six hundred mature, female, Dutch-belted rabbits and an albino strain derived from our Dutch-belted colony, along with a few Dutch–Polish cross-bred rabbits from the colony, were used. The true-breeding coloured and the albino lines were maintained as a means of identifying the source of young born following transfer. In addition, twenty mature Dutch-belted females 3½ to 5 years of age with complete breeding histories were purchased. All animals were kept at 21° C with 12 hr of artificial light. Each animal was caged individually and was fed a high quality pelleted diet containing a minimum of 17% protein. Periodically, a medicated diet containing 0·0055% furazolidone and 0·0125% sulphaquinoxaline was fed.

The main experiment was designed to compare development of embryos obtained from young does 5 to 7 months old with ageing does aged over a period from 5 to 35 months, after transfer to both young and ageing recipients. The ageing does were used repeatedly while new young does were continuously available. In a smaller trial, a group of old does were superovulated for the first time when they were at least 4 years old. They are referred to as old donors. Analysis of variance, Chi square, Duncan's multiple-range test and multiple regression were used to analyse the results of these experiments statistically.

Ageing donors

A group of sixty albino and true breeding coloured does, 20 to 25 weeks of age, were assigned as the 'ageing donors'. Body weight averaged 2·2 kg initially and was maintained at about 2·5 kg during the experiment. Each doe was superovulated and embryos collected six times at approximately 26-week intervals for

3 years. Each 20- to 25-week-old doe was injected with 0·3 mg FSH (FSH-P Armour) s.c. twice daily for 3 days and on the 4th morning, 2·5 mg LH (PLH, Armour) was injected i.v. (Maurer, Hunt & Foote, 1968). The FSH dosage was increased to 0·5 mg twice daily for 3 days for the second to the sixth superovulation, while the LH level remained at 2·5 mg. Does were artificially inseminated with 0·1 ml of semen containing more than 1×10^6 motile spermatozoa from a male of the same coat colour at the time that they received an ovulatory injection of LH. At the fourth, fifth and sixth superovulation, does previously ovulating poorly from the exogenous LH were injected with 50 to 100 i.u. of HCG.

Approximately 12 weeks after every superovulation, each doe was either mated naturally or inseminated and subsequently allowed to kindle and raise any resulting young to 4 weeks of age. Also, any doe kindling as a result of embryos not recovered at the superovulation cycle was allowed to raise the young to 4 weeks of age.

After the fourth superovulation, the number of does was reduced to thirty animals. Nine died before or during the fourth superovulation, nine were culled for adhesions or failure to ovulate and the remaining twelve does were randomly selected and used on a further study of uterine conditions.

Old donors

Six does 210 to 267 weeks of age and averaging seventeen previous litters were superovulated using 0·5 mg FSH and 2·5 mg of LH, as described for the ageing donors after the first superovulation. They were also inseminated. These 'old donors' had not been given any exogenous gonadotrophins previously.

Young donors

Six groups of twenty to thirty nulliparous does from albino or true-breeding coloured lines 5 to 7 months of age and averaging 2·1 kg in body weight were used. They were superovulated with 0·3 mg FSH s.c. twice daily for 3 days followed with 2·5 mg of LH, and inseminated at the same time as the ageing donors. An additional group of six 24-week-old does were superovulated at the same time as the old donors to provide embryos from young donors.

Males

Three albino and six coloured mature fertile males, 20 to 150 weeks of age, were maintained for natural mating and as semen donors for artificial insemination. They were checked periodically for fertility. Each semen sample collected was examined before it was used for insemination.

Three vasectomized males were used to induce ovulation in certain females. Seminal fluid was collected from these males and repeatedly found to be free from spermatozoa before the animals were assigned to the experiment.

Recipients

'Young' recipients were nulliparous does of 18 to 30 weeks of age. 'Ageing' recipients were parous does, 52 to 221 weeks of age, which aged during the experiment. 'Old' recipients were parous does, 222 weeks of age. Each recipient

was placed with a vasectomized male for 2 min and allowed to have two sterile copulations to induce ovulation. If copulation failed to occur, each recipient was injected with 0·5 mg LH/kg b.wt.

Embryo collection and transfer

Embryos were collected 26 to 33 hr after LH injection by the method of Maurer, Hunt & Foote (1968). The average interval for most groups was 29 to 30 hr. Ovulation points were counted on each ovary. The embryos and/or oocytes obtained were counted and examined with the aid of a stereomicroscope at ×40 to ×120 for stage of development and abnormalities. An ovum was considered to have been fertilized and was classified as an embryo if it contained two or more equal blastomeres and otherwise appeared to be normal morphologically.

The method of embryo transfer described by Maurer, Hunt, Van Vleck & Foote (1968) was used. The number of ovulation points for each recipient doe was counted. The genetic (coat colour) type, stage of development and number of embryos transferred to each oviduct were recorded. When possible, ten embryos, five from an ageing donor or old donor and five from a young donor were placed in opposite oviducts of each young, ageing and/or old recipient. The oviduct to receive each set of five embryos was chosen at random. The donors of the embryos transferred to opposite oviducts were of different coat colours so that the neonates could be identified at birth by their colour pattern. Every pair of recipients included in the results was required to have (a) received at least two embryos from an ageing or old donor as well as from a young donor, (b) ovulated within 36 hr preceding transfer, and (c) lived for at least 28 days after transfer.

Embryo survival

After birth, the neonates were counted, sexed, weighed and their genetic mothers identified by coat colour. All male neonates were immediately removed from the experiment. All ageing recipients raised their female neonates, but young recipients were removed from the experiment shortly after parturition.

To obtain information on implantation and embryo survival, embryos from a sample of ageing and young donors were transferred to ageing and young recipients. Laparotomy was performed 12 days later under ether anaesthesia. Controls consisted of similar animals but laparotomies were not performed. The number of implantations was counted and their diameters measured. At birth, the neonates were handled as described for the recipients not subjected to laparotomy.

Embryo culture

When more embryos than were needed for transfer were available, five embryos from each ageing and young donor were placed in culture dishes and incubated for 6 days. The method of Onuma, Maurer & Foote (1968), using heat-treated rabbit serum as the culture medium, was followed.

RESULTS

Embryo collection from superovulated donors

The results of repeated superovulation of ageing donors and single superovulation of six groups of young donors with the statistical significance of the differences are presented in Table 1. A high proportion of does ovulated. There was considerable individual variability in the number of ovulations with a distinct downward trend with age and superovulation in the ageing donors.

The six groups of young donors also differed ($P<0{\cdot}05$) in the number of ovulation points. The sixth group, which had the lowest mean, contained many Polish–Dutch-belted crossbreds.

Embryo recovery usually exceeded 80%, but it fluctuated among groups due to interference with gamete transport in a few animals, to possible errors in counting ovulation points in live does and to unknown causes. The number of

TABLE 1

OVULATORY RESPONSE AND EMBRYOS COLLECTED FROM AGEING AND YOUNG DONORS

Observation	*Age (weeks) ± S.E.*	*No. of does ovulating/ total does*	*Av. no. of ovulations/ doe ovulating ± S.E.*	*% of oocytes or embryos:*					
				Recovered	*Cleaved*	*Two-cell*	*Four-cell*	*Eight-cell*	*Abnormal*
AGEING DONORS									
1	21 ± 0·3	60/60	61·7 ± 2·6	97·3	87·7	17·4	60·4	9·9	3·8
2	46 ± 0·3	57/58	44·8 ± 2·1	88·3	87·6	19·1	62·9	5·6	2·2
3	72 ± 0·3	51/53	36·6 ± 2·5	80·9	90·5	24·1	64·9	1·5	1·5
4	94 ± 0·6	46/51	24·4 ± 2·0[a]	66·2	96·1	23·7	72·4	0	0·5
5	123 ± 0·4	29/30	20·9 ± 2·3[a,b]	80·5	81·9	20·5	60·0	1·4	2·1
6	148 ± 0·4	19/27	14·9 ± 2·4[b]	86·2	71·7	29·5	42·2	0	1·2
YOUNG DONORS									
1	20 ± 0·3	21/21	54·9 ± 3·9[a,b]	77·0	82·9	26·2	55·7	1·0	4·7
2	21 ± 0·4	28/28	49·8 ± 1·9[a,b,c]	77·9	90·3	20·4	68·7	1·3	1·8
3	25 ± 0·4	27/27	54·3 ± 2·8[a,b]	94·5	87·6	16·7	69·3	1·6	2·5
4	26 ± 1·1	19/19	56·4 ± 4·3[a]	85·4	88·8	24·8	63·8	0·2	2·4
5	26 ± 0·4	23/23	45·4 ± 3·9[b,c]	92·3	82·2	16·0	63·1	3·1	1·5
6	25 ± 2·2*	16/16	40·7 ± 5·0[c]	81·1	72·8	32·3	40·5	0	2·1

[a,b,c] Means with the same superscript are not significantly different ($P<0{\cdot}05$).
* Cross between albino Dutch and Polish females were used.

neonates, representing embryos which were not recovered from the oviducts of the donor, was less than 0·5% of the ovulation points in both groups.

The fertilization rate (Table 1) exceeded 80% in the first five observations in both the young and ageing donor groups. There was a noticeable decrease in both groups at the sixth observation for no apparent reason. Small differences in the stage of embryo development reached in the different donor groups were due to slight variation in the time that elapsed between LH injection and embryo recovery. The percentage of abnormal embryos was low and no trends were observed.

Six old does, superovulated for the first time when 229 weeks old, averaged only 14·2 ovulations. This was comparable to does 148 weeks old superovulated for the sixth time. Young (24-week-old) donors treated simultaneously averaged

62·3 ovulation points. The number of embryos recovered averaged 11·7 and 49·2 for the old and young does. Embryo development for the old does 29 hr after the LH injection was 5·7% one-celled, 32·9% two-celled, 61·4% four-celled and 0% abnormal-appearing ova. Corresponding results for the young does 30 hr after LH injection were 5·8, 38·0, 55·6 and 0·7%, respectively.

Natural mating of ageing donors between each superovulation

The average number of neonates and the sex ratio resulting from mating the ageing donors between each superovulation are presented in Table 2. The

TABLE 2

NUMBER OF YOUNG KINDLED BY THE AGEING DONORS BETWEEN EACH SUPEROVULATION

Age (weeks) ± S.D.	*No. of does kindling/ total does*	*Av. no. of young /doe kindling*	*Sex*	
			No. of males	*No. of females*
32 ± 6	42/60	5·7[a]	116	122
55 ± 11	55/58	5·5[a,b]	137	165
82 ± 12	45/53	4·6[b]	105	104
107 ± 5	27/30	4·7[b]	62	64
134 ± 4	27/27	3·6[c]	53	44
Total or mean	196/228	5·0	473 (48·7)	499 (51·3)

[a,b,c] Means with the same superscript are not significantly different ($P<0·05$). Numbers in parentheses are percentages.

analysis of variance of the number of neonates per doe kindling showed a significant decrease ($P<0·01$) with increased parity. The sex ratio of males to females did not deviate significantly from the expected figure.

Embryo transfer to old, ageing and young recipients

The results of transferring 3351 embryos from ageing and young donors to ageing and young recipients are presented in Table 3 and Text-fig. 1. The young recipients produced 48·1% neonates from all transfers as compared to 37·2% for the ageing recipients ($P<0·005$, $\chi^2 = 41·3$, 1 d.f.). The correlation between age of recipient and percentage of neonates was $-0·18$ ($P<0·01$), indicating a downward trend with age (Text-fig. 1). The time between embryo collection and transfer lengthened as age increased due to the greater difficulty in securing enough eggs from each animal to make the appropriate transfer. However, this did not affect the percentage of neonates, $r = -0·02$ ($P>0·10$). Multiple regression analysis revealed that only 3·4% ($P>0·01$) variation in neonates found was accounted for by age of recipient and time required for transfer.

The number of corpora lutea remained the same, $r = 0·02$ ($P>0·10$), in older recipients and this, together with the age factor, accounted for 9·1% of the variation in the proportion of neonates obtained ($P<0·01$). The correlation between number of corpora lutea of the recipients and the proportion of neonates was 0·15 ($P<0·01$).

Overall, embryos taken from ageing donors developed into 44·6% neonates in their hosts, compared to 40·7% for embryos taken from young donors ($P<0{\cdot}05$, $\chi^2 = 3{\cdot}9$, 1 d.f.). An interaction of recipient by donor age existed ($P<0{\cdot}10$, $\chi^2 = 3{\cdot}7$, 1 d.f.) as more embryos from ageing donors developed into neonates in the ageing recipients.

Two grossly abnormal young were produced in the ageing recipients. One

TABLE 3

DEVELOPMENT OF TRANSFERRED EMBRYOS INTO VIABLE YOUNG

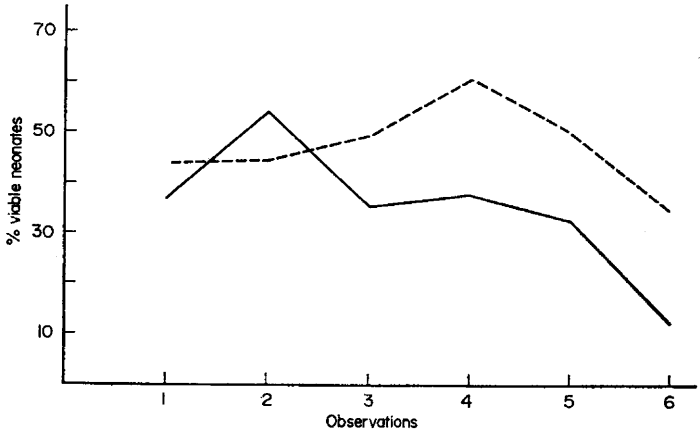
Av. age of donor (weeks)	*Recipients No. of pairs*	*Recipients Av. age (weeks)*	*Av.* time (min)*	*Av. no.† of CL ± S.E.*	*No. of young/ no. of embryos transferred*	*% development*
AGEING RECIPIENTS						
21 A	33	81	7	3·4 ± 0·44	63/165	38·2
20 Y	33	81	17	3·8 ± 0·30	57/162	35·2
46 A	33	101	15	3·3 ± 0·31	94/165	57·0
21 Y	33	101	23	4·1 ± 0·35	78/154	50·6
72 A	33	127	20	3·1 ± 0·33	59/162	36·4
25 Y	33	127	32	3·6 ± 0·44	56/164	34·1
94 A	33	141	29	3·7 ± 0·24	68/150	45·3
26 Y	33	141	26	3·9 ± 0·35	50/163	30·7
123 A	26	175	44	3·1 ± 0·39	40/121	33·1
26 Y	26	175	49	2·5 ± 0·31	41/128	32·0
148 A	16	196	47	3·0 ± 0·42	10/62	16·1
25 Y	16	196	59	2·9 ± 0·30	7/80	8·8
Total A	174	129	25	3·3	334/825	40·5
or mean Y	174	129	31	3·6	289/851	34·0
YOUNG RECIPIENTS						
21 A	33	21	8	3·4 ± 0·26	73/165	44·2
20 Y	33	21	9	3·3 ± 0·24	70/164	42·7
46 A	33	21	15	2·8 ± 0·28	70/165	42·4
21 Y	33	21	23	3·2 ± 0·31	72/154	46·8
72 A	33	24	20	3·1 ± 0·24	75/161	46·6
25 Y	33	24	28	2·8 ± 0·30	85/164	51·8
94 A	33	30	30	3·7 ± 0·28	97/149	65·1
26 Y	33	30	35	3·3 ± 0·31	91/162	56·2
123 A	26	27	44	3·6 ± 0·27	65/121	53·7
26 Y	26	27	52	3·0 ± 0·20	59/127	46·5
148 A	16	22	36	2·4 ± 0·42	22/63	34·9
25 Y	16	22	56	2·9 ± 0·30	27/80	33·8
Total A	174	24	24	3·2	402/824	48·8
or mean Y	174	24	31	3·1	404/851	47·5

* Average time between collection and transfer of the embryos.
† Represents one ovary or uterine horn/recipient.
A = ageing donor and Y = young donor.

came from a young donor while the other was from an ageing donor. The anomalies found were cranioschisis, hydrocephaly, cerebellum exencephalic, anophthalmia, ophthalmacrosis, acorea, ablephoron, apinna, cleft palate, external medial sagittal sinus, spina bifida, abrachia, brachymetacarpalia, brachymetapody, thoracogastroschisis and skin defects.

The results of transferring embryos from old donors superovulated for the first time at 229 weeks of age and young donors are given in Table 4. Embryos

obtained from old donors were less able to survive following transfer ($P<0{\cdot}05$, $\chi^2 = 7{\cdot}50$, 1 d.f.), but the 34·4, 38·7 and 41·9% survival rate of neonates in the young, ageing and old recipients did not differ ($P>0{\cdot}10$).



TEXT-FIG. 1. The proportion of embryos which resulted in viable neonates when transferred to ageing recipients (———) every 26 weeks for 3 years in comparison with survival in six groups of young recipients (– – – –).

TABLE 4

DEVELOPMENT OF EMBRYOS FROM OLD AND YOUNG DONORS TRANSFERRED TO YOUNG, AGEING AND OLD RECIPIENTS

Av. donor age (weeks)	*No. of recipients*	*Av. age of recipients (weeks)*	*No. of young/ no. of embryos transferred*	*% development*
229 O	7	30	5/21	23·8
24 Y	7	30	16/40	40·0
229 O	4	160	3/11	27·3
24 Y	4	160	9/20	45·0
229 O	7	222	6/22	27·3
24 Y	7	222	20/40	50·0
229 O	18	Total	14/54	25·9
24 Y	18	Total	45/100	45·0

O = old donor and Y = young donor.

Development 12 days after embryo transfer

Results are presented in Table 5. Young recipients had more implantations ($P<0{\cdot}01$, $\chi^2 = 7{\cdot}78$, 1 d.f.) and kindled more neonates ($P<0{\cdot}05$, $\chi^2 = 6{\cdot}59$, 1 d.f.) than ageing recipients. Laparotomy reduced the proportion of neonates resulting from transferred embryos in ageing does ($P<0{\cdot}05$). The normal implantations in the ageing recipients were significantly larger in diameter than in the young recipients ($P<0{\cdot}01$).

TABLE 5

EMBRYO DEVELOPMENT IN RECIPIENTS AT LAPAROTOMY 12 DAYS AFTER EMBRYO TRANSFER

Criteria	*Embryos transferred to ageing recipients from:†*			*Embryos transferred to young recipients from:†*		
	Ageing donors	*Young donors*	*Total or mean*	*Ageing donors*	*Young donors*	*Total or mean*
No. of embryos transferred						
Control does	137	137	274	130	135	265
Does at laparotomy	136	140	276	135	140	275
Implant at 12 days						
No. normal	65	73	138	82	84	166**
Diameter (mm)	18·7	17·9	18·3**	15·9	16·5	16·2
No. abnormal	5	8	13	9	8	17
Diameter (mm)	10·7	9·8	10·1	9·9	6·7	8·4
Neonates produced (%)						
Control does	54	42	48	52	56	54
Does at laparotomy	38	42	40	50	51	51*

† There were twenty-eight control and twenty-eight ageing recipients subjected to laparotomy (average age 138 weeks) with opposite oviducts receiving embryos from ageing and young donors. Likewise, there were twenty-eight young recipients subjected to laparotomy (average age 24 weeks) but only twenty-seven control young recipients.

* $P < 0.05$ for comparable values in the same row.

** $P < 0.01$ for comparable values in the same row.

Neonatal sex ratio and birth weights

These data are given in Table 6. The ratios of male to female neonates in the ageing and young recipient groups were 80·5 : 100 and 82·4 : 100, ($P > 0.10$).

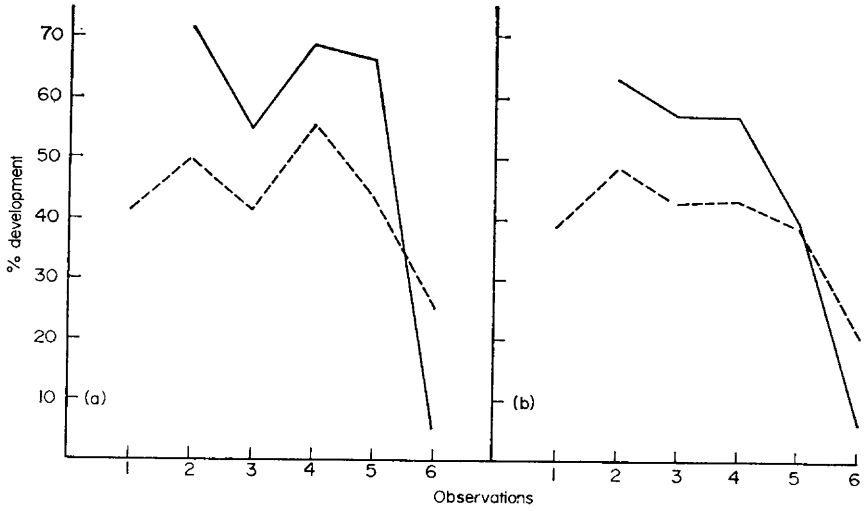
TABLE 6

SEX RATIO AND AVERAGE LITTER WEIGHT FOR VIABLE YOUNG DEVELOPED FROM TRANSFERRED EMBRYOS

Observation	*Embryos from ageing donors*			*Embryos from young donors*		
	No. of males	*No. of females*	*Average birth wt (g)*	*No. of males*	*No. of females*	*Average birth wt (g)*
AGEING RECIPIENTS						
1	26	37	49·7	20	37	48·2
2	41	53	49·4	41	37	48·2
3	28	31	52·4	26	30	48·8
4	31	37	47·9	26	24	49·2
5	16	24	46·8	11	30	46·2
6	7	3	54·7	5	2	46·4
Total or mean	149	185	49·5	129	160	48·2
YOUNG RECIPIENTS						
1	28	45	47·4	30	40	43·8
2	33	37	48·0	32	40	43·7
3	35	40	42·3	41	44	40·3
4	44	53	41·0	48	43	40·4
5	26	39	44·8	25	34	43·8
6	11	11	33·3	11	16	31·8
Total or mean	177	225	43·8	187	217	41·6

The overall sex ratio of 81·6 : 100 was lower ($P<0·05$, $\chi^2 = 4·86$, 1 d.f.) than the ratio of 104·1 : 100 found for this colony (Maurer, 1966). The sex ratios of 79·5 : 100 and 83·8 : 100 resulting from embryos obtained from the ageing donors did not differ ($P>0·10$) from each other. However, the ratio of 79·5 : 100 resulting from embryos obtained from the ageing donors was different from the sex ratio of 95 : 100 born to this group following natural mating ($P<0·10$, $\chi^2 = 3·1$, 1 d.f.).

The birth weights of neonates developing in ageing recipients were heavier than their counterparts which developed in young recipients. The ageing recipients generally had smaller litters which can account for the differences (Venge, 1950). Also, birth weights were lower in the crossbred young recipients used for the sixth observation.



TEXT-FIG. 2. The proportion of embryos recovered every 26 weeks from (a) ageing and (b) young donors which developed into viable neonates (– – – –) in comparison with those which developed *in vitro* into blastocysts (———). In the first observation, the cultures were halted before the embryos could reach the blastocyst stage.

Culture in vitro *of embryos from ageing and young donors*

The development *in vitro* of 726 embryos from ageing donors was 55·4% blastocysts, 24·4% morulae and 21·2% pre-morula stages. Corresponding results for 554 embryos from young donors were 32·5, 40·2 and 27·2%. A higher proportion of the embryos from the ageing than from the young donors developed into blastocysts ($P<0·005$, $\chi^2 = 8·006$, 1 d.f.), but embryos from young donors were held longer at room temperature before being placed in culture because more of them were usually available for transfer to recipients before residual embryos were cultured. Development of the embryos into blastocysts *in vitro* paralleled the development *in vivo* into neonates. This relationship for embryos from ageing and young donors is illustrated in Text-fig. 2.

DISCUSSION

Superovulation of donors

The significant decrease ($P<0{\cdot}005$) in the number of ovulation points with repeated superovulation of the ageing donors (Table 1) agrees with findings of previous workers (Sakuma, Ishijima & Ishida, 1964; Maurer, Hunt & Foote, 1968) who attributed this decline in the rabbit to antihormone production. Repeated surgery also may have had some effect. This decline in the numbers of ovulations with age is expressed by a regression equation $\log \hat{Y} = 1{\cdot}8842 - 0{\cdot}0048X$, where $\hat{Y}$ = the number of ovulations and X = the age in weeks. A good fit is shown by the correlation, r = 0·99, between observed and predicted values. Jones & Krohn (1961) showed that there was a continuous gradual decline in numbers of oocytes in mice with advancing age, which could be described by a similar regression equation. Recently Krarup, Pedersen & Faber (1969) concluded that the number of small mouse oocytes stimulated to grow at any time is a logarithmic function of the actual size of the pool of small oocytes and does not depend on other parameters. If the rabbit ovary functions similarly, the decrease in ovulations with successive superovulations is probably due, in part, to a decrease in the population of small oocytes. Complete failure of the does to ovulate, which can be partly overcome by administering other gonadotrophins (Maurer, Hunt & Foote, 1968), presumably involves the production of antihormone.

The lower number of ovulations in the last group of young donors may have been due to the crossbred strain used. Strain differences in oocyte populations have been reported for mice by Jones & Krohn (1961), and the ovulatory response to gonadotrophins may vary.

The fertilization rate and early development of embryos from the ageing and old donors were similar to the young donors in all respects. The generally good cleavage is similar to the findings of Blaha (1964) and Adams (1964), and contrary to the work by Boot & Mühlbock (1954). Additional problems might have arisen had the present study been continued longer, but cleavage rate was 94% in the does first superovulated at 229 weeks of age. During the sixth observation, the fertilization rate and subsequent development was reduced in both the ageing and young donor groups. Since there were no changes in the animal care or experimental procedures and both groups were affected, the reason for the change is unknown.

Embryo transfer to old, ageing and young recipients

The significant decrease ($P<0{\cdot}005$) in the number of transferred embryos surviving until birth in the ageing recipients (Table 4) is in agreement with the work of Boot & Mühlbock (1954), Adams (1964), Blaha (1964) and Talbert & Krohn (1966). The higher percentage ($P<0{\cdot}05$) of embryos from ageing donors developing into young was unexpected. The ageing donors were 3 years old at the conclusion of the experiment which is relatively young for rabbits. Also, they tended to be higher than other young donors from the start and may have been a more fertile group. However, when embryos were taken from donors 4½ years old and transferred to recipients of various ages, only 26% developed into viable neonates regardless of the uterine environment. The results of these experiments

suggest that first the uterine environment becomes less conducive to prenatal development as the rabbit ages and then the oocytes show the effects of the ageing process either directly or as a result of exposure of the oocyte of young embryos to the ageing oviduct before transfer.

The two severely deformed young kindled by the ageing recipients also suggest a decreasing suitability of the maternal environment. Similar deformities have been produced experimentally by reducing progesterone (Poulson, Robson & Sullivan, 1965) or blood flow to the uterus (Brent & Franklin, 1960).

Development 12 days after embryo transfer

The higher proportion of implantations and neonates produced in the young recipients, as compared to ageing recipients, following embryo transfer (Table 5) is consistent with other portions of this study. The difference between age groups of recipients was due to pre- or early implantation mortality, which was 45·3% in ageing recipients and 33·5% in young recipients ($P<0·01$). Senger, Lose & Ulberg (1967) showed that a reduced blood supply to the uterus in mice increased early embryonic death. Blood flow could have been lower in the ageing donors, but it was not measured. Adams (1964) found similar losses in young animals, but pre- and postimplantation losses of 56·7 and 41·8% in older does were higher than those found in the present study. Postimplantation losses in the present study were similar (14·8 and 16·0%) in ageing and young recipients. The age of the donor of the embryos had no effect on embryo or foetal mortality.

The implants were larger in diameter in the ageing than in the young recipients ($P<0·01$). This did not appear to be related to the fact that there were fewer implants because, in the six ageing recipients with eight to ten normal implants, the average diameter was also 18·3 mm. Degenerating embryos in ageing recipients were more often found in the middle portion of the uterine horns, while they were distributed randomly in young recipients.

The laparotomies caused no increased loss in the young recipients, but did decrease the number of viable neonates ($P<0·05$) in ageing recipients. This indicates embryos developing in an older maternal environment or the older does are more susceptible to stress.

Sex ratio of neonates from transferred embryos

The overall sex ratio in neonates developing from transferred embryos was lower ($P<0·05$) than expected for this colony of rabbits, and among transferred embryos from ageing donors was lower ($P<0·10$) than the sex ratio following natural mating of these ageing does. A slight preponderance of females was obtained from transferred embryos in a previous study (Maurer, Hunt, Van Vleek & Foote, 1968). If the deviation of the sex ratio is real, two possible explanations are (1) that female embryos tolerate the stress of transfer better than male embryos and (2) that the maternal environment in the donor doe is changed with superovulation so that it favours the X-bearing spermatozoa.

ACKNOWLEDGMENTS

This investigation was supported by Grant HD 03471 from the National

Institute of Child Health and Human Development, USPHS. The authors are grateful to Dr Hideo Onuma, Dr J. Hahn, Dr M. Kane, Bruce Hakes and Michael Simkin, for their assistance in collecting the data.

REFERENCES

ADAMS, C. E. (1964) The influence of advanced maternal age on embryo survival in the rabbit. *Proc. Vth Int. Congr. Anim. Reprod. A.I. Trento*, **2**, 305.

BIGGERS, J. D. (1969) Problems concerning the uterine causes of embryonic death, with special reference to the effects of ageing of the uterus. *J. Reprod. Fert.* Suppl. **8**, 27.

BLAHA, G. C. (1964) Effect of age of the donor and recipient on the development of transferred golden hamster ova. *Anat. Rec.* **150**, 413.

BOOT, L. M. & MÜHLBOCK, O. (1954) The ovarian function in old mice. *Acta physiol. pharmac. néerl.* **3**, 463.

BRENT, R. L. & FRANKLIN, J. B. (1960) Uterine vascular clampings: new procedure for the study of congenital malformations. *Science, N.Y.* **132**, 89.

CORNER, G. W. (1923) The problem of embryonic pathology in mammals with observations upon intrauterine mortality in the pig. *Am. J. Anat.* **31**, 523.

JONES, E. C. & KROHN, P. L. (1961) The relationship between age, numbers of oocytes and fertility in virgin and multiparous mice. *J. Endocr.* **21**, 469.

KRARUP, T., PEDERSEN, T. & FABER, M. (1969) Regulation of oocyte growth in the mouse ovary. *Nature, Lond.* **224**, 187.

KROHN, P. L. (1962) Review lectures on senescence. II. Heterochronic transplantation in the study of ageing. *Proc. R. Soc.* B, **157**, 128.

KROHN, P. L. (1964) The reproductive lifespan. *Proc. Vth Int. Congr. Anim. Reprod. A.I., Trento*, **3**, 23.

MAURER, R. R. (1966) *Repeated superovulation and ovum transfer, and the survival of transferred ova and newborn in the rabbit.* M.S. thesis, Cornell University, Ithaca, N.Y.

MAURER, R. R., HUNT, W. L. & FOOTE, R. H. (1968) Repeated superovulation following administration of exogenous gonadotrophins in Dutch-belted rabbits. *J. Reprod. Fert.* **15**, 93.

MAURER, R. R., HUNT, W. L., VAN VLECK, L. D. & FOOTE, R. H. (1968) Development potential of superovulated rabbit ova. *J. Reprod. Fert.* **15**, 171.

ONUMA, H., MAURER, R. R. & FOOTE, R. H. (1968) *In-vitro* culture of rabbit ova from early cleavage stages to the blastocyst stage. *J. Reprod. Fert.* **16**, 491.

POULSON, E., ROBSON, J. M. & SULLIVAN, F. M. (1965) Embryopathic effects of progesterone deficiency. *J. Endocr.* **31**, xxviii.

SAKUMA, Y., ISHIJIMA, Y. & ISHIDA, K. (1964) Repeated superovulation test by FSH on the same rabbits with special reference to antihormone production. *Jap. J. Fert. Steril.* **13**, 1.

SENGER, P. L., LOSE, E. D. & ULBERG, L. C. (1967) Reduced blood supply to the uterus as a cause for early embryonic death in the mouse. *J. exp. Zool.* **165**, 337.

TALBERT, G. B. (1968) Effect of maternal age on reproductive capacity. *Am. J. Obstet. Gynec.* **102**, 451.

TALBERT, G. B. & KROHN, P. L. (1966) Effect of maternal age on viability of ova and uterine support of pregnancy in mice. *J. Reprod. Fert.* **11**, 399.

VENGE, O. (1950) Studies of the maternal influence on the birth weight in rabbits. *Acta zool., Stockh.* **31**, 1.