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Comparative study of preimplantation development and embryonic loss in two rabbit strains

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Summary. The prolificacy of two rabbit strains (Californian and New Zealand) has been studied in parallel with ovulation rate and embryonic development. The number of mean ovulations was 11.6 in Californians and only 9.6 in New Zealands. In spite of the higher rate in the former, the number of young born was about 8 in both strains.

Californian and New Zealand blastocysts were transferred at 96 h post-coïtum by injecting them into the uterus of recipient does of the same strain or of the other strain (crossed transfer).

The percentage of unfertilized and delayed eggs was significantly higher (20 % *vs* 12 %) in Californians. The size of blastocysts recovered at 96 h p.c. was significantly higher in New Zealand than in Californian does (370 *vs* 319 μm). The difference was due to the larger proportion of small Californian blastocysts and of large New Zealand blastocysts.

The larger blastocysts were placed in the right horn of recipients and the small ones in the left horn. Number of foetuses at D27 showed that the survival percentage was always higher when large blastocysts were transferred. However the survival difference with small transferred blastocysts was not significant.

When transfer was within the same strain or crossed the results were not different. Since the New Zealand blastocysts were larger at D4 p.c. (one day after they arrived in the uterus), it could not be determined if this improved development was due to the conditions in the fallopian tubes or to the quality of the uterine secretion. The rate of embryonic loss during gestation indicated that the uterus of New Zealand does supported a gestation better than the uterus of Californians.

However other experiments are necessary to determine the real cause of the loss of normal embryos at D4 when compared to the number of ovulations.

Introduction.

Species prolificacy is determined by the number and quality of ovulated oocytes, the fertilization rate and the percentage of embryonic loss up to D27 of pregnancy. The relative importance of these parameters is more easily distinguished in species like the rabbit which has multiple ovulations and several embryos in the same uterine medium. A comparison of the performances of two strains of the same species may provide us with information on the factors influencing prolificacy.

Two rabbit strains (New Zealand A1077 and Californian A1066), selected since 1976 on litter size at weaning, differ from each other by the number of ovulations and the rate of embryonic survival estimated 4, 7 and 12 days after mating (Hulot and Matheron, 1981 ; Meunier *et al.,* 1983). California does ovulate two eggs more than New Zealand ones. However, the mean number of young born per litter is similar (Californian : 8.04 ; New Zealand : 8.09) (Hulot et Matheron, 1979).

The aim of the present study was to determine the causes of this difference by carrying out intrastrain and crossed transfer of blastocysts.

After transfer, the recipient does were killed either 17 days after mating to determine to rate and term of embryonic degeneration in the first half of pregnancy (first experiment), or 27 days after mating (near term) to estimate the ability of the uterus of each strain to ensure full development of the transferred blastocysts (second experiment).

Material and methods.

Experiment 1. — For crossed transfer 18 nulliparous Californian and 19 New Zealand does were used. One-half of these females in each strain (donors) were mated to males of the same strain ; the other half (recipients) were mated with vasectomized males so that the donor-recipient pairs were synchronized.

The donor rabbits were killed 96 h post-coitum (p.c.) ; each uterine horn was perfused with 10 ml of phosphate buffer (PBS) + 10 % of foetal calf serum (FCS) in an incubator at 38 °C. The diameter of the blastocysts was measured without the zona pellucida and the mucin coat ; the blastocysts were maintained under an atmosphere of air + 5 % CO_2 until transfer.

The recipient does were anesthetized with intramuscular injection of hypnorm (fentanyl + fluanisone UVA). After laparatomy, a Pasteur pipette containing the blastocysts was introduced at the top of each uterine horn by a small incision. Four blastocysts were transferred into each horn, the largest ones in the right horn and the smallest in the left horn. Unfertilized eggs were eliminated (= 100 µm). The recipient does were killed 17 days after mating.

The number and aspect of the corpora lutea, traces of implantation and the number of live embryos were noted. Degeneration times were estimated according to the aspect of the uterus :

— degeneration at D8 was detected by traces of decidual reaction ;
— when degeneration occurred at D10-12 the maternal placenta was already visible but no foetal placenta and foetuses were present ;
— when degeneration occurred at D14-15, the maternal and foetal placentae were still present but the foetus had disappeared.

Experiment 2. — Four types of transfer were carried out at 96 h p.c. :

— 16 New Zealand donors and 15 Californian recipients ;
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— 17 Californian donors and 16 Californian recipients.

The recipients were killed at day 27 of gestation and the ovaries, embryos and uterus were examined as before.

Results.

Ovulation and embryonic loss between D0 and D4.

In both experiments the total number of corpora lutea in Californian donors killed at 4 days p.c. and in Californian recipients killed at 17 and 27 days p.c. was 942 or 11.6 + 0.2 per doe. In New Zealand does killed at the same stages, the number was 793 or 9.7 $\pm$ 0.2 per doe. Thus ovulation rate was 2 units higher in the Californians ($P < 0.001$), as shown previously (Hulot et Matheron, 1981).

The percentages of unrecovered eggs, relative to the number of corpora lutea, were not significantly different in the two strains (table 1a). The percentage of unfertilized eggs (= 100 µm) relative to eggs recovered (table 1b), as well as the number of those with delayed cleavage (125 µm < $\bar{M}$ < 200 µm) (table 1c) were significantly higher in Californian does ($P < 0.05$). The ratio of the total number of unfertilized oocytes and delayed embryos to the number of recovered « eggs » (table 1d) was also significantly higher ($P < 0.005$) in Californians.

TABLE 1

Percentage of lost or unfertilized embryos, or those with delayed cleavage at 96 h p.c. in Californian and New Zealand donors.

	Californian		New Zealand
No. of does	41		41
a) Unrecovered eggs	13.2 %	NS	10.5 %
b) Eggs = 100 µm	5.2 %	*	2.2 %
c) Eggs 125 µm < M < 200 µm	14.7 %	*	9.7 %
d) Unfertilized + delayed « eggs »	20.0 %	***	12.0 %

a : relative to the number of corpora lutea.
b,c,d : relative to the eggs recovered.
* : $P < 0.05$.
*** : $P < 0.005$.

Blastocyst size

Experiment 1. The size of 94 Californian and 84 New Zealand blastocysts was calculated (table 2a) ; there was no significant difference in mean blastocyst size. However with a higher number of blastocysts (Experiment 1 + Experiment 2), the New Zealand ones (n = 359) were clearly larger than the Californian ones (n = 401) (table 2b).

Depending on the number of available recipients, 322 Californian and 302 New Zealand blastocysts were transferred ; the size distribution of these two populations is shown in figure 1.

TABLE 2

Mean number and size of recovered blastocysts.

	Californian		New Zealand
a)			
No. of does	9		9
No. of blastocysts	94		84
Mean size	329 µm	NS	341 µm
S.E.M.	9.92		13.33
b)			
No. of does	41		41
No. of blastocysts	401		359
Mean size	319 µm	***	370 µm
S.E.M.	5.84		8.96

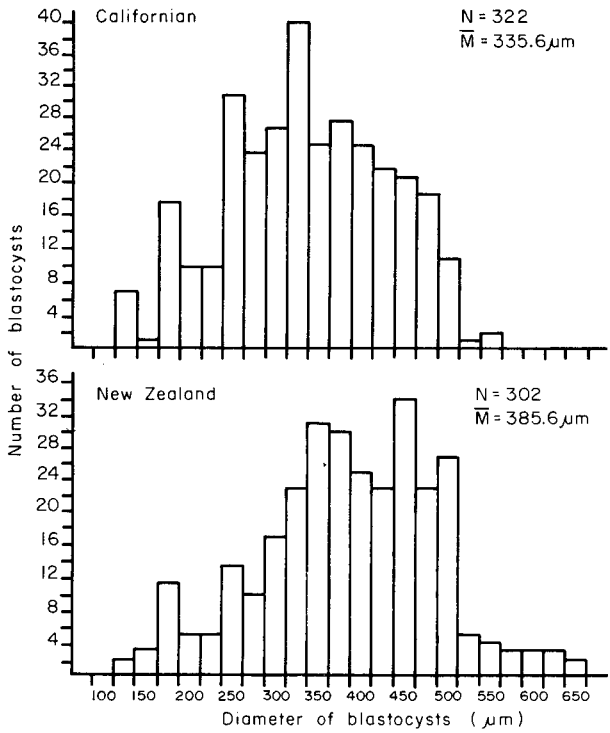

FIG. 1. — *Size distribution of transferred Californian and New Zealand blastocysts.*

Three size classes distinguished were : 125 µm to 275 µm, 300 to 475 µm and 500 µm to 675 µm (table 3). The proportion of small blastocysts was significantly higher in Californian does, while the percentage of large blastocysts was higher in New Zealand does.

TABLE 3

Size distribution of blastocysts.

Size	Californian		New Zealand
125 → 275 µm	31.4 %	***	16.2 %
300 → 475 µm	64.3 %	NS	68.2 %
500 → 675 µm	4.4 %	***	15.6 %

Experiment 2. In each type of transfer, the « large » blastocysts were selected in such a way that their size was significantly greater than that of the « small » ones (table 4).

TABLE 4

Mean size of transferred blastocysts.

		Donor					
		New Zealand			Californian		
Recipient		large		small	large		small
N.Z.	No.	64		57	58		57
	Mean dia.	441	***	345	392	***	275
	S.E.M.	9.73		12.17	8.41		9.51
Calif.	No.	60		55	64		63
	Mean dia.	451	***	315	402	***	287
	S.E.M.	8.49		11.22	7.22		8.97

Determination of blastocyst size-survival ratio.

The minimal size, below which eggs would be considered as non-viable, is given by pregnancy results. When, in a group of four transferred blastocysts one was 175 µm in diameter, 50 % of the recipient does gave four normal foetuses. Since one egg diameter was 125 µm in the transferred group, only 14 % of the recipient does gave four normal foetuses.

Aside from eggs with a diameter of less than 175 µm, a mean of 8.9 good embryos was recovered from Californians ; a mean of 8.2 was taken from the New Zealand.

Development after transfer.

Experiment 1. Out of 72 New Zealand blastocysts transferred into 9 Californian does, 54 normal embryos (75 %) were found at 17 days, while the 80 Californian blastocysts transferred into 10 New Zealand does gave 72 embryos (90 %). The difference between the two percentages was significant ($P < 0.05$).

The distribution of embryonic mortality (table 5) at D8 showed a difference between the two types of transfer. Seven New Zealand embryos had degenerated after implantation into the uterus of Californian does as against 0 when Californian embryos were transferred into the uterus of New Zealand females.

TABLE 5

Mortality distribution.

Transfer	No. implantations	Degeneration D8	D12	D15	Total
Californian ↓ New'Zealand	4	0	3	1	8
New Zealand ↓ Californian	4	7	5	2	18

Experiment 2. This experiment used a larger number of recipients, donors and blastocysts. Eight groups of transferred blastocysts were distinguished according to size (table 6). Although there was no statistically significant difference between the survival percentages of large and small blastocysts of one strain transferred into a recipient of the other strain, the survival rate of smaller blastocysts was always less than that of the larger ones. This situation was particularly evident when the donors and recipients were Californians (81 % *vs* 67 %). Leaving aside blastocyst origin and recipient strain, 216 large blastocysts out of 246 (88 %) gave viable foetuses, while the small blastocysts gave only 168 foetuses out of the 232 blastocysts transferred (72 %).

TABLE 6

Embryonic survival at D27 of pregnancy.

	Donors					
	New Zealand			Californian		
Recipients	large		small	large		small
New Zealand	57/64 (89 %)	NS	14/57 (72 %)	51/58 (88 %)	NS	42/57 (74 %)
Californian	51/60 (85 %)	NS	43/55 (78 %)	52/64 (81 %)	NS	42/63 (67 %)

Number of live foetuses/number of blastocysts transferred.

The mean number of foetuses at D27 in relation to the number of embryos transferred is shown in table 7. A certain homogeneity in litter size is evident, except for the Californian/Californian transfer where loss reached 26 %.

TABLE 7

Number of live embryos per doe rabbit at D27.

	New Zealand recipients		Californian recipients	
	No. of blastocysts transferred/doe	No. born alive	No. of blastocysts transferred/doe	No. born alive
NZ	7.6	6.1	7.7	6.3
Loss	19 %		18 %	
Calif.	7.7	6.2	7.9	5.9
Loss	19 %		26 %	

Discussion.

Ovulation rate is 2 units higher in Californian rabbits than in New Zealand ones. However the number of young born is similar. This situation may be due to differences in fertilization rates and/or in embryonic mortality. In order to determine the importance of uterine environment on embryonic loss, we carried out crossed and intrastrain blastocyst transfer at 96 h p.c.

Ratios between the number of living foetuses and the number of blastocysts transferred do not show significant differences between strains, however there is a tendency for embryonic loss to increase in Californian recipients (tables 5-6), particularly when Californian blastocysts were transferred into Californian foster mothers.

After elimination of abnormal eggs (unfertilized, delayed embryos), the average number of transferable blastocysts was 8.9 from Californian donors and 8.2 from New Zealand ones.

The uterus of Californian does offered less chance for good embryonic development than the uterus of New Zealand rabbits. Moreover, the small blastocysts had a lower potential for development than the larger ones (table 6). Thus smaller blastocysts put in a less favourable medium might degenerate, as in the Californian/Californian transfer.

However, the main difference was already visible at D4 since the number of normal blastocycts was lower in Californian donors and average blastocyst size lower. This was shown by Adams (1960) ; when blastocysts selected on size were transferred at 96 h p.c., embryonic loss was reduced by at least one-third (20 *vs* 30 %).

In mouse strains, Bradford (1979) has shown one selection of large litter size, via an increase in ovulation rate. Experiments of reciprocal embryo transfer support the conclusion that maternal genotype has a greater influence than the genotype of the embryo on prenatal survival. These conclusions fit with the hig-

her ovulation rate in Californian does and the characteristics of the New Zealand uterus, but do not explain the higher percentage of unfertilized and degenerated eggs in Californian does.

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Résumé. *Etude comparée du développement avant implantation et de la mortalité embryonnaire chez deux génotypes de lapines.*

La prolificité de deux souches de lapines californiennes et néo-zélandaises a été étudiée en mettant en parallèle le taux d'ovulations et le développement embryonnaire précoce. Le nombre moyen d'ovulations qui est de 11,6 chez les californiennes n'est que de 9,6 chez les néo-zélandaises. Malgré le taux d'ovulations supérieur chez les californiennes, le nombre de jeunes nés est environ de 8 dans les deux souches.

Pour savoir si la différence de prolificité vient de la qualité des œufs pondus ou des conditions plus ou moins favorables à la survie dans le tractus génital de chaque souche, nous avons fait des transferts croisés de blastocystes californiens dans des néo-zélandaises et inversement. Dans deux séries témoins, les blastocystes ont été transférés dans des receveuses de même souche. Les transferts ont été réalisés 96 h p.c., les lapines donneuses et receveuses étant synchronisées.

Le nombre d'œufs non fécondés et en retard de développement est significativement supérieur (20 *vs* 12 %) chez les californiennes. La taille des blastocystes récupérés à 96 h est significativement supérieure chez les néo-zélandaises par rapport aux californiennes (370 *vs* 319 µm). La différence réside dans la proportion plus importante de petits blastocystes californiens et de gros blastocystes néo-zélandais.

Au cours des transferts, les plus gros blastocystes sont déposés dans la corne droite des receveuses et les plus petits dans la corne gauche. Les résultats des gestations à 27 jours montrent que les pourcentages de survie sont meilleurs, bien que non significativement différents, quand on transfère des gros blastocystes.

L'examen des mortalités embryonnaires au cours de la gestation laisse penser que l'utérus de néo-zélandaises est plus apte à la poursuite de la gestation que l'utérus de californiennes.

Cependant, d'autres expériences sont nécessaires pour connaître les causes de l'augmentation du nombre d'œufs non fécondés et en retard de segmentation chez les californiennes.

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