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Effect of inbreeding on ovulation rate and foetal mortality in mice

By D. S. FALCONER and R. C. ROBERTS

Agricultural Research Council Unit of Animal Genetics, Institute of Animal Genetics, Edinburgh, 9

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INTRODUCTION

The reduction of the litter size of mice resulting from inbreeding was described in two earlier papers (Roberts, 1960; Bowman & Falconer, 1960). The number of live young born in first litters was taken as a measure of litter size, and this was found to decline in a very regular manner as inbreeding increased: at an inbreeding coefficient of 50% the reduction was about $2\frac{1}{2}$ young per litter. Crosses between lines inbred to 50% showed that the reduction was attributable in part to inbreeding in the litters and in part to inbreeding in the mother bearing the litter. The present paper is concerned with the developmental stage at which the reduction of litter size takes place. The reduction could arise from a reduced ovulation rate, an increased loss of eggs or embryos before implantation, or an increased mortality of embryos at any stage between implantation and birth. Dissections of pregnant females were made in order to identify the stage at which the losses from inbreeding take place. The females were dissected 16 days after insemination, and counts were made of (1) the number of corpora lutea, as a measure of the number of eggs ovulated, (2) the total number of implantation sites, and (3) the number of embryos alive at 16 days of gestation. The difference between (1) and (2) indicated pre-implantation losses and the difference between (2) and (3) indicated post-implantation mortality. Further, a comparison of the number of embryos alive at 16 days in the dissected females with the number of live young born to comparable females, allowed to bring their litters to term, provided a measure of perinatal loss, i.e. losses occurring in the last 2 or 3 days of gestation and between the birth and the recording of the litter. Our interest was primarily in the maternal contribution to the inbreeding depression of litter size, i.e. in the stage at which inbreeding of the mother reduces the size of the litter she bears. For this reason most of the comparisons made were between inbred and non-inbred parents, both with non-inbred embryos.

STOCKS AND TECHNIQUES

Three series of dissections were made, all on females of the same basic stock. The dissections and counts of the first series were made by Roberts, those of the second and third series by Falconer. The details of the three series were as follows:

Series I. The mice came from the inbred lines described by Roberts (1960).

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Twenty-one independently inbred lines were represented among the females dissected. Five groups of females were arranged according to whether the mother, the father, or the embryos were inbred or non-inbred. The inbreeding coefficients of the mice used in the five groups are given with the results in Table 1. The inbreeding coefficients were either 50% or 59%, or zero.

Series II. The mice came from an experiment on selection for sex-ratio (Falconer, 1954). Nine independently inbred lines were represented among the females dissected. The mice for dissection were 50% inbred and they were mated to males similarly inbred but of a different line. The embryos were thus non-inbred. Comparisons were made with a contemporary group of non-inbred females, produced from line-crosses and mated to similar males from a different line-cross. Thus the only difference between the two groups compared in the series-II dissections was in the inbreeding of the parents.

Series III. The inbred mice were derived from six of the independently inbred lines described by Bowman & Falconer (1960). The females were mated to males from a different line, in the same way as in Series II, so that only the parents and not the embryos were inbred. The coefficient of inbreeding of the dissected females was 63%. The non-inbred mice for comparison came from three sources: a line selected for large litters, a line selected for small litters, and a control line maintained without selection. All three lines had been maintained with minimal inbreeding, and were in the fourth generation of the selection experiment.

All the dissections were made 16 days after insemination, indicated by the presence of a vaginal plug. The uteri were opened and the numbers of implantation sites and of live embryos were noted. Few embryos were found that had died at an identifiable stage of development. For this reason the different stages of post-implantation death were not distinguished in the analysis of the results. The numbers of corpora lutea were counted by examination of the ovaries under a low-power binocular microscope. Exact correspondence between the number of corpora lutea counted and the number of eggs shed was not to be expected, because it was difficult, particularly when the corpora lutea were numerous, to distinguish between one large corpus luteum and two adjacent and partially confluent ones. On the whole the number counted is probably an underestimate rather than an overestimate of the ovulation rate. The corpora lutea counted in some mice of the first series of dissections were compared with egg counts made on a comparable group of females by Dr A. W. H. Braden. The mean number of corpora lutea among 38 females was $10{\cdot}6 \pm 0{\cdot}27$ and the mean number of eggs among 32 females was $10{\cdot}1 \pm 0{\cdot}29$. The close correspondence between the two suggests that, in this series at least, the corpora lutea counts gave a good estimate of the ovulation rate. Among the non-inbred mice of series II and III, 9 out of 74 pregnancies (12·2%) showed an excess of implants over corpora lutea (see Table 3). This, however, is an underestimate of the percentage of errors because about 60% of pregnancies showed some loss of eggs before implantation, and an error of counting would only be revealed when there was no loss. As a very rough estimate we may say that probably about 30% of the counts were too low.

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Since, however, we are to compare groups of mice counted in the same way, the bias introduced should not seriously affect the conclusions to be drawn.

There were a few mice with corpora lutea but no implantations. These were excluded, on the grounds that a total loss of eggs would be followed by another ovulation before the dissections were made, so the corpora lutea counted would not represent the eggs that had been lost.

RESULTS

The mean numbers of corpora lutea, implants, and live embryos are given in Table 1. Inspection of these figures shows clearly that inbreeding does not reduce the number of corpora lutea but it does reduce the numbers of implants and of live embryos. Let us, however, examine the results more closely. The five groups of females dissected in Series I are shown separately at the top of Table I. None

Table 1. *Mean numbers of corpora lutea, implants, and live embryos at* 16 *days' gestation* ($\pm$ *standard errors*). *N is the number of females dissected*

Group	Inbreeding coefficients (%) ♀♀	♂♂	Embryos	N	Corpora lutea	Implants	Live embryos
Series I							
A	50	50	59	27	10·0 $\pm$ 0·34	8·4 $\pm$ 0·48	7·1 $\pm$ 0·48
B	50	50	0	29	9·9 $\pm$ 0·32	7·6 $\pm$ 0·54	6·7 $\pm$ 0·52
C	59	0	0	30	10·2 $\pm$ 0·51	8·8 $\pm$ 0·50	7·8 $\pm$ 0·53
D	0	59	0	28	9·9 $\pm$ 0·26	8·9 $\pm$ 0·37	8·1 $\pm$ 0·52
E	0	0	0	30	10·3 $\pm$ 0·33	9·1 $\pm$ 0·40	7·8 $\pm$ 0·54
A+B+C	50–59	—	—	86	10·0 $\pm$ 0·23	8·3 $\pm$ 0·29	7·2 $\pm$ 0·30
D+E	0	—	0	58	10·1 $\pm$ 0·21	9·0 $\pm$ 0·27	7·9 $\pm$ 0·37
				t =	*0·22*	*1·79*	*1·57*
				P =	*0·9*	*0·1*	*0·2*
Series II	50	50	0	13	10·9 $\pm$ 0·58	8·5 $\pm$ 0·69	7·3 $\pm$ 0·74
	0	0	0	15	11·7 $\pm$ 0·46	11·3 $\pm$ 0·57	9·8 $\pm$ 0·66
				t =	*1·09*	*3·15*	*2·54*
				P =	*0·3*	*0·01*	*0·05*
Series III	63	63	0	17	12·5 $\pm$ 0·82	7·9 $\pm$ 0·82	6·4 $\pm$ 0·80
(H)*	0	0	0	23	10·0 $\pm$ 0·41	9·4 $\pm$ 0·28	8·2 $\pm$ 0·41
(L)*	0	0	0	18	11·9 $\pm$ 0·80	9·9 $\pm$ 0·71	8·9 $\pm$ 0·69
(C)*	0	0	0	18	9·1 $\pm$ 0·29	8·0 $\pm$ 0·42	7·4 $\pm$ 0·47

* H, L and C are the lines selected for high and low litter size and unselected control, respectively.

of the differences between the groups, considered separately, are clear enough to allow us to draw firm conclusions about the different effects of inbreeding in the mother, the father, or the embryos. In particular, the only comparison that contains information about the effect of inbreeding in the embryos (groups A

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and B) shows no reduction of the numbers of implants or embryos. The apparent absence here of any effect of inbreeding in the embryos cannot, however, be given much weight because the numbers are not very large and because an increase of the numbers born from crosses between highly inbred lines has often been observed (see, for example, Eaton, 1953). Our chief interest here is in the effect of inbreeding in the mother of the litter, and for this purpose we may combine the groups according to whether the mother was inbred or non-inbred. This gives the same type of comparison as is made in the dissections of Series II and III. The combined groups are given also in Table 1. The numbers of corpora lutea are now almost identical, showing that the ovulation rate is not affected by inbreeding. The numbers of implants and of live embryos are both lower in the inbred females than in the non-inbred, though neither of these differences reach a fully convincing level of significance.

The results of the Series II dissections are quite clear. There is a small but non-significant difference in corpora lutea. Both the implants and the live embryos are fewer in the inbred than in the non-inbred females, and both differences are significant at the 5% level. In the Series III dissections the inbred females have more corpora lutea, but again fewer implants and live embryos. The three non-inbred groups, however, differ significantly between themselves and therefore cannot be combined. These differences were associated with differences of body weight.

The significance tests of the differences between group-means given in Table 1 and also in Table 2 followed the method given by Snedecor (1956, pp. 97–98) which does not assume equality of variance. In fact, the variances of the inbred and non-inbred groups differed significantly in almost every comparison, the inbreds being the more variable. It is not possible, however, to draw genetical conclusions from this fact because the expected changes of the genetic variance at intermediate levels of inbreeding cannot be predicted unless all the variance is additive (Robertson, 1952). For this reason the variances will not be further discussed.

From the three series of dissections we may conclude at this stage that inbreeding does not reduce the ovulation rate as measured by the number of corpora lutea. The reduction of litter size at birth must therefore arise from losses of eggs or embryos either before or after implantation.

Losses

Consideration of the losses of eggs or embryos makes the picture clearer. The pre- and post-implantation losses in the three series of dissections are given in Table 2. The dissections of Series I are here grouped according to the inbreeding of the female parent. The three groups of non-inbred females in Series III are here combined because the differences between them, though still just significant, are much less. All three series agree in showing a greater pre-implantation loss in inbreds than in non-inbreds, but no difference in the post-implantation losses. The significance of the difference of pre-implantation loss does not quite reach the 5% level in Series I, but in Series II and III it reaches the 1% level. There can be

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Table 2. *Mean numbers of pre- and post-implantation losses (± standard errors). The percentage losses show respectively the percentage of corpora lutea not represented by implantation sites and the percentage of implants not represented by live embryos at 16 days*

Series	Group	N	Loss: Pre-implantation		Loss: Post-implantation	
I	A+B+C (♀♀ inbred)	86	1·77 ± 0·245	17·6%	1·07 ± 0·179	12·9%
	D+E (♀♀ crossbred)	58	1·12 ± 0·219	11·1%	1·04 ± 0·159	11·5%
			t = 1·99			
			P = 0·05			
II	Parents inbred	13	2·46 ± 0·765	22·5%	1·15 ± 0·355	13·6%
	Parents outbred	15	0·47 ± 0·336	4·0%	1·47 ± 0·390	13·0%
			t = 2·39			
			P = 0·05			
III	Parents inbred	17	4·65 ± 1·010	37·1%	1·47 ± 0·355	18·7%
	Parents outbred	59	1·24 ± 0·237	12 0%	0·93 ± 0·159	10·2%
			t = 3·29			
			P = 0·01			

little doubt that the difference is real in all three series. The conclusion is, therefore, that inbreeding in the female parent increases the loss of eggs or early embryos between ovulation and implantation, but it does not increase the mortality of embryos after implantation.

If the groups of Series I are arranged according to the inbreeding of the male parent, the pre-implantation losses are a little greater with inbred than with non-inbred fathers (1·70 against 1·28), but the difference is insignificant. The pre-implantation loss from inbred mothers is therefore more probably attributable to the females themselves than to a failure of fertilization caused by inbreeding of the male parent.

The distributions of pre-implantation losses are given in Table 3. The higher mean loss in inbreds is due to a larger number of large losses rather than to a difference in the modal loss: in other words, to a drawing out of the upper tail of

Table 3. *Distributions of pre-implantation losses, i.e. numbers of dissections exhibiting each degree of loss. 'Negative' losses represent deficiencies of corpora lutea due to miscounting*

	Number of eggs lost (= excess of corpora lutea over implants)															
	−2	−1	0	1	2	3	4	5	6	7	8	9	10	11	12	13
Series I																
Inbred ♀♀		1	30	18	14	12	5	0	1	0	1	3	1			
Outbred ♀♀	1	1	24	16	7	4	1	3	0	1						
Series II and III																
Inbred ♀♀			6	7	2	2	3	2	1	1	2	1	1	1	0	1
Outbred ♀♀	1	8	23	23	7	5	4	0	0	2	1					

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the distribution. The skewed distributions make the exact level of significance of the *t*-tests a little uncertain, but the reality of the difference between inbred and non-inbred females can hardly be questioned.

Nothing has yet been said about the perinatal losses. Not all the groups of dissected females had suitable females available for comparison of the number of young born alive. Those available for valid comparison are shown in Table 4.

Table 4. *Comparison of live embryos at* 16 *days and live young at birth in litters of comparable females*

		Live embryos		Live births	
Series	Parents	N	Mean ± s.e.	N	Mean ± s.e.
I B	Inbred	29	6·7 ± 0·52	107	6·2 ± 0·067
E	Outbred	30	7·8 ± 0·54	147	8·5 ± 0·034
II	Inbred	13	7·3 ± 0·74	21	7·4 ± 0·70
	Outbred	15	9·8 ± 0·66	27	8·3 ± 0·38

The evidence, as far as it goes, points to the perinatal losses being very small, and substantially the same in inbred as in non-inbred mothers.

Connexion between ovulation rate and body weight

The connexion between ovulation rate and body weight has an important bearing on the interpretation of the fact that ovulation rate did not decline with inbreeding. This will be explained below, in the Discussion, but the data are given here. The influence of body weight on the number of corpora lutea was examined in the dissections of Series I. The females were weighed at 6 weeks of age and were mated soon after, so that the ovulation corresponding to the corpora lutea counts took place between the ages of about 7 and 9 weeks. The regression of corpora lutea on 6-week weight was 0·244 ± 0·063 corpora lutea per gram. (The regressions did not differ significantly between the groups and this is the pooled value.) There is, thus, a positive association between body weight and ovulation rate. Comparisons of ovulation rate between inbred and outbred females should therefore take into account any differences of body weight. The mean weights of the females in the three series of dissections are given in Table 5, together with the mean ages at ovulation and the numbers of corpora lutea, from Table 1, to facilitate comparison. It will be seen that the weights of the inbred and outbred females of series I were very nearly the same, and so were the ovulation rates. The inbred females of Series II were 2 g. lighter than the outbred females at the time of ovulation (the weights were not recorded at 6 weeks) and the ovulation rate was lower. In Series III, as in Series I, the mean 6-week weight of the inbred females was not less than that of the outbred females. The differences of weight between the groups were, however, rather larger than in Series I, and the inbred females were substantially older than the outbred ones at the time of ovulation. The comparisons of ovulation rates in this series are therefore less reliable.

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Table 5. *Weights and ages of the females dissected*

Series	Group	Corpora lutea	Weight at 6 weeks (g.)	Weight at insemination (g.)	Age at insemination (days)
I	Inbred ♀♀ (A + B)	10·0	21·7	—	53
	(C)	10·2	21·7	—	59
	Outbred ♀♀ (D + E)	10·1	21·4	—	58
II	Inbred ♀♀	10·9	—	27·1	93
	Outbred ♀♀	11·7	—	29·0	95
III	Inbred ♀♀	12·5	22·9	—	103
	Outbred ♀♀ (H)	10·0	20·6	—	59
	(L)	11·9	23·8	—	69
	(C)	9·1	22·0	—	60

DISCUSSION

The fact that the ovulation rate, measured by the number of corpora lutea, was not affected by inbreeding calls for some comment. It would be reasonable to expect that body weight would decline on inbreeding, and, since ovulation rate is positively correlated with body weight, a reduction of ovulation rate on inbreeding might reasonably be expected. In fact, however, the weights of the mice used in this work, except those of Series II, did not decline on inbreeding for the following reason (see Roberts, 1960). The weights of mice, both at weaning and subsequent ly, are inversely correlated with the number reared in the litter, so that mice reared in small litters are larger than mice reared in large litters. (See, for example, Falconer, 1955.) No adjustment of the litter size at birth was made during the inbreeding of the mice dissected in Series I and III, the mothers being left to suckle all the mice to which they gave birth. But the numbers born declined as inbreeding proceded, and consequently the weights tended to increase. Presumably there was a contrary tendency for the weights to decrease from reduced pre-weaning nutrition and slower growth. These two opposing tendencies counterbalanced each other and the weights remained substantially constant. By this fortunate coincidence we were able to assess the effects of inbreeding on the ovulation rate without the disturbing influence of associated changes of body weight. The mice dissected in Series II, however, were differently treated. Litters were standardized at birth to four young during the inbreeding, and the compensatory effect of declining litter size was here absent. The inbred females were lighter than the outbred females and their ovulation rate was lower, though neither difference was statistically significant.

Thus the conclusion that inbreeding does not influence ovulation rate requires qualification because it refers only to the rather special circumstances where the effect of inbreeding on growth is not apparent. Under other circumstances, which allow body size to decline with inbreeding, the ovulation rate would almost certainly decline too. The conclusion, translated into genetic terms, is that genes that

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influence ovulation rate without affecting body size do not show directional dominance, but genes that influence ovulation rate as a consequence of their effect on body size may show directional dominance.

It is interesting to note that the effect of inbreeding on the ovulation rate of pigs appears to be different. The ovulation rate is reduced by inbreeding (Squiers *et al.*, 1952; King & Young, 1957), and this—rather than pre- or post-implantation loss—is the chief cause of the reduction of litter size. One cannot invoke a reduction of body size as an explanation because the ovulation rate was not found to be significantly correlated with body weight (King & Young, 1957). An explanation of the difference between pigs and mice might perhaps lie in their past histories of selection. The directional dominance exhibited by the genes affecting the ovulation rate of pigs may be the consequence of selective pressure in the past directed toward increased litter size; there has probably been much less selection of this sort in the past history of laboratory mice.

Reverting now to mice: the conclusion reached from the work described here is that the reduction of litter size in inbred mothers is mainly due to an increased loss of eggs or embryos before implantation. This loss could be caused by failure of fertilization or by failure of the fertilized eggs to implant. Since the inbreeding of the male did not influence the pre-implantation loss, any failure of fertilization would have to be attributed to the female. Losses attributable to the female might arise from (i) the production of abnormal eggs, (ii) impaired transport of the sperm to the site of fertilization, or (iii) failure of implantation in consequence of an impairment of endocrine function. The first seems unlikely because Braden (1957) found the incidence of abnormal eggs was no higher in inbred than in non-inbred females. The second is possible because only quite a small number of sperm reach the site of fertilization (Braden, 1958) and it would not be unreasonable to suppose that impaired transport might reduce their number enough to leave some eggs unfertilized. Nevertheless, failure of the fertilized eggs to implant, in consequence of an impaired endocrine function, seems to be the more likely cause of the increased pre-implantation loss in inbred females.

SUMMARY

Dissections were made of 16-day-pregnant female mice with the object of discovering the developmental stage at which litter size is reduced by inbreeding. Counts were made of the numbers of corpora lutea, implantation sites, and live embryos, and comparisons were made between females with inbreeding coefficients of 50–60% and non-inbred females. Except in one group the embryos were all non-inbred, so that the comparisons showed the effect of inbreeding in the mother of the litter. No influence of inbreeding in the male parent was found.

The only difference found between inbred and non-inbred females was in the number of eggs or embryos lost before implantation. The greater pre-implantation loss in inbred females was enough to account for the smaller number of young born alive in their litters.

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There was no difference between the inbred and non-inbred females in the ovulation rate, measured by the number of corpora lutea, or in the post-implantation mortality of the embryos.

There was a positive correlation between ovulation rate and weight at 6 weeks. For reasons explained in the Discussion, the inbred females did not differ in weight from the non-inbred females. If, under other conditions, the weight declined on inbreeding, the ovulation rate would be expected to decline also.

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