The effects of different rack systems on the breeding performance of DBA/2 mice

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Summary

Housing systems for laboratory animals have been developed over a long time. Microenvironmental systems such as positive, individually ventilated caging systems and forced-air-ventilated systems are increasingly used by many researchers to reduce cross contamination between cages. There have been many investigations of the impact of these systems on the health of animals, the light intensity, the relative humidity and temperature of cages, the concentration of ammonia and CO_2, and other factors in the cages. The aim of the present study was to compare the effects of different rack systems and to understand the influence of environmental enrichment on the breeding performance of mice. Sixty DBA/2 breeding pairs were used for this experiment. Animals were kept in three rack systems: a ventilated cabinet, a normal open rack and an individually ventilated cage rack (IVC rack) with enriched or non-enriched type II elongated Makrolon cages. Reproduction performance was recorded from 10 to 40 weeks of age. In all three rack systems there was a similar breeding index (pups/dam/week) in non-enriched groups during the long-term breeding period, but the coefficients of variation in the IVC rack were higher for most parameters. This type of enrichment seems to lead to a decrease in the number of pups born, especially in the IVC group. However, there was no significant difference in breeding index (young weaned/female/week).

Keywords Inbred mice; breeding performance; ventilated cabinet; individually ventilated cage; environmental enrichment

Housing systems for laboratory animals have been developed over a long time. Recently, microenvironmental systems such as positive, IVC systems and forced-air-ventilated systems are increasingly being used by many researchers, to reduce cross contamination between cages.

For example, at the Jackson Laboratory pressurized, individually ventilated (PIV) cages have been used to house weaned mice. This system has reduced cross contamination between cages and the transmission of pneumonia virus of mice and ammonia production, while it has increased the number of cages for a given floor area up to 40%, due to the decreased space between shelves (Cunliffe-Beamer & Les 1983, Les 1983).

Similar designs have been developed, mostly to reduce microbiological contamination. There have been many investigations of the impact of these systems on the health of animals, the light intensity, the air exchange rate, the sound level, the relative humidity and the temperature within cages, the concentration of ammonia, CO_2, acetic acid, and sulphur dioxide in the cages, the containment level, the biomass, the airborne dust particles or the airborne bacteria

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(Keller *et al.* 1983, Wu *et al.* 1985, Yamauchi *et al.* 1989, Corning & Lipman 1991, 1992, Iwarsson & Norén 1992, Lipman *et al.* 1992, Huerkamp 1993, Kurosawa *et al.* 1993, Clough *et al.* 1995, Perkins & Lipman 1995, Yoshida *et al.* 1995, Perkins & Lipman 1996, Haseggawa *et al.* 1997, Reeb *et al.* 1997, Ishii *et al.* 1998, Reeb-Whitaker *et al.* 1999, Höglund & Renström 2001, Renström *et al.* 2001).

Moreover, these microenvironmental systems have also increasingly been used for breeding purposes in animal facilities, especially for transgenic animals, but there is very limited information on the impact of these systems on reproduction performance.

The present study was focused on the breeding performance of animals, to compare the effects of different rack systems: an IVC rack, a ventilated cabinet and a normal open rack system, and to understand the influence of environmental enrichment in these different rack systems.

Fig 1 Enriched cage

Material and methods

Animals and housing

Animals Sixty DBA/2 mice breeding pairs (Charles River Company, Sulzfeld, Germany) were used for this experiment. At 10 weeks of age animals were marked (using ear puncture) and randomly distributed to the three rack systems with 20 breeding pairs per system, 10 pairs each for enriched and non-enriched cages. For synchronization of oestrous cycles (Whitten-effect) some bedding from the male cages was transferred to all the female cages one day before the animals were re-grouped to breeding pairs.

Environment The animals were kept in three different rack systems: a ventilated cabinet (Scantainer, Scanbur Company, Køge Denmark), a normal open rack, and an individually ventilated cage rack (VR-IVC, Charles River Company, Sulzfeld Germany). All rack systems were kept in the same animal room under specific pathogen free (SPF) conditions at a room temperature 22 ± 1°C, with $55 \pm 10\%$ relative humidity, a 12/12 h light/dark cycle and a light intensity of 120–150 lux (measured 100 cm above the floor).

Housing All cages were type II elongated Makrolon cages ($32.5 \times 16.5 \times 14$ cm, Charles River Company, Sulzfeld Germany). The enriched cages contained a nest box ($12 \times 7 \times 4.5$ cm), a wood bar (13 cm $\times$ 7.5 cm, pine) for climbing, and nesting material (nestlets, cotton fibre, 5×5 cm, EBECO Company, Castrop-Rauxel Germany) (modified from Scharmann 1993). An enriched cage is shown in Figs 1 and 2.

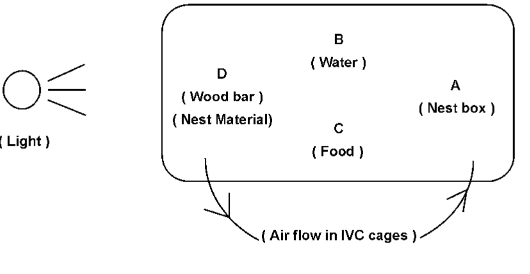

Fig 2 The structure of enriched cage. (After changing cages, wood bar, nest box and nest material were always placed in the same area, as the figure shows, although animals might move nest box and nest material into other areas)

Food and water Tap water in drinking bottles and pelleted food containing 22.5% protein, 5.0% fat, 4.5% fibre and 6.5% ash (Altromin No. 1310, Altromin GmbH, Lage, Germany) were given *ad libitum*.

Bedding 70–80 g wood shavings were used for bedding (Altromin Type 3–4, Altromin GmbH, Lage, Germany). Cages and bedding were changed once a week.

Health monitoring As infections could be the reason for differences in breeding performance and variance, at the end of the experiment the health of retired breeders was monitored as recommended by FELASA (Kraft *et al.* 1994).

Experimental design

Following 4 weeks of adaptation, at 10 weeks of age the animals were marked and randomly separated to the three rack systems described above. Breeding pairs were kept together (one pair/cage) during the experimental period from October to June. After regrouping to one breeding pair per cage, reproduction performance factors were recorded until 40 weeks of age, including litter size, number of pups weaned and body weight at weaning.

Statistics

Data were analysed by StatView 4.5 software (Abacus Concepts, Inc., Berkeley, CA, 1994) to calculate the mean values and the coefficients of variation of each group. All parameters were compared using a two-factorial analysis of variance with the factors 'rack system' and 'housing' (significant level 5%), to analyse the effect of the rack systems, the housing and the rack systems × housing interaction (Lee 1999).

During the experiment some females died while giving birth, and in one male the testes did not develop. These data are not included in the statistical analysis.

The total number of pregnancies included: (1) the number of females that gave birth (N1) and (2) the pregnancies estimated according to the weight development curve when a female's body weight continuously increased over 2 weeks and showed a clear decrease in the third week (N2), or when a female's body weight increased continuously over one week and showed a clear decrease in the following week (N3). The abortion rate was estimated by the relative number of pregnancies (N1 + N2 + N3) and litters for each female.

Results

Breeding performance

Total number of litters per dam Similar results were found in the non-enriched groups of the Scantainer and the open rack. There was a slightly lower number in the IVC rack, but no significant rack difference was found ($F_{2,24} = 0.528$; $P = 0.5963$; Table 1).

In comparison to non-enriched groups, enriched groups showed a decrease in the total number of litters per dam ($F_{1,47} = 3.681$; $P = 0.0611$), especially in the IVC rack (Table 1). A significant rack difference was also found in the enriched groups ($F_{2,23} = 3.554$; $P = 0.0452$).

Total number of pups born per dam There was no significant rack difference in the housing conditions. But although there were no differences in the Scantainer and the open rack, in the IVC rack there was a higher number in the non-enriched group and a smaller number in the enriched group (Table 1). Moreover, enriched groups had a significantly smaller number of pups born per dam ($F_{1,47} = 6.055$; $P = 0.0176$; Table 1).

Breeding index per dam Similar data were found in non-enriched groups. In comparison to non-enriched groups, enriched groups showed a decrease in this variable, especially in the Scantainer and the IVC rack, but this did not reach a significant difference for housing. Nevertheless enriched groups showed an increased coefficient of variation (Table 1).

Pups' body weight Even though a significant difference was found between racks under non-enriched housing conditions ($F_{2,404} = 5.043$; $P = 0.0069$), enriched groups

Table 1 Breeding performance in different rack systems and housing conditions

		Scantainer	Open rack	IVC	Rack difference	Housing difference
Total No. of litters/dam	Non-enriched (CV)	5.9 (23%)	5.6 (29%)	4.9 (61%)	$P = 0.5963$	$P = 0.0611$
	Enriched (CV)	5.6 (37%)	4.6 (54%)	2.8 (71%)	$P = 0.0452$ s	
Total No. of pups born/dam	Non-enriched (CV)	24.4 (31%)	24.1 (47%)	26.2 (71%)	$P = 0.9370$	$P = 0.0176$ s
	Enriched (CV)	17.6 (86%)	17.3 (69%)	13.4 (84%)	$P = 0.7315$	
Breeding index (young weaned/female/week)	Non-enriched (CV)	0.539 (47%)	0.490 (51%)	0.527 (77%)	$P = 0.9412$	$P = 0.2038$
	Enriched (CV)	0.386 (100%)	0.469 (82%)	0.348 (103%)	$P = 0.7804$	
Body weight of pups weaned (18 days, g)	Non-enriched (CV)	6.9 (16%)	6.4 (22%)	6.8 (21%)	$P = 0.0069$ s	$P < 0.001$ s
	Enriched (CV)	7.4 (18%)	7.5 (16%)	7.4 (17%)	$P = 0.5331$	
Age of dam at first birth (day)	Non-enriched (CV)	89.5 (4%)	92.2 (9%)	96.1 (27%)	$P = 0.7188$	$P = 0.1653$
	Enriched (CV)	98.0 (14%)	95.4 (13%)	104.2 (21%)	$P = 0.5400$	
Age of dam at first weaned (day)	Non-enriched (CV)	131.3 (39%)	129.8 (29%)	156.3 (47%)	$P = 0.8993$	$P = 0.2658$
	Enriched (CV)	178.4 (54%)	156.3 (51%)	165.4 (49%)	$P = 0.6443$	
Litters interval (day)	Non-enriched (CV)	31.8 (24%)	28.5 (17%)	36.3 (70%)	$P = 0.5706$	$P = 0.0498$ s
	Enriched (CV)	33.2 (27%)	41.6 (38%)	46.7 (33%)	$P = 0.1872$	

$N = 9$ in all non-enriched groups; $N = 7$, 9, 10 in Scantainer, open rack and individually ventilated cage (IVC) enriched group
CV: the coefficients of variation (SD/mean, %)
s: significant difference

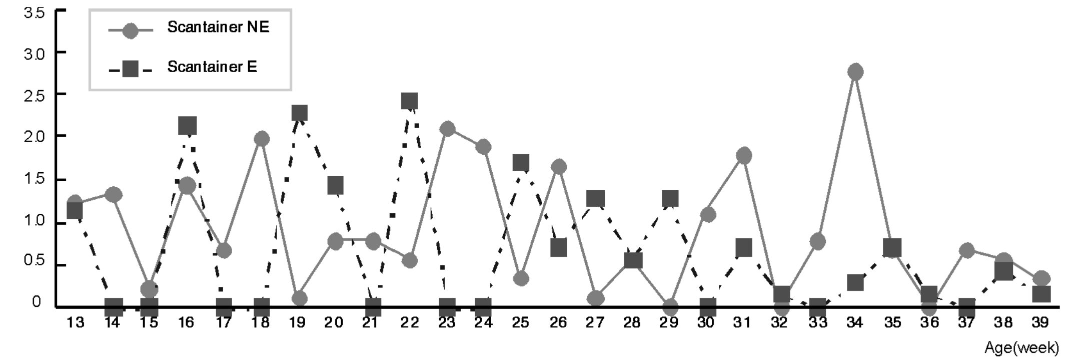

Fig 3 The number of pups born/dam in the Scantainer

had a similar body weight of pups at weaning (Table 1). The pups of enriched groups also had significantly higher body weights than those in non-enriched groups ($F_{1,705}=59.466$; $P= <0.0001$).

Age of dam at first birth On average the dams in the IVC rack under both housing conditions produced first litters later than the others, and all enriched groups delivered later than non-enriched groups. However there was no significant difference due to housing and rack (Table 1).

Age of dam at first weaning In non-enriched groups the pups in the IVC rack were also the last one to be weaned, but those in the Scantainer in the enriched group were the last (Table 1). In comparison to non-enriched groups enrichment caused a delay in the age of dam at first weaning in all rack systems, although this did not reach a significant housing difference.

Litter interval The results for the interval between litters were similar, as the IVC rack produced the longest interval, and enrichment led to an increase ($F_{1,45}=4.4063$; $P=0.0498$) in the litter interval in all rack systems (Table 1).

Rack × housing interaction A significant effect of rack systems × housing interaction was found only with regard to the body weight of weaned pups ($F_{2,705}=4.097$; $P=0.0170$).

The number of pups born and weaned per dam versus breeding age

The number of pups born/week/dam and the number of pups weaned/week/dam versus breeding age are shown in Figs 3–8. The

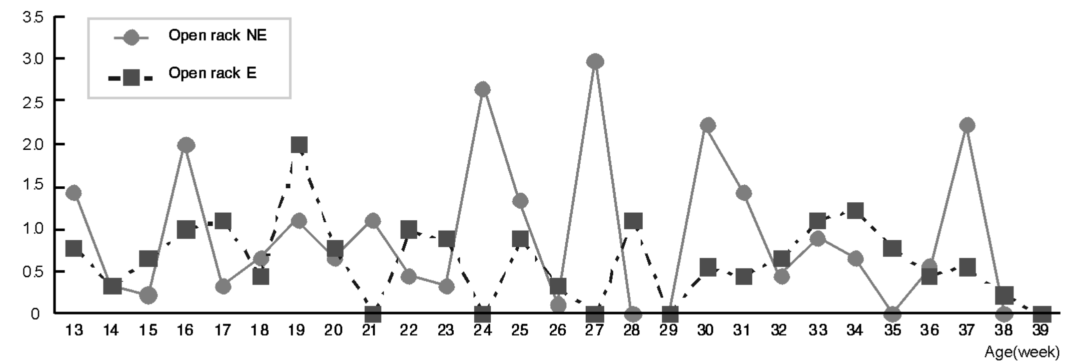

Fig 4 The number of pups born/dam in the open rack

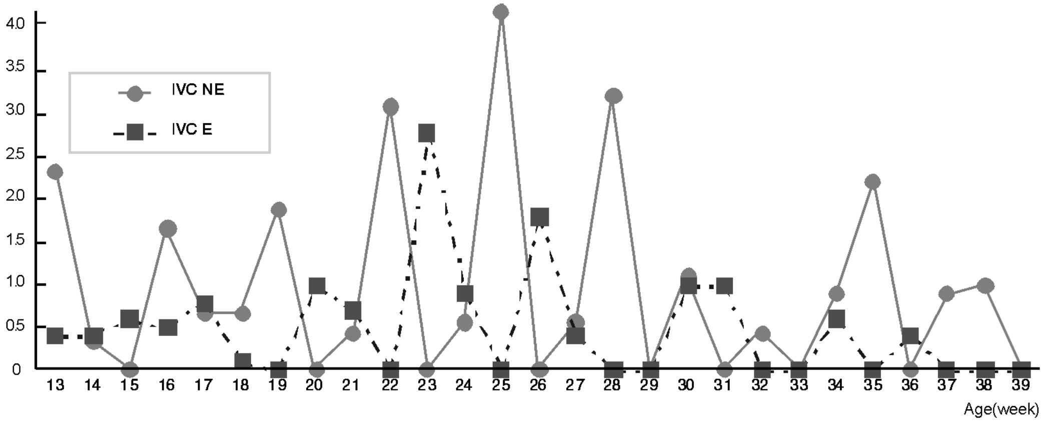

Fig 5 The number of pups born/dam in the IVC rack

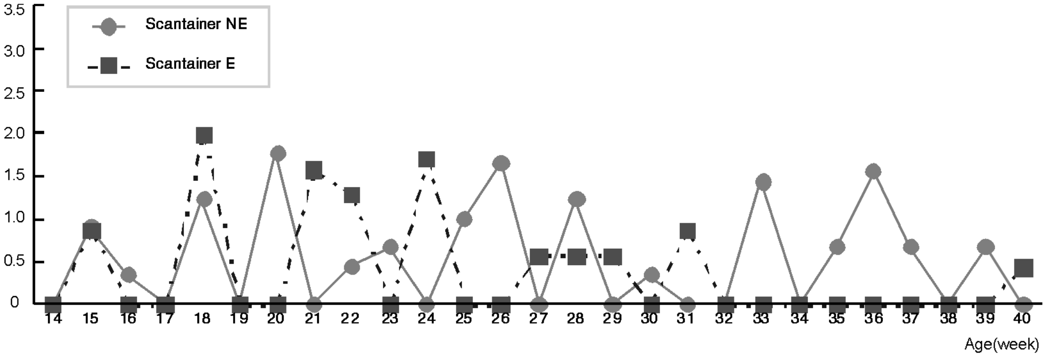

Fig 6 The number of pups weaned/dam in the Scantainer

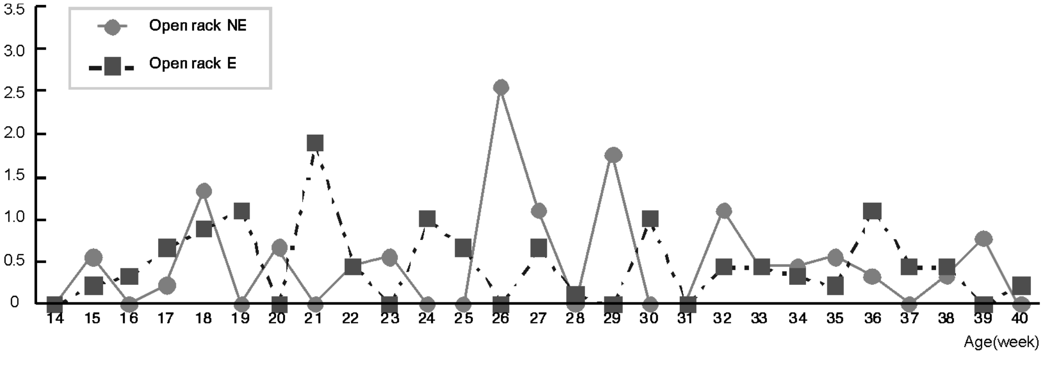

Fig 7 The number of pups weaned/dam in the open rack

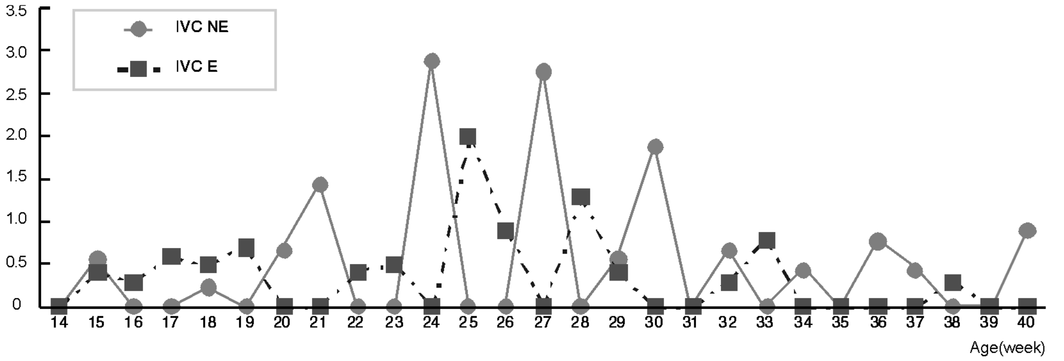

Fig 8 The number of pups weaned/dam in the IVC rack

breeding period was divided into three equal phases for analysis of the differences between phases (Tables 2 and 3).

The number of pups born/dam/week versus breeding age In general a significant difference between phases was found in non-enriched groups ($F_{2,72}=3.6513$; $P=0.0309$), but not in the enriched groups ($F_{2,72}=2.8793$; $P=0.0629$). The development of breeding results of open rack and IVC rack for non-enriched groups is similar, showing an increase in the middle phase and a decrease from the middle phase onwards till the end of this experiment, while the Scantainer showed a slight decrease during the whole experiment (Table 2).

Of the enriched groups, the Scantainer and IVC groups had a higher number of pups born in the middle phase than in the beginning and the end phases, while the open rack enriched group had a decrease in the middle phase and a slight increase from the middle phase onwards till the end of this experiment (Table 2). Moreover, from the middle phase onwards all enriched groups had a significantly lower number of pups born than in the non-enriched groups ($F_{1,47}=4.6477$; $P=0.0362$ at the middle phase; $F_{1,47}=6.4404$; $P=0.0145$ at the end).

The number of pups weaned/dam/week versus breeding age The open rack and IVC rack non-enriched groups showed higher numbers in the middle phase, while in the Scantainer the non-enriched group had a slightly lower number in the middle phase (Table 3).

For the enriched groups, the number decreased in the Scantainer and the open rack over time, while the group in the IVC rack showed higher numbers in the middle phase

Table 2 The number of pups born/female/week in different breeding periods under different rack systems and housing conditions

	Phase	Scantainer	Open rack	IVC	Rack difference	Period difference
Non-enriched	Beginning (CV)	0.951 (49%)	0.877 (62%)	0.889 (70%)	$P=0.9538$	$P=0.0309$ s
	Middle (CV)	0.926 (51%)	1.1235 (50%)	1.1498 (71%)	$P=0.3568$	
	End (CV)	0.840 (60%)	0.691 (71%)	0.605 (100%)	$P=0.6473$	
Enriched	Beginning (CV)	0.778 (100%)	0.790 (74%)	0.500 (69%)	$P=0.4718$	$P=0.0629$
	Middle (CV)	0.889 (82%)	0.531 (113%)	0.767 (105%)	$P=0.5961$	
	End (CV)	0.286 (138%)	0.605 (89%)	0.222 (149%)	$P=0.1489$	

$N=9$ in all non-enriched groups; $N=7$, 9, 10 in Scantainer, open rack and individually ventilated cage (IVC) enriched group
CV: the coefficients of variation (SD/mean, %)
s: significant difference

Table 3 The number of pups weaned/female/week in different breeding periods under different rack systems and housing conditions

	Phase	Scantainer	Open rack	IVC	Rack difference	Period difference
Non-enriched	Beginning (CV)	0.593 (81%)	0.420 (133.2%)	0.321 (104%)	$P=0.4685$	$P=0.0234$ s
	Middle (CV)	0.469 (95%)	0.728 (59%)	0.975 (75%)	$P=0.1721$	
	End (CV)	0.556 (62%)	0.321 (92%)	0.284 (169%)	$P=0.2806$	
Enriched	Beginning (CV)	0.635 (107%)	0.617 (88%)	0.378 (98%)	$P=0.5172$	$P=0.0187$ s
	Middle (CV)	0.476 (100%)	0.432 (132%)	0.544 (122%)	$P=0.9158$	
	End (CV)	0.048 (264%)	0.358 (99%)	0.122 (216%)	$P=0.0860$	

$N=9$ in all non-enriched groups; $N=7$, 9, 10 in Scantainer, open rack and individually ventilated cage (IVC) enriched group
CV: the coefficients of variation (SD/mean, %)
s: significant difference

(Table 3). Although in the beginning phase all non-enriched groups had a higher number of pups born per dam than in enriched groups, the groups in the Scantainer and open rack under non-enriched conditions had a lower number of pups weaned than in the enriched groups.

A significant difference between phases was found in both housing conditions ($F_{2,72}=3.9561$; $P=0.0234$; $F_{2,72}=4.2169$; $P=0.01887$), although a significant housing difference was found only at the end ($F_{1,47}=5.1164$; $P=0.0187$).

Discussion

The data showed that the dams in the IVC rack had a lower total number of litters per dam and that enrichment led to a significant decrease in all three rack systems, especially in the open rack and IVC rack. For this reason the abortion rate of females in all rack systems could be compared (Fig 9).

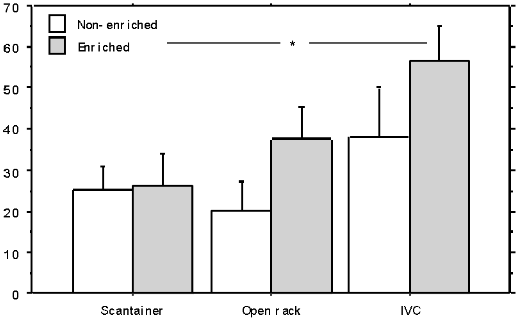

Fig 9 Abortion per dam of different rack systems and housing conditions (Abortion = the total number of litters/the total number being pregnant, %)

By comparison with the Scantainer and the open rack, in the IVC rack there was an increase in the estimated abortion rate under both housing conditions. Rack difference was found in the enriched group ($F_{2,23}=3.383$; $P=0.0442$), mainly due to the significant difference between the Scantainer and IVC rack (Fig 9). In the open and the IVC racks, enriched groups had a higher abortion rate than non-enriched groups, although overall no significant housing difference was found ($F_{1,47}=3.174$; $P=0.0813$), due to the very small difference between enriched and non-enriched groups in the Scantainer. In addition there was a high correlation between the total number of litters per dam and the estimated abortion rate ($P<0.001$). Since N2, but not the N3, could be also detected when animals were weighed, another statistical analysis was performed in which N3 was not included in the number of pregnancies. Similar results were also found in the additional analysis, which seems to indicate that abortion could be a possible reason for the lower number of litters per dam during the experiment.

In the present study the results of non-enriched groups were similar to those reported by Reeb-Whitaker *et al.* (2001). Their data on the breeding performance of C57BL/6J mice (non-enriched cages changed every week) in non-ventilated racks and IVC racks showed that the number of pups born per dam in the IVC rack was slightly higher than in the non-ventilated rack, but the preweaning mortality was higher in the IVC rack and the overall number of mice weaned per dam was similar in both racks.

There are several factors to explain these results. First, in both studies racks were kept in the same animal room, so that the noise which is produced by IVC racks would also affect other racks. Secondly, the breeding performance data in both experiments were collected for at least 7 months, so the animals had more time to adapt to the IVC rack and to increase their breeding capabilities. Thirdly, due to the protection of a closed cabinet, the noise effect on the animals in the Scantainer may be decreased, leading to a better reproduction rate in this experiment. Thus the difference between the IVC rack and the open rack may have been decreased, resulting in the similar breeding performance in the Scantainer and the open rack.

Table 3 shows that the Scantainer and open rack enriched groups had a higher number of pups weaned than did non-enriched groups in the beginning phase, even though the number of pups born in non-enriched groups was higher. It seems that enrichment may have a positive effect on raising pups in the first breeding phase. This might be the reason why a significant housing difference was found in the total number of pups born, but not in the total number of pups weaned.

Since females which had fewer pups often raised them better than those with more pups, the effect of enrichment was not clear at second and third phases, due to the fact that enriched groups had a lower number of pups born.

Although in the beginning phase the numbers of pups born of the non-enriched group in the IVC rack were similar to those in the Scantainer, the difference between the Scantainer and IVC rack in the total number of pups weaned increased over time (Tables 2 and 3). As this phenomenon was not observed in the middle phase, this may indicate that animals need some time to adapt to the environment of the IVC rack. The animals which were kept in the open rack also showed similar results. This phenomenon might be due to the noise which was produced by the IVC rack. Moreover, the coefficients of variation of groups in the IVC rack were often higher (Table 1); and this may suggest that there are individual differences in the capability of animals to adapt to the IVC rack.

Nevertheless the main effect of enrichment was a decrease in the number of pups born per dam. The reason for the lower number of pups born was the higher abortion rate in the open and the IVC racks and a lower number of pups born per litter in the Scantainer. Perhaps the effect of airflow was increased, as the air passed through the nest box, possibly leading to the lower number of pups born per dam. The higher abortion rate may have been due to the fact that some of the animals which were kept in enriched cages were more active. Furthermore, the cotton nest material may have influenced breeding performance negatively, as has been described for cellulose bedding material (Iturrian & Fink 1968). However, further study is needed.

In summary, it seems the mice used in this study needed more time to adapt to the IVC rack than to the Scantainer and the open rack, and that there is an individual difference in the capability of animals to adapt to the IVC rack, although over a long breeding time there were similar breeding indices for non-enriched groups in all three rack systems.

The type of enrichment used in this study seems to lead to a decrease in the number of pups born, especially in the IVC group. However, there were no significant difference in breeding indices (young weaned/female/week).

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