Scand. J. Lab. Anim. Sci. No. 3. 2001. Vol. 28

Cross-fostering in the rat performed shortly after delivery: description of the procedure

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Additional information and illustrations can be obtained at: http://oslovet.veths.no/teaching/rat/techniques/fostering.

Introduction

In a series of toxicological studies we needed to create groups of offspring that were exposed to a lipophilic test chemical exclusively *in utero* or through lactation, in addition to a control group. When animals or humans take in a lipophilic chemical, it will diffuse along with fat from plasma into different body compartments including the gravid uterus and be stored in the body fat and excreted with milk during lactation. Thus, the pups had to be cross-fostered before they started to suckle.

The rat is considered to be an excellent foster-mother, but disturbances in connection with the delivery may elicit cannibalism by the dam (*Weihe*, 1987). In previous studies, we had experienced that disturbances shortly after delivery such as sudden noise or handling by unfamiliar persons elicited cannibalism by the dams. Additionally, we have seen that dams have eaten weak or cold and cyanotic pups.

In reproduction studies, the number of litters is particularly crucial and it is desirable to minimise the risk of losing litters by cannibalism. We searched the available databases (Advanced MedLine Research) and literature for information about how to perform cross-fostering in rats shortly after delivery. Results derived from cross-fostering procedures are commonly referred to regarding drugs, but we found no detailed information about how to perform it. However, we carefully planned the cross-fostering procedure and carried it through.

This short communication is written in order to share our experiences with other in the same situation and give some guidelines, which may be of benefit for a successful cross-fostering procedure.

Materials and Methods

The study was approved by the local competent person under the surveillance of the National Animal Research Authority (NARA) («Utvalg for forsøk med dyr»). The experiment was performed and conducted in accordance with The Norwegian Regulation on Animal Experimentation of January 15th 1996.

Test animals and treatment:

Forty DA/OlaHsd female rats time-mated with LEW/SsNHsd males (both strains barrier-bred of SPF quality from Harlan, UK) arrived on gestation day (GD) 4. The rats were virgins prior to the study. The day the vaginal plug was noted by the breeder was assigned GD 0.

The dams were weighed and stratified according to weight and then block-randomised into three groups on arrival. The dams were housed individually in Macrolon type III cages. Room temperature was kept at 20° ± 1° C with a relative humidity of approximately 55 to 70 %. The light/dark cycles were 12:12 hours, with lights switched on at 08.00 and off at 20.00. The animal room was ventilated with 15-18 air changes per hour.

The cages were placed on the middle and lower levels of three rows of shelves on both sides of the animal room. Each cage was equipped with nesting paper (non-bleached cellulose paper) and the rats had free access to water and a commercial pelleted diet from arrival to birth (Beekay Rat & Mouse Standard Diet No. 1), and during lactation

(Beekay Rat & Mouse Autoclavable Diet). Both diets were obtained from B&K Universal, UK. The bedding used was a wood chip bedding from Norwegian aspen trees (B&K GLP bedding, B&K Universal, UK).

Only persons involved with the experiment were allowed in the room during the gestational period. The first six days after arrival the dams were not handled, in order to be habituated to the new environment. From GD 10 to 20 the dams were weighed every second day and subjected to treatment by gavage, which was performed in a separate room. One person was responsible for carrying the cages to the treatment room and for weighing the rats, while another person was responsible for the treatment. The same two people always performed these tasks. These persons were therefore familiar to the animals before the cross-fostering procedure started.

Deliveries and cross-fostering:

Three empty cages were placed in the middle of the animal room on GD 21. Two of the empty cages were to be used as temporary holding cages for the dams and were equipped with bedding, nesting paper and some food pellets. The third one was equipped to take care of the pups and contained an artificial nest made of soft paper placed on the top of two rubber gloves filled with warm water (Fig. 1). New-born rat pups are hairless and easily lose body heat if left alone. We have seen in earlier experiments that cold pups turn cyanotic and become passive and are more readily rejected by the dam than warm ones. Thus, it was important to refill the rubber gloves with warm water regularly.

Delivery started after working hours on GD 21. The door to the animal room was left open in order to minimise disturbances due to the observer frequently entering the room. The lights in the room were turned on manually at the time they were due to be switched off automatically and manually switched off by midnight. This interruption of normal light cycle did not seem to affect the animals.

The observer entered the room regularly and looked for traces of blood on the nesting paper and/or for pups while noting the time of delivery

Fig. 1

and the identity of the dam. Dams were only observed through the transparent walls of the cages. They were never disturbed or handled until about three hours after delivery. The dams generally delivered pups every 10 to 15 minutes. However, some dams spent more than three hours completing their delivery because of large litters containing ten or more pups.

During delivery, pups were scattered around in the cage while the dam was busy delivering, eating the placentas and cleaning the new-born young (Fig. 2). Delivery was over when the dam started to gather the pups beneath her for nursing. This was found to be the optimal time to perform cross-fostering. Similar size litters of dams from different treatment groups were crossed.

Two cages with dams and their litters were gently removed from their shelf and placed near the three

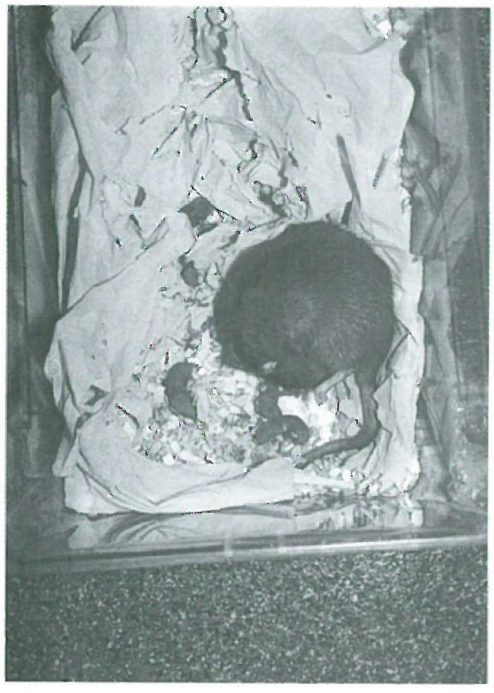

Fig. 2

empty cages (Fig. 3). The dams were carefully removed from their home cages and temporarily placed in the empty cages. Coloured identification papers were used to keep track of the dams. One litter was placed in the artificial nest while the other one was inspected and handled. The pups were counted and sexed and the information was noted both on the dam's identity card and on a separate sheet. The litter was held in the observer's hand for two to three minutes and the pups were cleaned if necessary. Some pups had bloodstained paper and/or bedding attached to their bodies and this was removed by means of a warm, wet cloth. Soft paper was used to dry the pups. Then the clean and warm litter was placed in the cage of the foster-mother and covered by her nesting paper. The other litter, which in the meantime had been in the artificial nest, was then subjected to the same procedure. When both litters had been placed in the cage of their foster-mothers, the dams were returned to their home cages. The cages were then carefully transferred back to the shelf. The time period of the exchange of the dams lasted five to ten minutes, depending on how much the pups needed to be cleaned.

In general, the dam spent the first minutes after returning to her home cage exploring the cage and reorganising the litter by carrying pups around. After a few minutes the dam calmed down and began to nurse the young (Fig. 4).

Results

Twenty-nine litters were successfully cross-fostered using the procedure described above. The remaining eleven dams were either not pregnant or excluded from the study due to small litter size. Each group consisted of more than eight litters and each litter consisted of seven to eight pups. The cross-fostering procedure resulted in three groups of offspring: Pups prenatally exposed to the test chemical (*in utero* exposed), pups postnatally exposed to the test chemical (through mother's milk) and controls.

Discussion

Time-mated rats are mated but not necessarily pregnant. However, increased body weight towards GD 20 indicated pregnancy. Dams whose body weight increased more than 30 % from GD 10 to 20 proved to be likely to deliver litters of eight or more pups.

The animals were probably not used to handling on arrival to the laboratory unit. All the dams became familiar to handling during the treatment period with only a few exceptions. The non-pregnant dams were far more aggressive and difficult to handle than the pregnant ones.

One dam delivered ten pups with obvious difficulty. Her pups were scattered around in the cage. A few were cleaned but several had dried, bloodstained paper attached to their bodies. The dam did not start to gather her pups. The observer considered it necessary to take the chance of eliciting cannibalism by intervening. Cross-fostering was performed and the pups were carefully cleaned and warmed. The exhausted dam accepted the new, clean and warm pups, which immediately started to suckle.

This study shows that cross-fostering of new-born

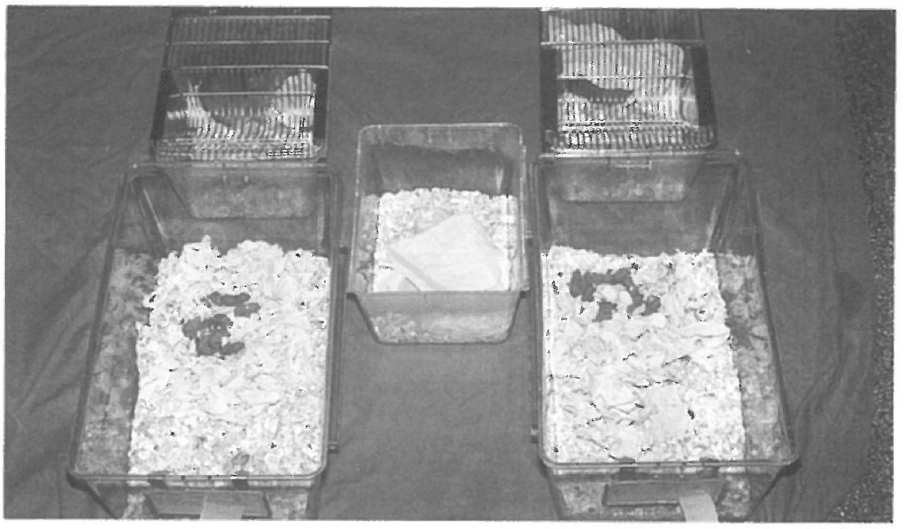

Fig. 3

rats is easily performed providing that some precautions are taken. Above all, the animals must be used to handling and the person carrying out the procedure should be familiar to the animals beforehand. This cross-fostering technique has been repeated successfully with rats of the same strain in our unit.

Summary

This paper describes a cross-fostering procedure in rats performed shortly after delivery. The aim of the procedure was to create groups of offspring that were exposed to a lipophilic test chemical exclusively *in utero* or through lactation, in addition to a control group. Time-bred dams were watched during delivery and their litters were exchanged before the young started to suckle. All dams accepted their role as foster-mothers. We had no previous experience in performing this kind of study. The following precautions taken have probably contributed to make a successful cross-fostering: It was restricted access to the animal facilities during the gestation and lactation periods and only a limited number of persons performed the experimental procedure. The dams became used to handling during the gestation period. Dams were temporarily removed from their litter and

Fig. 4

cages about three hours after delivery and just before they started to nurse the young. The newborn pups were kept warm and cleaned during the switch of cages. Dams were returned to their home cages containing foster-pups. It was a minimum of physical disturbances during the delivery and the person who performed the cross-fostering was familiar to the animals through previous handling. Twenty-nine litters were successfully cross-fostered.

Sammendrag

Forløpet av en kryssfostring utført rett etter fødsel hos rotte er beskrevet. Hensikten var å lage grupper av avkom som var eksponert for et fettløslig testkjemikalium enten utelukkende i fosterlivet eller gjennom morsmelken, i tillegg til en kontrollgruppe. Hunnrotter parret på samme tidspunkt ble holdt under oppsikt under fødselen og kullene byttet mellom mødrene før de nyfødte begynte å die. Alle rottemødrene godtok fosterungene sine. Vi hadde ingen erfaring i å utføre denne typen forsøk. De forholdsreglene som ble tatt kan ha bidratt til den vellykkede gjennomføringen av kryssfostringen. Under drektighets- og laktasjonsperiodene var det begrenset adgang til oppstallingsrommet og kun få personer deltok i selve eksperimentet. Rottemødrene ble håndtert jevnlig under drektigheten. Rottemødrene ble midlertidig tatt vekk fra buret og kullet sitt cirka tre timer etter fødselen og før de begynte å amme ungene sine. De nyfødte ungene ble holdt varme og rengjort før de ble lagt ned i fostermorens bur. Rottemødrene ble satt tilbake til sine egne bur som nå inneholdt fosterunger. Det var minimalt med fysiske forstyrrelser under fødselen og dyrene var godt kjent med personen som utførte kryssfostringen. Tjueni kull ble tilfredsstillende kryssfostret.

Acknowledgement

The financial support from the Norwegian Research Council is gratefully acknowledged.

Reference

Weihe W.H: The laboratory rat. In: T. Poole. (ed.). The UFAW handbook on the care and management of laboratory animals, 6th edition, p. 309-30. Longman Scientific and Technical, 1987.