The Effects of Two Different Supplemental Gels on C57BL/6J Pup Survival and Weights

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Abstract

It is well known that certain strains of mice such as C57BL/6, have a tendency to be poor mothers. Certain transgenic or targeted mutation strains of mice can exhibit this as well. Some strains may not be able to care for their pups because of phenotypic feeding deficits. This causes frustration with planning experiments, colony maintenance, and increased animal numbers which negatively impacts time and financial resources. Often, nutritional supplements are added to breeding cages in an attempt to assist dams with raising their litters. We conducted a pilot study, comparing supplemental gels to regular rodent chow and breeder chow in C57BL/6J nulliparous mice starting at E13 to determine if there was any difference in pup survival, pup weight, and general thriftiness. There were five study groups including: Regular Chow only, Regular Chow plus Gel A, Regular Chow plus Gel B, Breeder Chow only, and Breeder Chow plus Gel B. Rodent or Breeder Chow was given ad libitum. Cages were changed weekly and gel was added to the cages in the gel groups three times per week (once at cage change). The number of pups per litter was recorded on P1. Pups were weighed at P7, P14, P21, and P28. Dead pups were recorded on the days when fresh gel was added to the cages or during health rounds. Dams that were fed rodent chow plus Gel A consistently had larger pups with good body and coat condition and had the fewest pup deaths as compared to the other groups. There was only a 7% mortality rate and pups were up to 17% larger compared with the other groups. Gel B left all of the mice with greasy, unkempt hair coats and did not improve pup survival or weight gain. Due to low surviving pup number in the breeder chow groups we were unable to make any meaningful comparisons among these groups. The low pup mortality rate and upward trend in pup weight of the pups in cages with the Gel A group shows there may be some potential for its use as a supplement to increase pup weight and decrease pup mortality. This could, in turn, increase colony production in C57BL/6 mice and other strains that can be challenging to maintain.

Introduction

A common complaint among those who maintain certain breeding colonies of mice is that the mothers will either neglect or cannibalize their litters. It is common for C57BL/6 nulliparous mice to lose their first litter[1,2]. This can also be seen in certain genetically modified lines of mice[2]. Nutritional supplements have been provided to assist female mice with gestation and feeding of their pups which, in turn, can lead to better mouse production and improved colony maintenance[2]. Recently, several supplemental nutritional gels have become available that can be used to aid rodent breeding. We examined 2 of these nutritional gels along with standard rodent chow and rodent breeder chow to determine if either of these gels or the type of chow provided would improve survival of litters and increase weight gain in pups. We started supplying the gels to nulliparous C57BL/6 time-pregnant mice (E13) upon arrival and continued the supplements to the females and pups until the pups were weaned at Day 28. Comparisons were made between the use of standard rodent chow and rodent breeder chow in combination with the supplemental nutritional gels.

Materials and Methods

Animals

All animals were used under an approved protocol by Tufts University Institutional Animal Care and Use Committee and cared for in accordance with the Guide for the Care and Use of Laboratory Animals[3] and the Public Health Service policy[4]. The mice were housed in an AAALAC, International accredited facility. Mice were housed in a rodent room with a 14/10 hour light cycle. 25 C57Bl/6J timed pregnant females at day E13 (Jackson Laboratories, Bar Harbor, ME) were received and randomly placed into five study groups. They were housed in ventilated caging (Thoren, Hazelton, PA) on autoclaved corncob bedding (Harlan, Madison, WI), provided nestlets (Ancare, Bellmore, NY), given acidified water and either standard irradiated rodent chow (Harlan Teklad 2918, Harlan, Madison, WI) or irradiated rodent breeder chow (Harlan Teklad 7904 Harlan, Madison, WI) ad libitum depending on the study group. Cages were changed once per week in an animal transfer station (Ancare, Bellmore, NY).

Experimental design

There were five study groups that were created for the purpose of this study as defined in Table 1. Each group was randomly assigned 5 timed pregnant females. Immediately upon arrival and housing, "do not disturb" cards were placed on the cage so that only those who were involved in running the study would be manipulating the cages which included cage changes, gel addition, litter checks, and health checks. Supplemental nutritional gels (DietGel®76A or DietGel®BOOST, ClearH2O, Portland, ME) were added to cages three times per week. The cages that were not getting gel were still taken off of the rack, opened, and closed up again so that they were being treated similarly to the cages that were having gel added to them each time.

Group	Rodent Chow	Supplemental Nutritional Gel
Group 1	Standard	None
Group 2	Standard	DietGel®76A
Group 3	Standard	DietGel®BOOST
Group 4	Breeder	DietGel®76A
Group 5	Breeder	DietGel®BOOST

Table 1.
Summary of study groups

Females were checked twice per week after arrival. On day E21 females were checked and all were found to have given birth on the same day. Total number of pups born, number of pups dead, and number of pups alive were recorded. Records of pups found dead were also recorded at each cage change and gel addition. Pups were then weighed at days P7, P14, P21, and P28. Any females that had their entire litters not survive were removed from the study. One-Way ANOVA statistical analysis was performed to determine if there was any statistical significance to our results.

Figure 2a.
Pups at P28 on Standard Rodent Chow

Figure 2b.
Pups at P28 on Standard Rodent Chow plus DietGel®76A

Figure 2c.
Pups at P28 on Standard Rodent Chow plus DietGel®BOOST

Figure 3a.
Pups at P28 on Rodent Breeder Chow

Figure 3b.
Pups at P28 on Rodent Breeder Chow plus DietGel®BOOST

Results

Pups from all groups were all born on the same day and every female produced a litter. As expected, any pups that died did so within in the first week of birth. In the Standard Rodent Chow only group all but one female had surviving litters. In the Standard Rodent Chow plus DietGel®76A groups every female had a successful litter. In the Standard Rodent Chow plus DietGel®BOOST and the Breeder Chow only groups, only one female had a litter that survived. In the Breeder Chow plus DietGel®BOOST, two females had litters that survived.

When we looked at the survival rate of pups, we totaled the number of pups born and compared that to the total number of pups that survived to weaning age for each group (Table 2).

Experimental Groups	Pups Survived/ Total Number of Pups	Percent Survival
Standard Rodent Chow only	24/36	67%
Standard Rodent Chow plus DietGel®76A	26/28	93%
Standard Rodent Chow plus DietGel®BOOST	6/22	27%
Rodent Breeder Chow only	2/32	6%
Rodent Breeder Chow plus DietGel®BOOST	8/21	38%

Table 2.
Survival rate of pups in each group

Pups were weighed starting at P7 and it was noted that even before they were mobile enough to start feeding on the gel, there was a weight difference among the groups. Pups in the Standard Rodent Chow plus DietGel®76A group had a higher average weight which continued throughout the course of the study and averaged about 17.5% higher when compared to the other groups as shown in Figure 1.

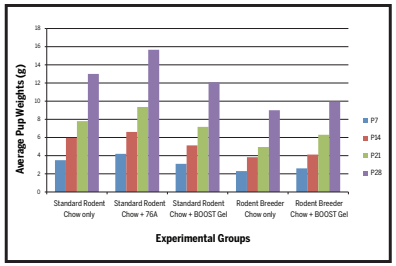

Figure 1.
Pup weights at P7, P14, P21, and P28

At the second addition of gel to cages it was noticed that the mice who received DietGel®BOOST in their cages had a greasy, unkempt looking coat while mice receiving DietGel®76A had normal looking coats similar to the cages that received no gels. Figures 2a,b,c and Figures 3a,b were taken at the time of weaning, P28, and illustrate the quality of hair coat among the different groups. All of the mice consumed both gels and didn't seem to have an aversion to it. A few of the females also attempted to incorporate some pieces of gel into their nests.

None of the results were statistically significant but this is likely due to the small N number in each group. However, we did see trends toward significance in several of the groups. When comparing survival rate of the litters, it came very close to statistical significance that the Standard Rodent Chow plus DietGel®76A had a better survival rate than the Standard Rodent Chow plus DietGel®BOOST litters.

Discussion

Despite the fact that they are notoriously poor first time mothers, C57BL/6 mice are commonly used in lab animal research. There will also be those mutant strains that are difficult to maintain due to poor mothering or phenotypic issues. The results of our study suggested that DietGel®76A supplemental nutrition gel may help breeding females successfully keep and raise their pups. It was also very interesting to us that the groups that had Rodent Breeder Chow versus Regular Rodent Chow seemed to have poorer outcomes. Mice were randomized into each group so this might have been by chance or there it is possible that there is some significance to this. Due to the poor coat condition of both the pups and the mothers, the lower survival rate of the pups and the lower average birth weight of the pups, we determined that DietGel®BOOST was probably not suitable for use with breeders and pups. A larger scale study with larger numbers of mice will need to be undertaken in order to further explore this and to determine if we can achieve statistically significant results as we would expend to see from the trends in this study. Based on the results of our study, our facility has begun to use DietGel®76A in breeding situations where we are having problems with litters being lost or pups having a phenotype that may benefit from it. We believe that we have seen more evidence in everyday use that may support the results of this study.

Acknowledgments

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