UNIVERSITY OF PADOVA
Department of Comparative Biomedicine and Food Science

First Cycle Degree (B.Sc.)
in Animal Care

Pup health: an observational analysis from birth to weaning

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Abstract

31 mice litters of different strains from Queen Mary University of London were analyzed using a score sheet with observational parameters which are non-invasive, to follow their development and record their appearance, activity, development and health status. Moreover, these data will be compared to the dataset given me by the research center on mice health concerns of the previous 3 years, to see if there are common or recurrent health concerns and to have an overall view of the status of the colonies.
The data were recorded each day and put into an excel file, to analyze the recordings using the R software.

Introduction

During my traineeship in the Biological Services at the Charterhouse Campus of Queen Mary University of London me and my company tutor Jordi Lopez Tremoleda developed a small research project about mice reproductive health.
Since the research facility started to see some health concerns related to reproduction and breeding, they wanted me to do some research on the health concerns data of the previous years, and to develop a score sheet to follow some litters from birth to weaning to see if pup health and survival was determined by something in particular, and if those results related somehow to the health concerns dataset of the past years. The dataset comprehended all the health alerts of some protocols put on the ARMIS mice management system in the past 3 years, and the score sheet was utilized on 31 different litters; the mice were followed from the first days of life till weaning at 21 days, and some observational parameters were recorded about the mother and the pups. Statistical analysis using R software was done on all the data recorded, to compare with the statistical data from the health concerns dataset.

In this facility, many health concerns are related to the breeding like hydrocephalus, malocclusion, dystocia, prolapse and others. This can be due to bad breeding and inbreeding, because sometimes researchers keep old pairs for too long or they have bad breeders, because by accident some characteristics were bred into the line and keep appearing, or simply because it is a characteristic of a particular strain. We have seen also a lot of eye and skin problems, which could be also a breeding problem in the sense that that particular health concern was bred into the genetic line. There also are genetic lines which are known to be more prone to some adverse phenotypic effects, and so have consequently more health concerns.
They asked me to look into the reproduction side to have a clearer mind on what is happening and which problems are more recurrent and also which lines need more help, so they can take action on it and refine the breeding strategies.

The project created consisted on the observational health data gathering of some mice litters, without using too invasive methods to avoid stressing the mother and lowering the chances of her cannibalizing the pups. I created a score sheet to evaluate the mice pups of 31 litters from day 1 to 7, at day 10, day 14 and at weaning at 21 days; yes/no or numbers from 0 to 3 were used to score the parameters chosen. What was included in my sheet for the first week of life was the color of the pups, the fur condition, the milk spot color or absence, eyes, ears, if there were any abnormalities, and the activity level. At day 10 I was looking at the fur condition, eyes, ears, activity level and any possible abnormalities; at day 14 I added a quick judgment on size, to see if I

could spot runts. Lastly, at weaning I checked eyes, ears, fur, weight and if they were ready for weaning depending on weight.
To be less invasive, I chose to not mark or move the pups too much from day 1 to 14, so the analysis was not perfectly done on every individual each time, but a litter analysis was made; for example, if two pups were having a light milk spot I would record it, but on the next measurement I would not be able to recognize who had a light milk spot yesterday.
The only handling I did was at day 21 in which I weighted each and every pup one at the time and recorded if suit for weaning or not, though some smaller weanlings were weaned anyway with the help of wet mash, which is soft food made with pellets' dust, and a longer tip of the water bottle cap was added to ease drinking for smaller mice.
Besides the pups, I gathered some info on the mother too like age, strain, how many litters she had in her cage and how many she had in her life, if she had had any health concerns, and what their breeding strategy was so if it was a trio (2F, 1M) or a duo (1F, 1M).

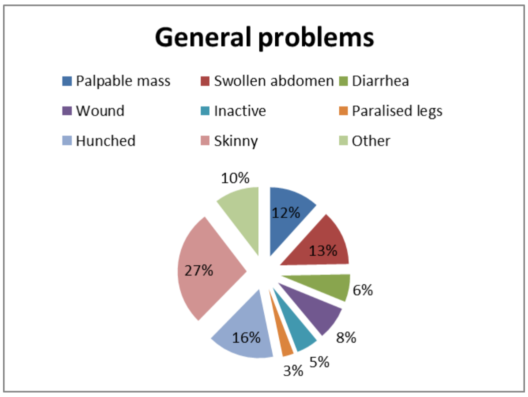
Other than the score sheet, the company gave me an excel file with all the health concerns of the last 3 years of some protocols in the lab facility. In this file I modified the data and grouped the health concerns into behavioral, breeding, eye problems, dermatitis, and general clinical signs. Then I did statistical analysis with excel and R to look into the dataset and understand more about the major mice health concerns and the most sensible strains. This helped me for the analysis of the score sheet data I have gathered, to understand if there are common results and similarities between the two.

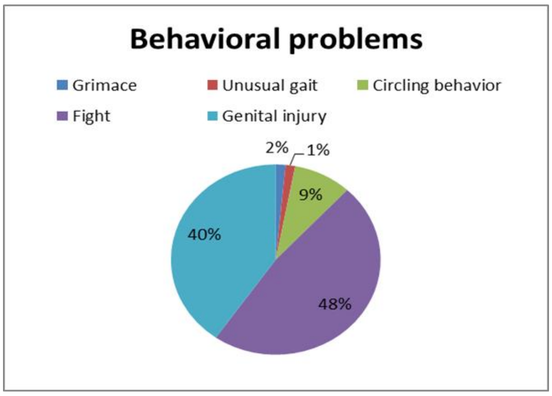
I used the R software to analyze all my data, especially doing descriptive statistics on the health concerns dataset and most importantly to create models for my score sheet data to see if there is anything significant which can tell us if we can spot future health concerns from birth and seeing what strains have which problems, and more useful info that could be of help to ameliorate the breeding system in the facility.

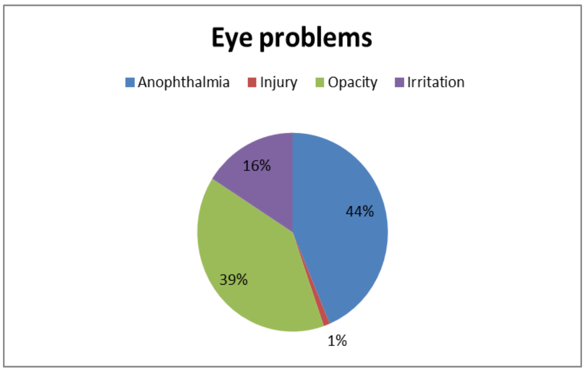
Materials and methods

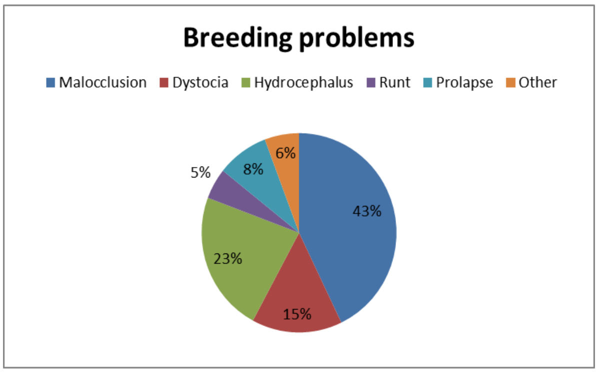
HEALTH CONCERNS DATASET

The health concern dataset comprehended 392 observations on a three year span regarding the two lab facilities at Charterhouse square and Whitechapel. Here are comprehended 16 project licenses and 52 different strains; age, sex, and date are recorded too.
The health concerns are grouped in breeding, behavioral, dermatitis, eye conditions, and general issues; those are again subdivided into other major groups as shown below.









It is clear some health concerns could have more than one possible cause (ex. Malocclusion can be caused by inbreeding, not wearing teeth, hitting the head, aging and others), and this will be taken into account in the conclusions drawn at the end. The categories assigned are related to the most plausible cause; having anophthalmia the first days of life is more likely due to a breeding problem, while if seen in adult age it could be the result of eye infection or cataracts.

After assigning categories to health issues, I started the descriptive statistics analysis using excel and R together. I created some graphs and tables to have a clearer view of which years were the worst based on health alerts and which problems were more encountered each year, which strains were more fragile and what were the main issues, which were the most occurring problems, the sex ratio in health concerns, the age in which most health concerns occurred also divided by issue category.
Unluckily, the company did not have the absolute number of animals available at that moment as recording systems on pups and litters have been revised and modified during the last years , so I

cannot compare the health alerts to the whole population and have a good estimation of which year was actually the best based on the ratio of healthy and sick animals.

<u>SCORE SHEET</u>

Taking into consideration the developmental stages of the newborn pup, I developed a score sheet to evaluate pup health and correct development. Pups were not moved or touched too much from day 1 to 14, and were only touched with cage bedding and/or scrubbing my gloves with bedding to have a known smell which was not alarming the mice too much. If the mother appeared stressed like running in circles and moving the pups, I would close the cage and wait a bit before completing my scoring. Pups were not marked, so every pup was not followed day by day but the whole cage was scored each day, not keeping track of which pup is who; this was made to lower even more the chances of stressing the mother too much.
30 cages and 31 litters were scored, and the parameters used are shown in the table below.

DAY 1-7	DAY 10	DAY 14	DAY 21
Day	Day	Day	Day
Pup	Pup	Pup	Pup
Alive/dead	Alive/dead	Alive/dead	Alive/dead
Color	Fur	Fur	Fur
Fur	Eyes	Eyes	Eyes
Milk spot	Ears	Ears	Ears
Eyes	Abnormalities	Size	Weight
Ears	Activity level	Abnormalities	Weaning
Abnormalities		Activity level	Comments
Activity level			

Each parameter had a yes/no or numerical/symbolical scoring, to which a written characteristic or developmental stage was assigned. Here is the description of each parameter:

- Alive =1, Dead=0
- Color: 1 red/ pink, 2 light pink, 3 pale/grey
- Fur: 0 no fur, 1 fuzz, 2 full, 3 alopecia/dermatitis
- Milk spot: 1 white, 2 pale, 3 not visible
- Eyes: V open, X closed, / anophthalmia
- Ears: 1 attached, 2 detaching, 3 fully erect
- Active: 1 active, 2 active if stimulated, 3 inactive
- Size: Yes – they are the right size, No – too small/smaller than the rest
- Weight: V – Big enough for weaning, X – Underweight/runt

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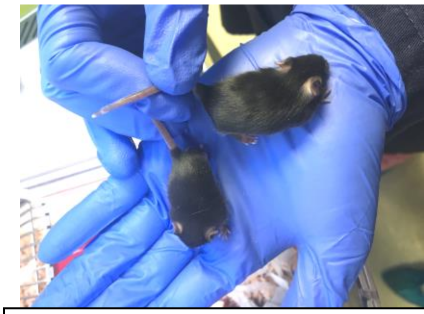
Pup has a score 1 milk spot and color.

Pup has a score 3 color and score 3 milk spot.



Runt (left) vs normally developed mouse (right) at 14 days.

Pup has a score 2 milk spot and score 1 color.

The mother and the cage were also taken into account in my study, and the information I chose to transcribe are:

- Day of birth of the litter
- Strain
- Mother’s age
- How many pregnancies the mother had in her lifetime
- Mother’s health concerns
- Cage barcode
- Cage rack location
- Room
- How many litters were in the cage
- Breeding pair type

Some were needed just to recognize the cages and find them easily, some gave me information about the mother’s health and breeding experience. The strain is also very useful to understand why for example some mice look smaller or have any particular problems.
One problem I found in trios is that I cannot have the number of pregnancies per mice but just an overall number of the two, so I don’t clearly know how many each had.
Also, the parameters are purely observational and subjective, so there can be a misjudgment error and also some bias on scoring.

STATISTICAL ANALYSIS OF THE SCORE SHEET USING THE R SOFTWARE

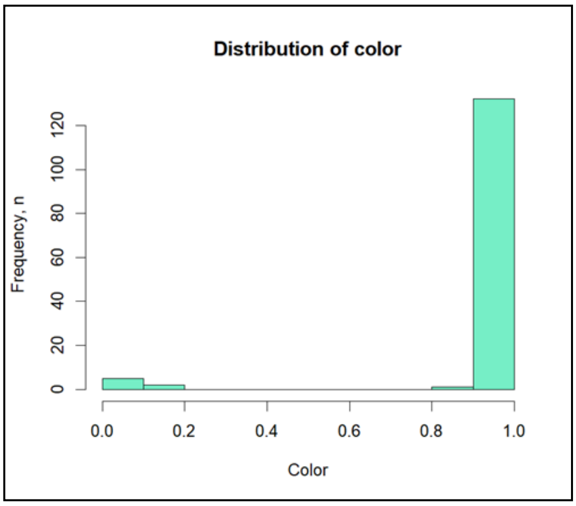
For the statistical analysis, the dataset on excel was adapted to diminish the noise and making the analysis have more power. Since the animals could not be recognized each day, the experimental unit was changed to litter, and every value was made a mean value or a percentage of a specific score.

- Litter
- Birth
- Barcode
- Strain
- Litters in cage
- Pair type
- Age mum
- Pregnancy number
- Mum health concerns
- Day
- Mortality %
- Number of pups
- Color s1 (% how many pups had a score of 1)
- Fur
- Milk spot ½ (% how many pups had a score of 1 or 2)
- Eyes
- Ears
- Size NO 14D (how many pups were too small at 14 days)
- Mean weight 21D
- Weaning NO (how many could not be weaned)
- Active s1 (% how many pups had a score of 1)
- Abnormalities
- Room

The variables were classified in response variables and explanatory variables. The explanatory variables were divided in random effects, covariates, and fixed effects.

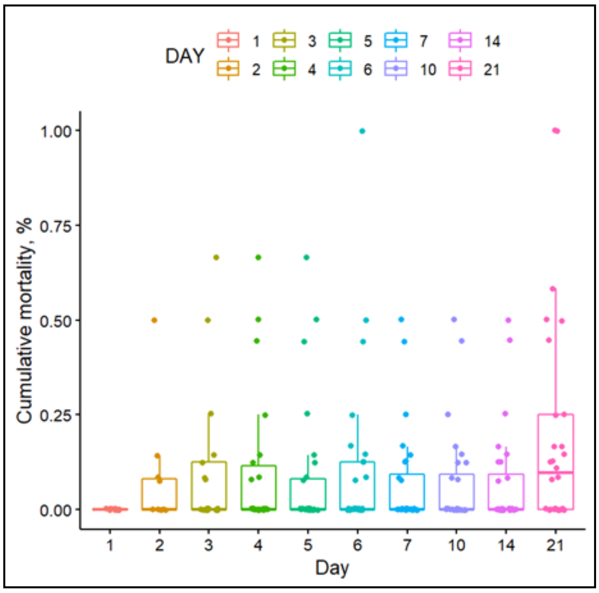
RESPONSE VARIABLES	RANDOM EFFECTS	FIXED EFFECTS	COVARIATE
CUMMORT	LITTER	LITTERS_IN_CAGE	AGE_MUM
LITSIZE		PAIR_TYPE	PREG_N
FUR		DAY	
MILK_SPOT_s12		Age_cl	
EYES		Preg_cl	
EARS			
ACTIVE_s1			
WEIGHT			

Strain and color were not analyzed, because I did not have enough litters per strain and I did not have enough variability in the color scores to be good enough to analyze.



Mortality was not used and cumulative mortality was used in its place, which is the total number of pups at birth minus the ones left each day divided by the n pups at birth.

(Npupsbirth – Npupsday)/Npupsbirth



Abnormalities were too many and also the mothers' health concerns, so they were changed in:

- 1=does have something wrong;
- 0=is healthy.

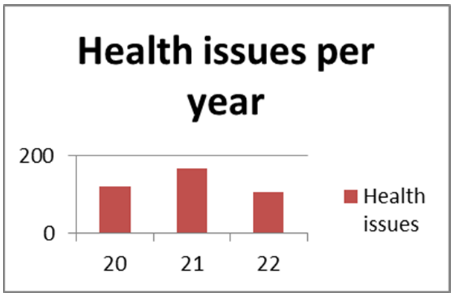
Litters 26 and 27 were removed due to too few observations, so we are left with 29 litters total.

Results

<u>HEALTH CONCERNS DATASET</u>

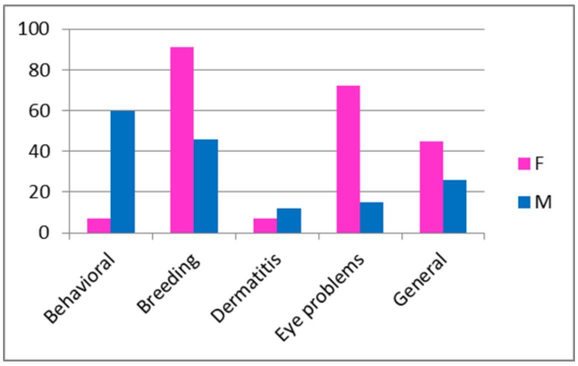
During the three years, there were more health issues in 2021, but we do not know how many animals there were in total so we can't do a good comparison on the yearly proportion of health/sick mice.

Most problems were related to breeding, followed by eye and behavioral problems. 2021 was the year with the most breeding problems.

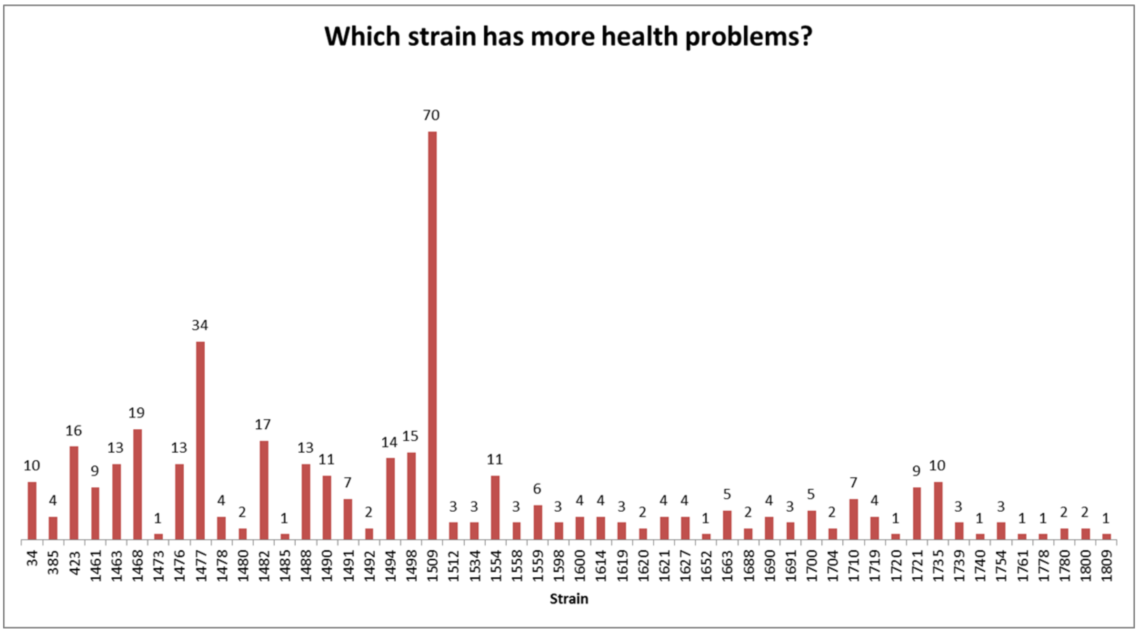


Health concern	2020	2021	2022
Breeding	32	60	48
Behavioral	22	25	20
Eye problems	26	41	27
General	35	29	7
Dermatitis	4	12	4
TOT	119	167	106

Females were the most affected in breeding and eye problems, but males as expected have the most behavioral problems.



In the next graph you can see the distribution of the health concerns by strain, with 1509 and 1477 being the most prone to phenotypic adverse effects of the ones represented.



By strain, I also looked into who has lots of a peculiar problem, and here we can see that strain 423 has lots of eye problems, 1477 has lots of breeding and eye problems, 1482 has breeding problems, and 1509 has lots of problems in general with many breeding and eye problems.

	34	385	423	1461	1463	1468	1473	1476	1477	1478	1480	1482	1485
Behavioral	0	0	1	6	1	3	0	5	4	0	0	0	1
Breeding	5	2	1	1	4	6	0	1	13	3	1	14	0
Dermatitis	0	0	0	0	1	1	0	0	1	0	0	0	0
Eye problems	4	0	14	0	1	6	0	6	13	1	1	1	0
General	1	2	0	2	6	3	1	1	3	0	0	2	0

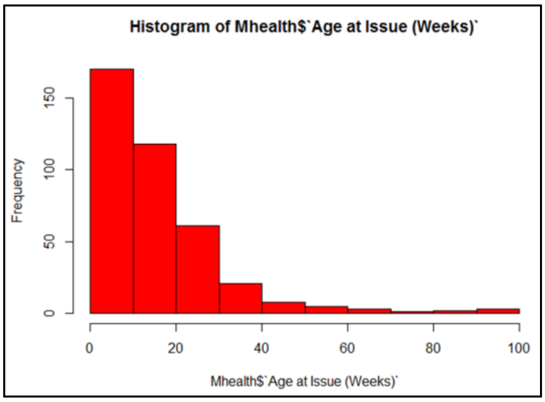
	1488	1490	1491	1492	1494	1498	1509	1512	1534	1554	1558	1559
Behavioral	6	1	1	2	0	2	7	0	1	3	1	1
Breeding	5	2	2	0	5	7	22	2	0	5	1	2
Dermatitis	1	0	0	0	2	2	2	1	0	0	0	0
Eye problems	0	1	1	0	4	3	22	0	0	1	1	3
General	1	7	3	0	3	1	17	0	2	2	0	0

	1598	1600	1614	1619	1620	1621	1627	1652	1663	1688	1690	1691
Behavioral	0	2	0	0	0	0	0	1	2	1	0	0
Breeding	1	1	1	0	1	4	2	0	2	0	3	1
Dermatitis	1	0	1	1	0	0	0	0	0	0	0	0
Eye problems	0	0	1	0	1	0	0	0	1	0	0	0
General	1	1	1	2	0	0	2	0	0	1	1	2

	1700	1704	1710	1719	1720	1721	1735	1739	1740	1754	1761	1778
Behavioral	0	1	0	0	1	6	4	2	0	0	0	0
Breeding	4	0	1	3	0	2	5	0	0	2	0	1
Dermatitis	0	0	5	1	0	0	0	0	0	0	0	0
Eye problems	1	0	1	0	0	1	1	1	0	0	1	0
General	0	1	0	0	0	0	0	0	1	1	0	0

	1780	1800	1809
Behavioral	1	0	0
Breeding	1	0	1
Dermatitis	0	0	0
Eye problems	0	2	0
General	0	0	0

Regarding the age, most health problems came up at 0 to 20 weeks of age, and in particular breeding issues and eye problems develop between 3 to 7 weeks of age.

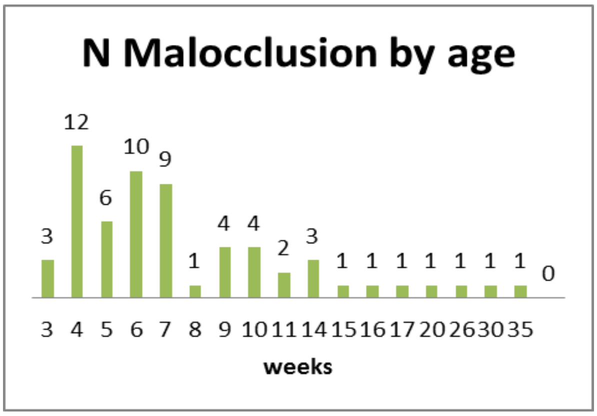


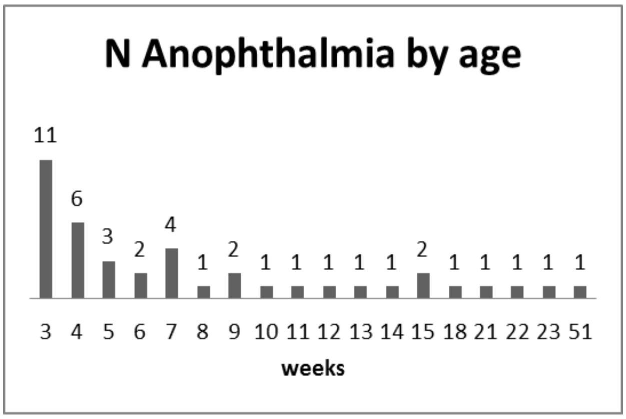
	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Behavioral	1	0	0	4	5	4	2	6	5	7	1	4	0	6	0	4	1	3	1	1	1
Breeding	10	18	19	14	11	3	4	5	4	0	2	5	1	4	1	2	3	6	2	3	2
Dermatitis	0	0	0	0	0	1	1	0	1	0	0	0	0	0	1	0	1	0	0	0	0
Eye problems	14	10	4	6	5	5	5	2	4	3	3	4	5	1	1	4	0	0	3	2	2
General	0	0	1	1	2	4	2	1	9	6	0	5	1	1	1	5	1	2	3	0	1

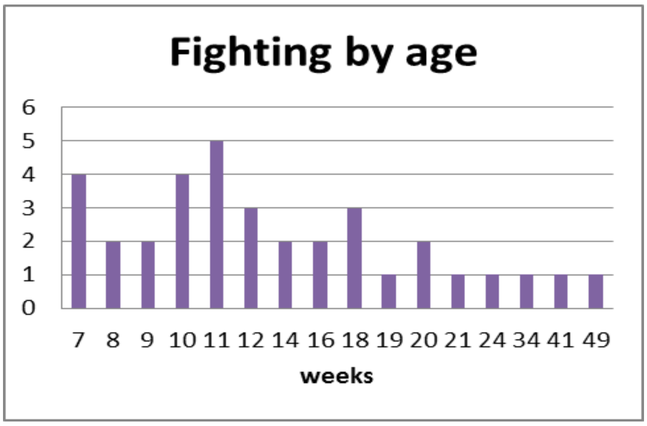
	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	41	42	44	48	49	50
Behavioral	1	0	0	1	3	1	0	0	0	0	1	0	1	0	0	1	0	1	0	1	0
Breeding	2	1	4	1	1	0	3	1	0	0	0	2	0	0	0	0	1	0	1	1	0
Dermatitis	0	1	2	0	0	1	0	1	1	1	0	0	0	2	1	0	0	0	0	0	1
Eye problems	0	0	0	0	4	1	0	0	1	1	0	1	0	0	1	0	1	0	0	0	0
General	3	1	4	2	1	1	1	2	1	0	0	0	2	1	0	0	0	0	0	0	0

	51	53	54	64	65	72	87	91	92	94
Behavioral	0	0	0	0	0	0	0	0	0	0
Breeding	1	2	0	0	0	0	0	0	0	0
Dermatitis	0	0	1	2	0	1	0	0	0	0
Eye problems	1	0	0	0	0	0	0	0	0	0
General	0	0	0	0	1	0	2	1	1	1

Also, malocclusion and anophthalmia were mostly in the early weeks of life, while aggression is more present between 7 and 20 weeks.
When anophthalmia is recorded later in life, it could easily be due to cataracts and eye problems which close up the eye; same for malocclusion, which other than because of genetics could be due to teeth overgrowth, injuries and old age.



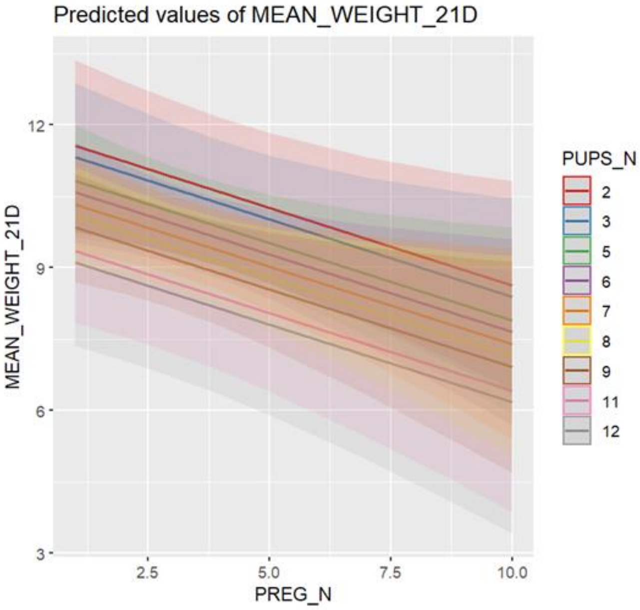


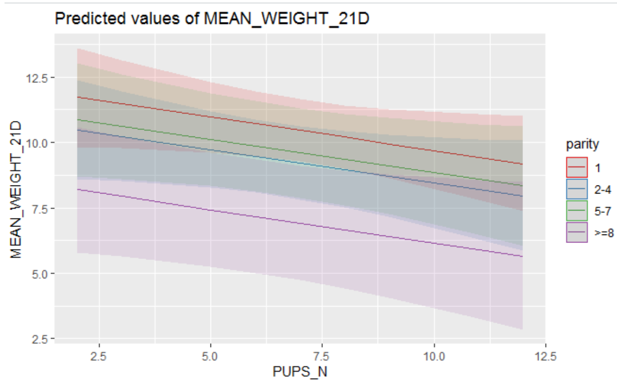


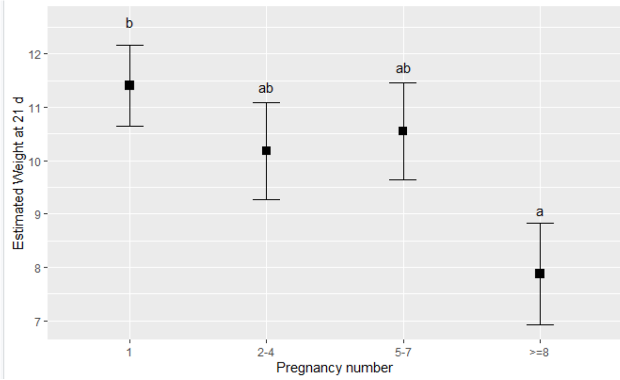
<u>SCORE SHEET</u>
In our analysis, the response variables tested are the weight, the litter size, milk spot, eyes, fur, ears, cumulative mortality and the activity level.

1. Weight

The weight was a mean of all the weights of the pups in the litter at 21 days. We run a linear model and the number of pregnancies turned out to be significant in determining the weight of the pups, decreasing with more pregnancies.

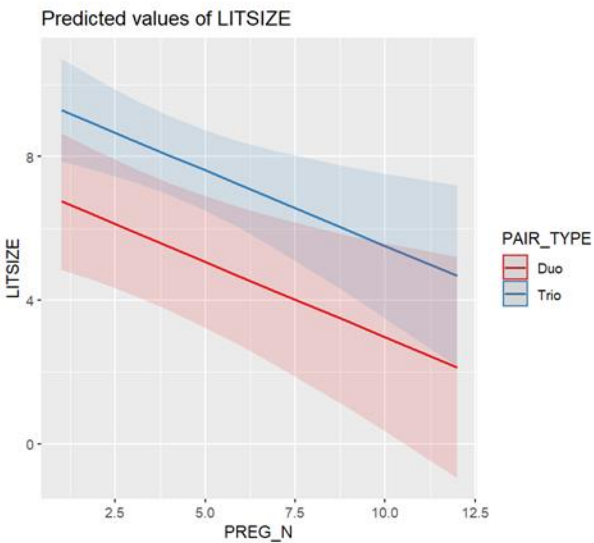


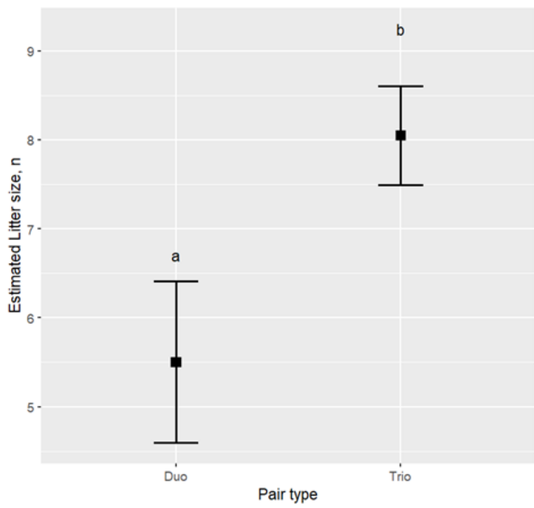




2. Litter size

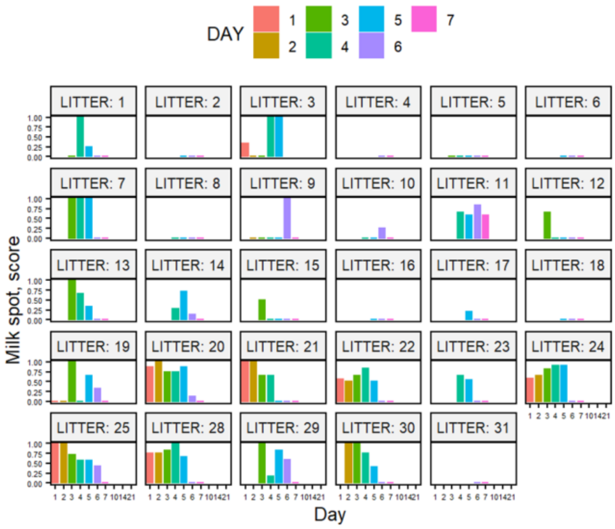
Running a linear model, we can see the number of pregnancies and the type of pair influences the litter size. Of course, the trios will have a larger litter size because if the two mothers give birth at the same time we will have no way of knowing, and assume those were the pups of just a litter.





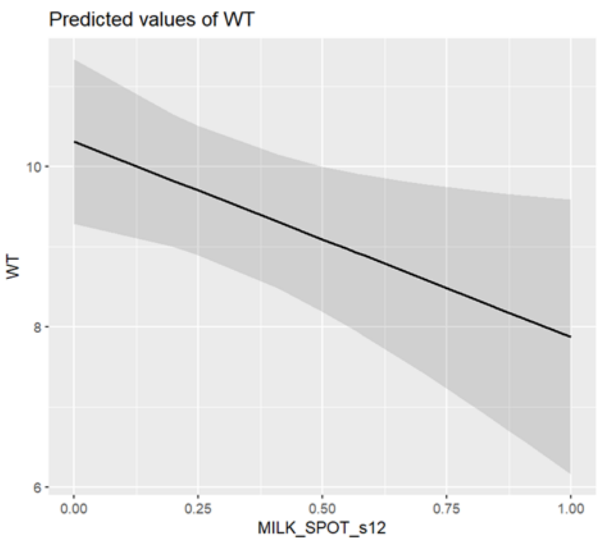
3. Milk spot

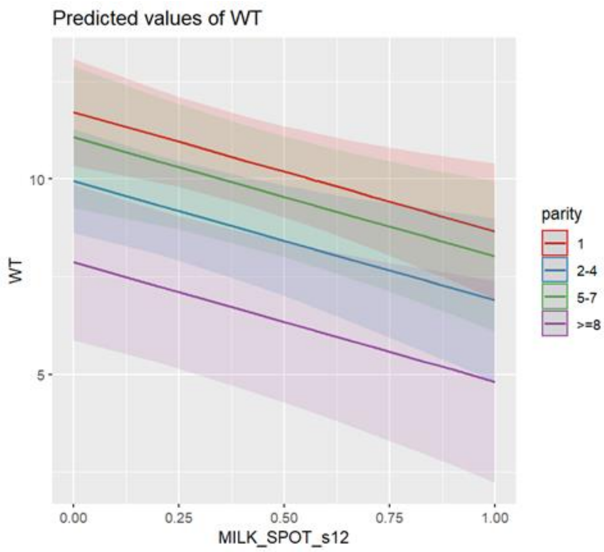
In the ordinal regression done pups number, weight, day 6 and 7, and cumulative mortality were significant. Those two days are significant because they are the days in which most of the litters lose the milk spot.



The weight response was re-analyzed adding the milk spot, since in the model run with it the weight was significant.

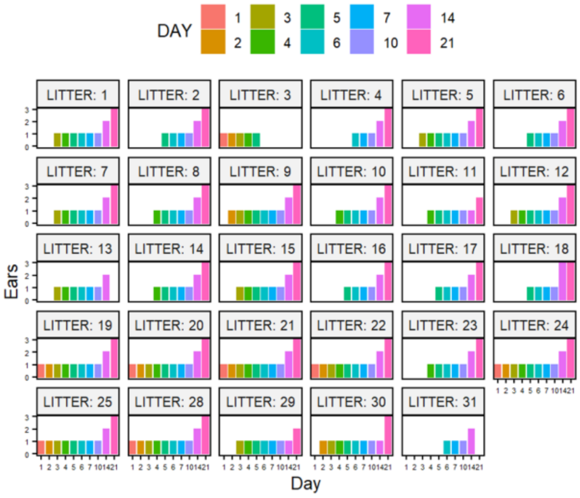
In those graphs we can see how with lower weight we have a less evident milk spot (score 1 was best, score 3 worst).

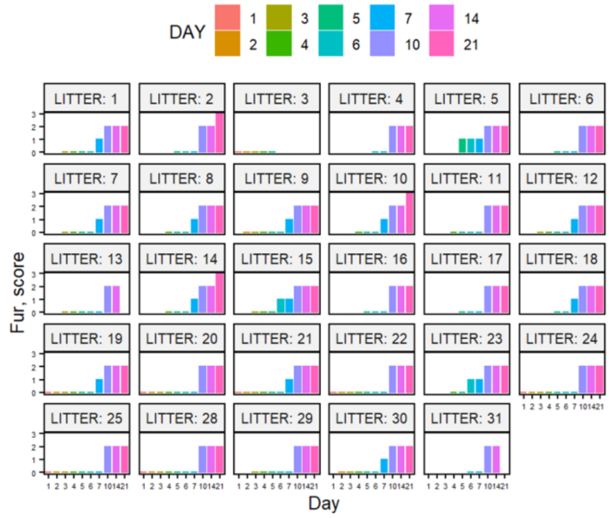


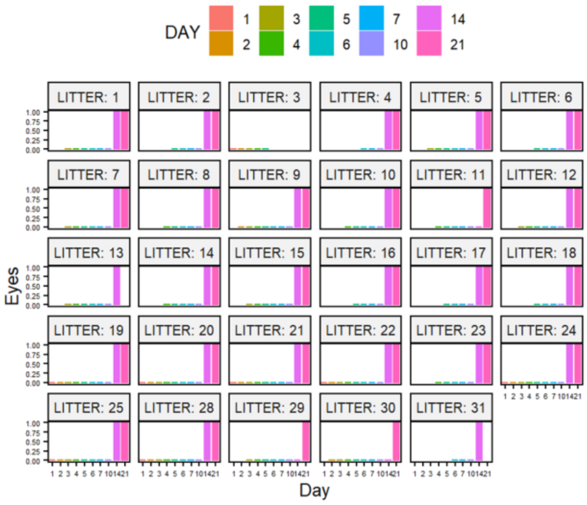


4. Eyes, fur, ears

Those variables were not possible to analyze, because of the low variability. All the pups open eyes between day 10 and 14, the fur growth and appearance is almost the same in all litters and ears detach between day 14 to 21 in all pups.

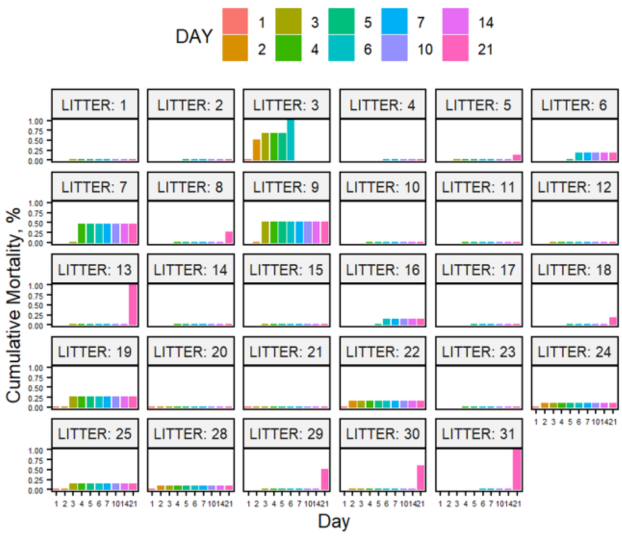






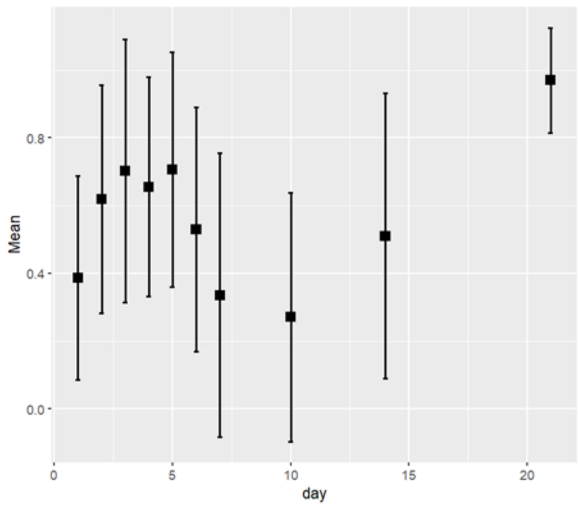
5. Cumulative mortality

Here we had again not enough variability, so the analysis was not very helpful. The mother health concerns were almost significant (P=0.09), maybe with more data we could have clearer results.



6. Activity level

I decided to try out this response variable too even though at the beginning I discarded it because the score is a bit subjective and also the measurements were taken at different times of the day, creating some noise.



Running the model the age of the mum and the weight turned out to be significant, and the number of pups was almost significant (P=0.06). Rather than believing those results, I would prefer to re-take the scores using a more precise scoring system and measuring them at the same time each day to be sure to have less noise.

Discussion

Analyzing the health concerns data given to me regarding the last 3 years, we can confirm the majority of the health problems seem to be related to the reproduction. Many cases of malocclusion and eye problems also come up early in life, between 0 and 20 days, which could be a sign of a reproductive problem or a problem passed down by genetics. Other causes are also plausible, because malocclusion could be also a result of a head trauma for example, but given the early onset we could hypothesize reproduction being the cause of it.

Many strains are very complicated to obtain and consequently more prone to some adverse phenotypic effects, so they are more keen on having health problems too; strains 1509, 1477, 423, and 1468 are all genetically engineered models used in leukemia research, which are complex to obtain and have a very specific genotype.

Keeping old breeding couples was a recurrent problem for some researchers in that facility due to the very difficult process of keeping a transgenic genetic line hard to obtain. They are resolving the problem refreshing the breeding pairs, and I think this is really important because of the results I obtained in my score sheet analysis. Weight and litter size are strongly influenced by the number of pregnancies the mother had, so keeping old pairs or pairs with lots of pregnancies does not benefit the breeding because you will obtain weaker and fewer pups. In the case of a strain which is hard to get and complicated to obtain, having health young couples could make easier the keeping of the line providing more and healthier pups.

Some parameters I wanted to test like the strain, color, mortality and activity level were not variable enough or I did not have enough observations; getting more data and refining the project could give us very interesting information on how the strain influences survival for example, and if the color is significant in determining future mortality or how evident is the milk spot.

I got some answers on it by working in the facility, because if I saw a pup out of the nest, with a dull pink skin color and a non-visible milk spot, usually the next day it was found dead.

To refine my project, it would be useful to mark the pups in some way, to have more precise information on the exact individual instead of having a mean of the whole litter.

Refining the definition of activity level and measuring it at the exact time each day also provides stronger information with less noise, to be more precise in the statistical analysis.

In conclusion, I can say that this analysis of the mice colonies and of my score sheet underlines how important is the breeding side of the laboratory mice, which can give better and more pups to use without losing many, which is a refinement and a reduction.

Healthy, young mice give birth to bigger and stronger pups, and in the right environment with good enrichment the stress levels are low and the mothers less likely to not take care of their litter.

Good health and good science go hand in hand, and we need to educate every researcher in the culture of care to have better research and happier healthier mice.

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