

## Using TM Lids to Improve Efficiency of Timed Mating in Mice

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### Abstract

Timed-mating (TM) procedures are required to achieve specific pregnancy dates in Mice that are designated for prenatal experiments. Traditional mating methods for mice yielded low rates of successful pregnancies, increasing the cost and number of animals used to achieve the specified target of pregnant female mice. The introduction of a new specialized TM Lids to replace conventional cage lids for mice aims to increase the success rate of pregnancy in mice selected for timed mating. A set of Standard Operating Procedures (SOP) tailored to the use of the new TM lids introduces the method of teasing the mice before mating, separating the male mouse from the female with a wire lid within the same cage to provide tactile and olfactory interaction. As per the Whitten effect, the limited interaction between male and female mouse triggers the oestrus cycle in female mice, improving ovulation rate at the time of mating. The implementation of the new TM lids has shown a significant increase in Plug Seen (PS) within mice when checked for proof of mating. It has also led to the increase pregnancy rates in female mice with PS on the morning after mating and fewer animals have to be used for mating to achieve the required number of pregnant females on a specific date.

### Introduction

Prenatal studies in mice more often than not require an accurate determination of the age of the fetuses within the pregnant female mouse. This means that a precise method of determining the time of copulation is essential for such studies. The traditional method of acquiring such pregnant mice involved putting the male and female mice to mate overnight within the same cage and then checking the female mouse for a vaginal plug in the morning. The plug is a clear indication that mating has occurred. However, it is not a reliable guide as to whether the female mouse would or would not be pregnant. Pregnancy can only be determined, at the earliest, 11 to 12 days after the plug-seen date. However, if by some unfortunate chance the mice were not pregnant, then the experiments would have to be delayed while the new pairs of mice were mated once again.

The introduction of the new TM lids (Figure 1) and SOP aims to improve the reliability of the timed-mating procedure, essentially increasing both the frequency of plug seen as well as pregnancy rates within the mice. This is achieved by taking advantage of the Whitten effect, increasing ovulation within the female mice before being put to be mated with their male counterparts. The newly designed cage lids come with a wire mesh barrier which compartmentalizes the male from female mouse within the same cage, while at the same time allowing of limited physical contact between the mice.

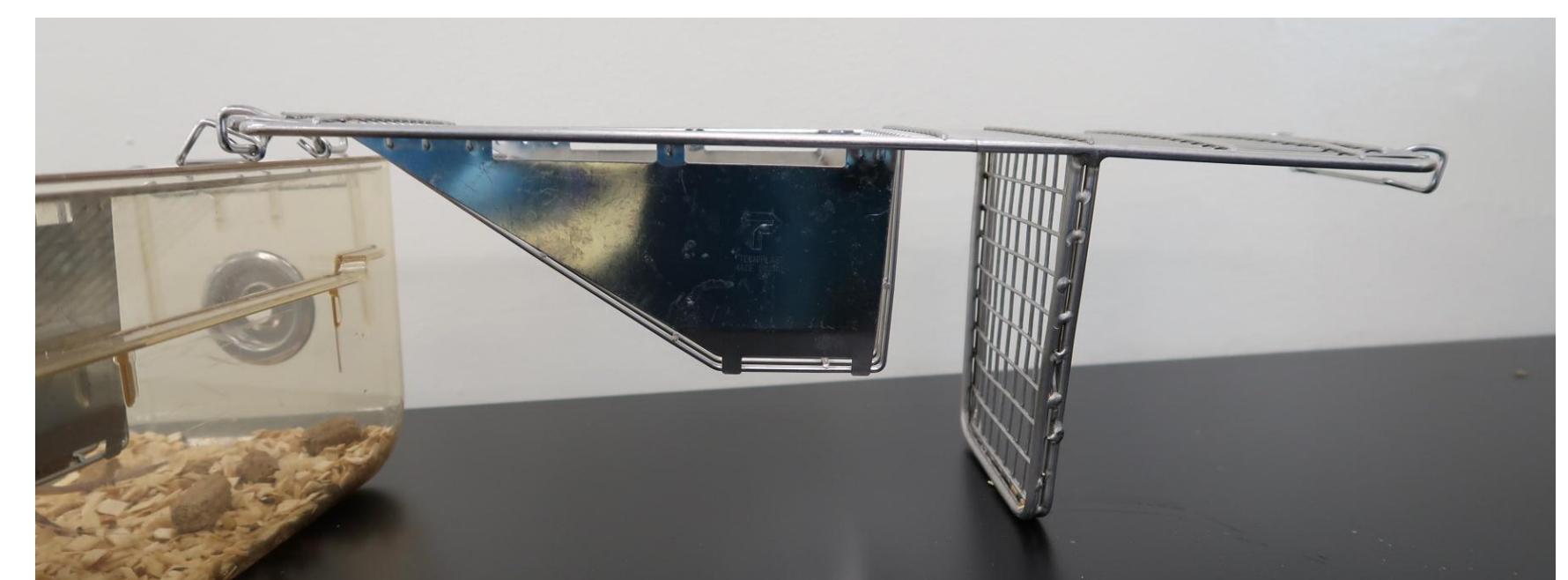


Figure 1. Timed-Mating Lid



Figure 2. Timed-Mating Setup

### Methodology

- I. Using the newly designed TM lid, add a virgin 7 to 8 week old female into the smaller compartment of a cage that is already housing a proven male. This teasing step is done in the morning and left for 2 days with each mouse separated by the wire mesh.
- II. In the late afternoon 2 days post teasing, the female mouse is introduced into the same compartment as the male mouse to allow for mating overnight.
- III. The next morning after having been placed together for mating, the female mouse is taken out and checked for a vaginal plug. The female mice are then separated according to whether there was a plug seen or not.
- IV. This new method of teasing utilising the TM lids was introduced in 2015 as compared to the traditional method of simply placing the animals within the same cage for mating in 2013 and 2014.

### Numerical Statistics

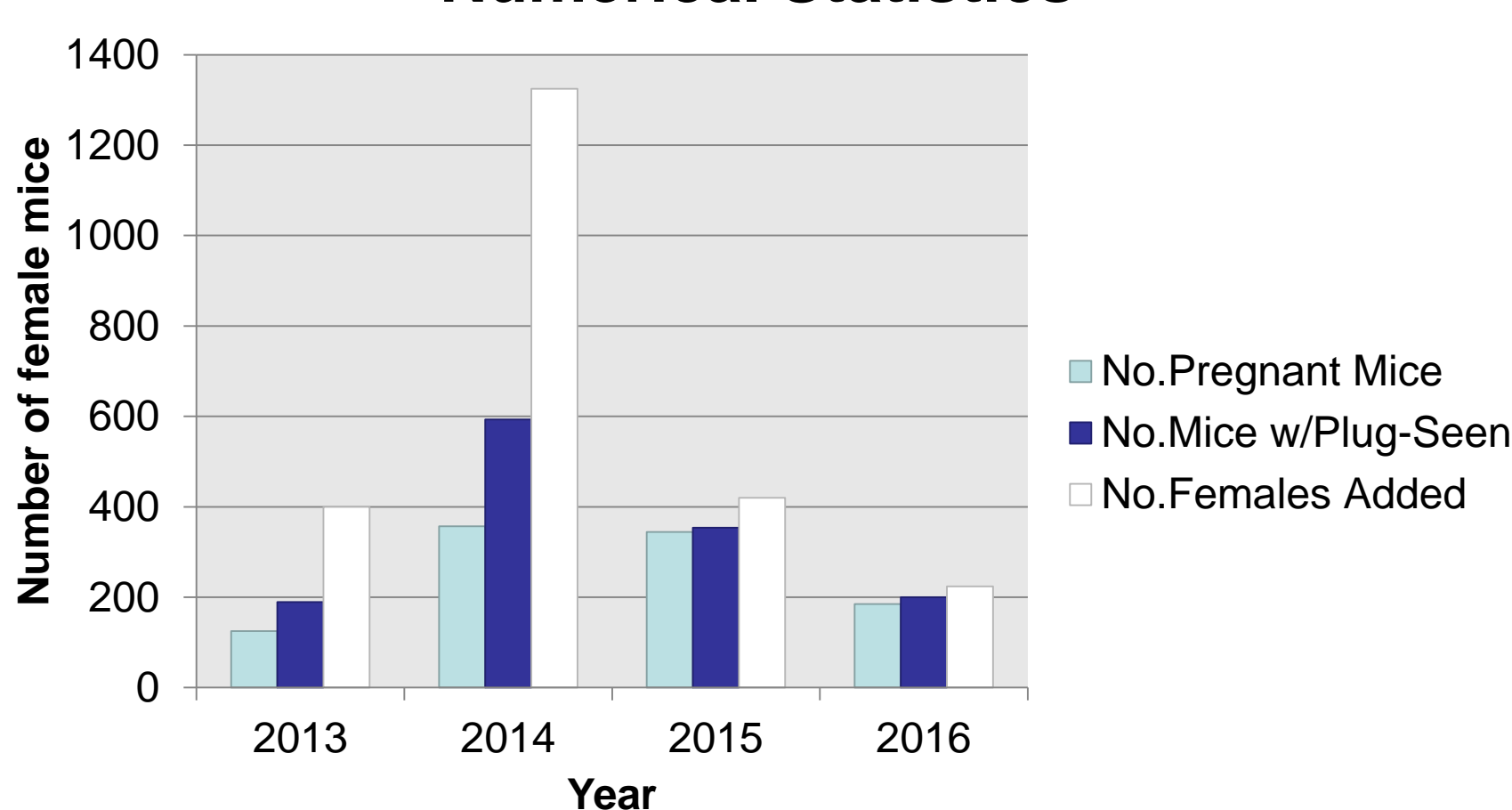


Figure 3

Year	2013	2014	2015	2016
<b>Pregnant</b>	125	357	344	185
<b>Plug-Seen</b>	189	593	354	200
<b>Females Added</b>	400	1325	420	224

Table 1

### Breeding Efficiency

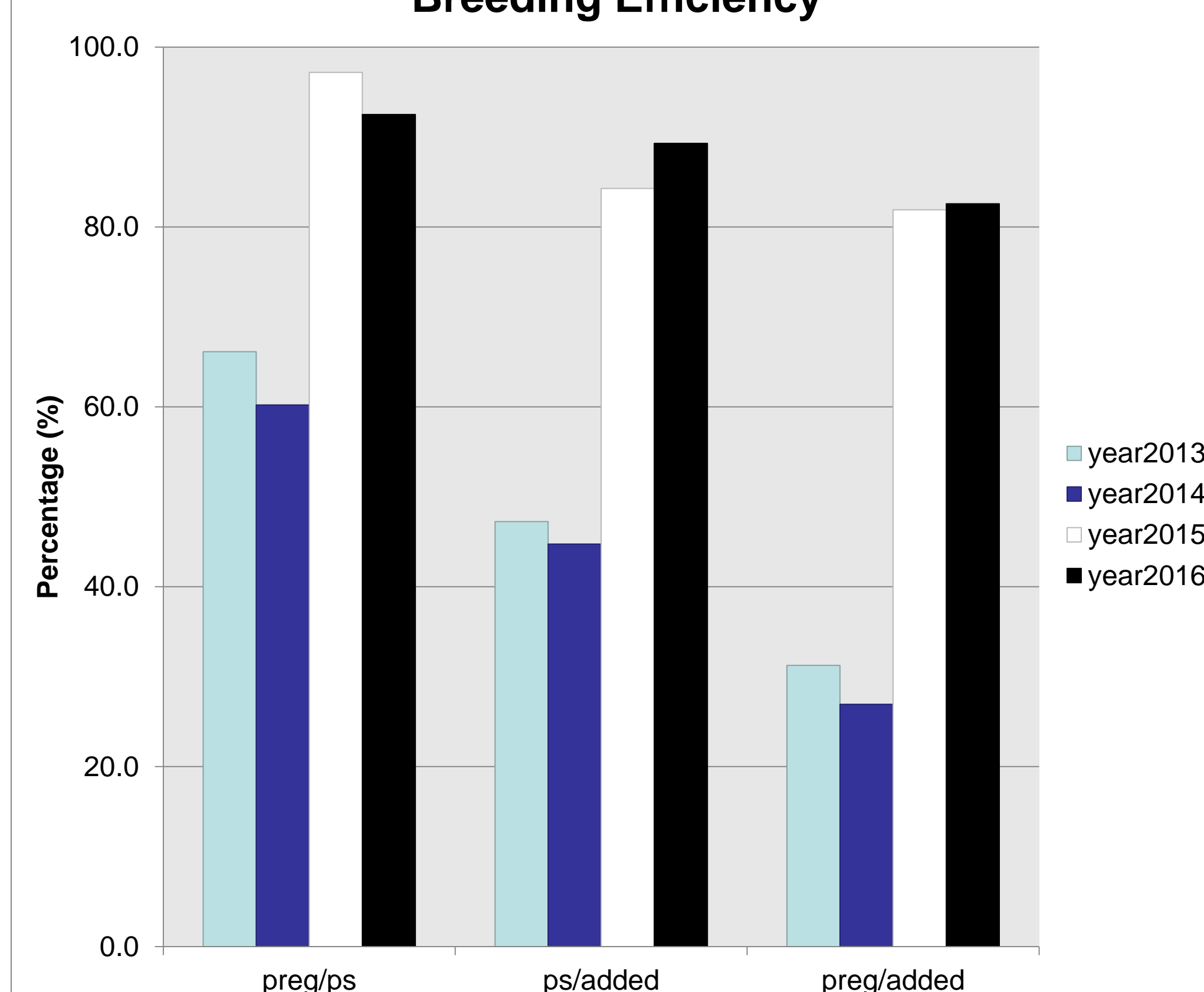


Figure 4

### Results

Figure 3 shows actual numbers of mice involved per year. It can be clearly seen that for 2013 and 2014, the number of plug seen is drastically lower than the number of female mice added for timed mating as compared to 2015 and 2016 after the new breeding method was introduced.

It is also the same for the number of mice that actually become pregnant out of those that are plugged. Figure 4 shows the various percentages as the years progresses. Notice that after the introduction of the TM lids and SOP in 2015, there is a sudden significant increase in all 3 percentage comparisons, especially in the percentage of pregnant/added which has more than doubled with the new method.

### Discussion

As seen in Figure 3 and Table 1 which shows the number of female mice used in Timed Mating over the course of 4 years, there is a significant reduction in the number of female mice added for mating to achieve the target number of pregnant mice. Just by comparing years 2014 and 2015, the number of pregnant mice (cyan bars) for each year is approximately the same. However, the number of female mice added for mating (white bar) is three times as many in 2014 as compared to in 2015. This means that fewer animals are required to achieve similar results with the introduction of the new lids and protocol.

Furthermore, when comparing the percentage of PS mice that become pregnant, it also shows an increase by at least 30%. As seen in Figure 4, there is an increase in efficiency of breeding across the board after the new TM lids and protocol were introduced in 2015. This indicates that exploiting the Whitten effect is a viable option in aiming to improve mating efficiency in mice. The open wire mesh allows for pheromones to pass through the compartments of the cage freely, hence triggering the estrus cycle within the doe. The teasing period of 2 days is sufficiently long enough for the majority of does to reset their estrus cycle, allowing for not only increased chance for mating to occur, but also for improved pregnancy rates.

### Conclusion

In conclusion, the use of the new TM lids and the new protocol has led to a significantly improved breeding efficiency in mice. This allows for a reduction in animals used to achieve the required target number of pregnant does, supporting the principle of 3Rs in animal production.

### References

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