

## Reproductive Output in Antigen Treated Male Mice

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### Abstract

Modulation of reproductive output by antigen-induced immunoenhancement is a significant component of reproductive adaptations behavior. To investigate this concept, we studied non-replicated antigens stimulation and reproductive output of male mice. We found that the activation of the immune system by non-replicated antigens increases their reproductive efficiency: antigen-treated males had higher reproductive output in comparison with control males and produced more progeny as a result.

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### Introduction

A trade-off between reproduction and survival is a characteristic of many living organisms. Competition for these two functions is widely discussed in frame of the trade-off concept in biology [1]. As postulated by this concept, the reciprocal distribution of resources between the generative and immune systems implies the suppression of reproduction during antigenic stimulation of immune system. However, there is also an alternative trade-off hypothesis of reproductive compensation [2]. According to this hypothesis, antigen recognition by the immune system, as a signal of an increased risk of mortality, triggers compensation mechanisms that are implemented through an increase in reproductive output. One of the approaches for demarcation between these alternative points of view can be a study of reproduction when introducing non-replicated antigens to one of the parents, that is, at treatments that are equivalent to vaccination.

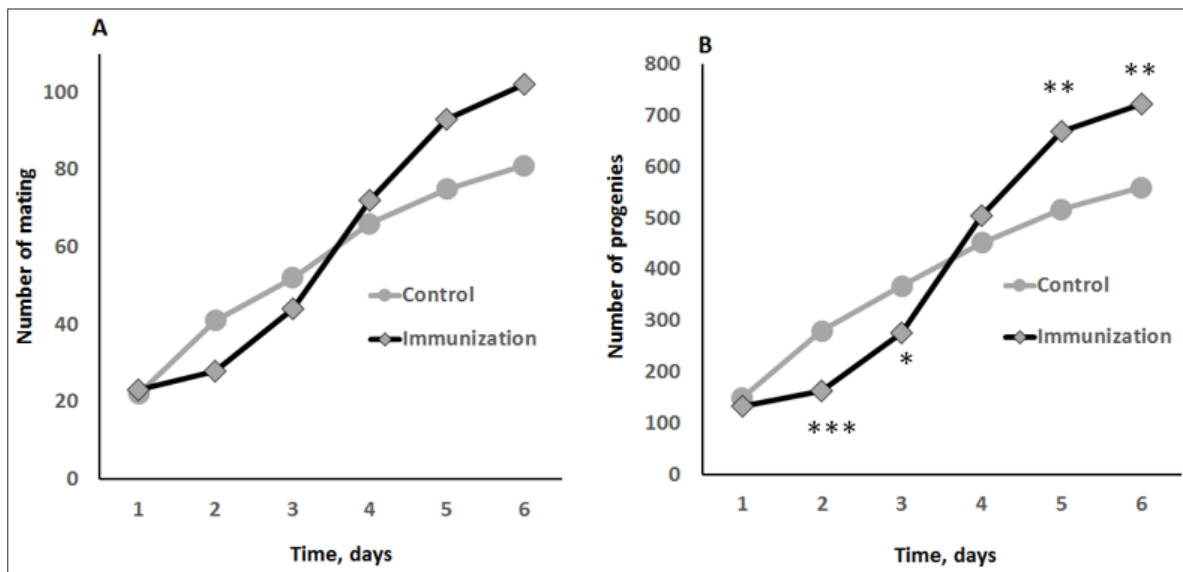
### Discussion

Since 2010 [3], we have been studying the reproductive properties of mouse males during breeding under antigen stimulation. The experiments were carried out on Specific Pathogen-Free (SPF) CD1, C57Bl/6J and BALB/c mice. In order to activate the adaptive immune system, the mice were injected with the following non-replicated antigens: Sheep Red Blood Cells (SRBC) [3] and immunogenic protein Keyhole Limpet Hemocyanin (KLH) [4-7]. The males injected with antigens or with saline (control) were housed with two virgin females in individually ventilated cages. The females were inspected daily for the presence of a vaginal plug, an indicator of fertile mating. Pregnant females were replaced to single cage. Then one group of these females was sacrificed by craniocervical dislocation on the 16<sup>th</sup> day of pregnancy, and the number of living embryos were recorded. In the second experimental group the number of newborns were recorded. The number of living embryos and the number of newborns were used as an indicator of male reproductive output in the immunized male group and the control group.

The differences between the immunized and the control males were similar in all experiments (Figure 1), which have been done on investigated strains of mice, when using antigens SRBC or KLH, and at different interval from the immunization of males to their co-housing with females (from 3 hours to 9 days). Half of the total number of fertile mating (50.6±5.6%) occurred in the control male on the first two days which significantly exceeded that in the immunized male group (27.4±4.4%, t=3.26, p<0.01) (Figure 1A). The immunized males not only caught up, but even exceeded control males in the number of females mated by them in the next 3-6 days. The consequent analysis of the cumulative number of conceived progenies demonstrates the intergroup differences more evidently. The immunized males lagged behind the control ones up to 3 days in terms of the number of conceived offspring

(Figure 1B). However, the immunized males conceived 723 offspring at the end of co-housing with females, which was almost 30% higher than in the control group ( $p < 0.01$ , Chi-square=10.2,  $p = 0.0016$ , when compared with the theoretically expected equal

reproductive output). Differences in reproductive output were achieved by summing the trends for a larger number of fertile mating (81 vs 102) and a larger litter size ( $6.9 \pm 0.30$  vs  $7.1 \pm 0.25$ ).



**Figure 1:** Cumulative curves of fertile mating (A) and number of progenies (B).

\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  statistically significant differences from equal values in control and experimental (immunization) groups-Yates corrected Chi-square.

## Conclusion

Thus, our results show that the activation of the immune system by non-replicated antigens not only does not suppress the generative system of males, but, on the contrary, increases their reproductive efficiency. The positive effect of antigenic stimulation may be used for the development of technologies for optimizing the reproduction of multiple domestic animals by combining vaccination and reproduction schedules. In addition, the study of the reproductive function of immunized males is of undoubted interest for the current debate about vaccination against COVID-19, which affects all segments of the population, including men of reproductive age.

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## Conflict of Interest

The authors declare that there is no conflict of interest.

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