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ABSENCE OF FERTILITY EFFECTS IN PARACETAMOL AND IBUPROFEN PRE-TREATED MICE

AUSÊNCIA DE EFEITOS NA FERTILIDADE DE CAMUNDONGOS PRÉ-TRATADOS COM PARACETAMOL E IBUPROFENO

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Abstract
Mice treatment with anti-inflammatory medications can be a routine procedure in mouse facilities. In some cases, these animals can be used to perform breeding pairs, and nothing is known about any deleterious pre-treatment effect on their fertility. Our goal was to evaluate anti-fertility effects of paracetamol and ibuprofen if administered before mating. Female Swiss mice were treated either with paracetamol or with ibuprofen, prior to mating. After mating, the number of pregnant females, fetuses, and spontaneous fetal reabsorptions were counted. Neither pre-mating treatments produced measurable differences in the proportions of pregnant females, number of fetuses, or fetal reabsorptions when compared to the non-treated control or vehicle-treated (PBS) groups. Thus, continuous treatments of either paracetamol or ibuprofen may not induce permanent physiological changes that interfere with the female fertility if the treatments are interrupted during the mating period.

Resumo
O tratamento de camundongos com medicamentos antiinflamatórios pode ser um procedimento de rotina em biot´érios de camundongos. Em alguns casos, esses animais podem ser usados para formar acasalamentos, e nada se sabe sobre qualquer efeito deletério prévio destes tratamentos em sua fertilidade. Nosso objetivo foi avaliar os efeitos anti-fertilidade do paracetamol e do ibuprofeno, quando administrados antes da cópula. Fêmeas de camundongos Swiss foram tratadas com paracetamol, ou com ibuprofeno, antes da cópula. Após a cópula, o número de fêmeas grávidas, fetos e reabsorções fetais espontâneas foram contados. Nenhum dos tratamentos pré-cópula produziu diferenças mensuráveis nas proporções de fêmeas grávidas, número de fetos ou reabsorções fetais, quando comparados aos grupos controle não tratados ou tratados com veículo (PBS). Assim, tratamentos contínuos com paracetamol ou ibuprofeno não induziem alterações fisiológicas permanentes que interfiram na fertilidade feminina, desde que os tratamentos sejam interrompidos durante o período de cópula.

Keywords: over-the-counter medications; pain relievers; pregnancy; implantation

Palavras-chave: medicamentos isentos de prescrição; analgésicos; gravidez; implantação

Introduction

Mouse fertility is an important issue when we consider animal facilities; under specific circumstances, it is possible that animals being treated with over-the-counter medications for minor health ailments such as pain, fever, and inflammation could be further used for mating. Among these medications, paracetamol and ibuprofen are commonly used and, therefore, previous knowledge about the effects of these medications in mouse fertility is needed.

Initial experimental studies of paracetamol demonstrated deleterious effects in mouse litters, with reduced mouse numbers and birth weights when compared with

control mice from nontreated breeding pairs (REEL, Jerry R.; LAWTON, A. Davis; LAMB, James C., 1992). In male rats, paracetamol have deleterious effects on libido, sexual stamina and performance, while in mating females, fertility was reduced (RATNASOORIYA; JAYAKODY, 2000), most probably by a decrease in gonocyte numbers, which can persist until F2 females (ARENDRUP et al., 2018; HOLM et al., 2016). It is interesting to note that male mice from treated mothers also showed a decrease in the number of germ cells, but later recovered to normal numbers at adulthood (DEAN et al., 2016).

Taking under consideration ibuprofen, a reduction in testosterone plasma levels in mice was observed (STUTZ et al., 2000), as well as abnormal sperm cell parameters, like motility, morphology, count, DNA integrity and chromatin condensation (ROODBARI; ABEDI; TALEBI, 2015). Fewer mechanisms were described to explain these results, but some authors have shown that ibuprofen could inhibit the maintenance of an acidic microenvironment on Sertoli cells, which is essential for spermatogenesis (AUZANNEAU et al., 2008). Since ibuprofen is a nonsteroidal anti-inflammatory drugs (NSAIDs), it may also interfere with spermatogenesis in other levels, inhibiting PGE_2 synthesis, which is responsible for the production of glia cell line derived neurotrophic factor (MAYERHOFER, 2019; REY-ARES et al., 2018). In females, studies with NSAIDs have shown the occurrence of luteinized unruptured follicle syndrome (MICU, M. C.; MICU, R.; OSTENSEN, 2011), and could interfere with ovary development inside the womb, during the first trimester of pregnancy (LEVERRIER-PENNA et al., 2018).

According to the results described above, it seems that paracetamol and ibuprofen would have deleterious effects on fertility when administered in animals dedicated for breeding. However, nothing is known or reported about the pre-treatment of these animals with these two drugs and their subsequent use in breeding pairs. Therefore, we decided to address this question, treating female mice with paracetamol or ibuprofen before breeding.

Material and Methods

1. Animals

Swiss female and male mice (7-week-old) were used in our experiments. All animals were kept in micro-isolated cages, with sterile water and food *ad libitum*.

Female mice were divided into 4 groups (n = 10 females/group): control (without any treatment), PBS (received buffered-saline solution), Paracetamol, and Ibuprofen (each treated with the respective medication). All experimental procedures were approved by the institutional committee for animal experimentation (protocol number 494-2018).

2. Treatments with paracetamol and ibuprofen, and pregnancy evaluation

Mice were treated either with paracetamol (10 mg/kg) or with ibuprofen (5 mg/kg), 0.5 ml/mouse, by gavage. These treatments were performed for 10 days, with two days-interval between 5 days. At day 14 after the beginning of mouse treatments, all females were mated with males, during a period of 5 days. These females were euthanized 10 days after removing the males (pregnancy at days 10 to 14). In each group we evaluated the proportion of pregnant females, the numbers of fetuses, and the amount of spontaneous fetal reabsorptions.

3. Statistical analysis

We presented the experimentally observed values in terms of mean ± standard deviation (SD). The statistical correlation between the proportions of pregnant females and the applied treatment was studied with a Pearson's χ^2 test based upon a four-categories contingency table. The numbers of fetuses and amount of fetal reabsorptions between groups were compared by ANOVA, followed by pair-wise comparison with Tukey *post hoc* test, by a GraphPad Prism software, version 7.0 for PC (GraphPad Software, San Diego California USA). A P-value less than 0.05 was considered significant.

Results and Discussion

Figure 1 shows the numbers of pregnant females (Figure 1A), fetuses (Figure 1B), and spontaneous fetus reabsorptions (Figure 1C and Figure 2), in groups of mice that were either treated or not with paracetamol and ibuprofen. As shown, there was no differences among these data of pregnant females across all groups tested (numbers of pregnant females: $\chi^2 = 2.76$, df = 3, P = 0.429; fetuses per females: ANOVA: $F_{3,19} = 1.56$, P = 0.232; numbers of fetal reabsorptions: ANOVA: $F_{3,19} = 1.543$, P = 0.236). Thus, these results show that mice pre-treated with paracetamol or ibuprofen do not show a measurable effect in the fertility of mice. In comparison with

data published by other authors, where the amount of pregnancies were reduced when animals were treated with these medications (MICU, M. C.; MICU, R.; OSTENSEN, 2011; RATNASOORIYA; JAYAKODY, 2000; REEL, J R; LAWTON, A D; LAMB, J C, 1992), our results show that paracetamol and ibuprofen probably do not appear to interfere with normal follicular development when the medication was administered to the female mice prior to the mating period. While data from other studies show deleterious effects of paracetamol on fertility of F2 females treated mice (ARENDRUP et al., 2018; HOLM et al., 2016), we could not conclude that pre-treatment with paracetamol causes any measurable effect on F2 offspring since we did not test these F2 females from our treated mice.

Taken together, our results indicate that pre-treatment with these over-the-counter medications do not interfere with fertility and any negative residual effects of these drugs are quickly dispersed after treatment interruption.

Declaration of Conflicting Interests

The author(s) declared no research, authorship, and/or publication conflicts of interest.

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Figures

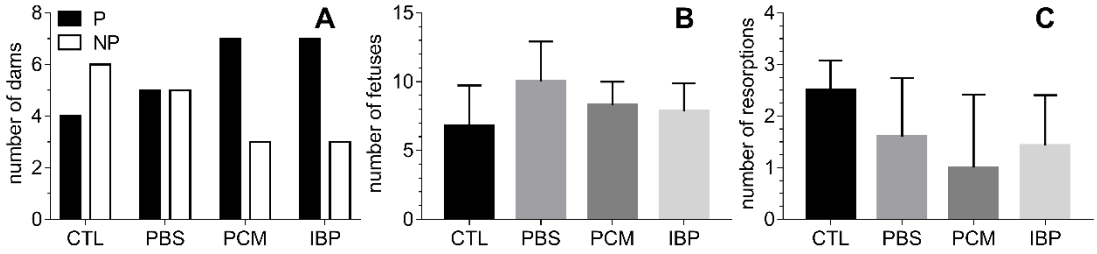

Figure 1 – Number of pregnant (P) and non-pregnant (NP) Swiss mice (A). Number of fetuses (B) and number of fetal reabsorptions (C) in pregnant Swiss mice. Results are shown as mean values with the associated standard deviation. CTL = control, PBS = PBS-treated, PCM = paracetamol-treated, IBP = ibuprofen-trated mouse groups.

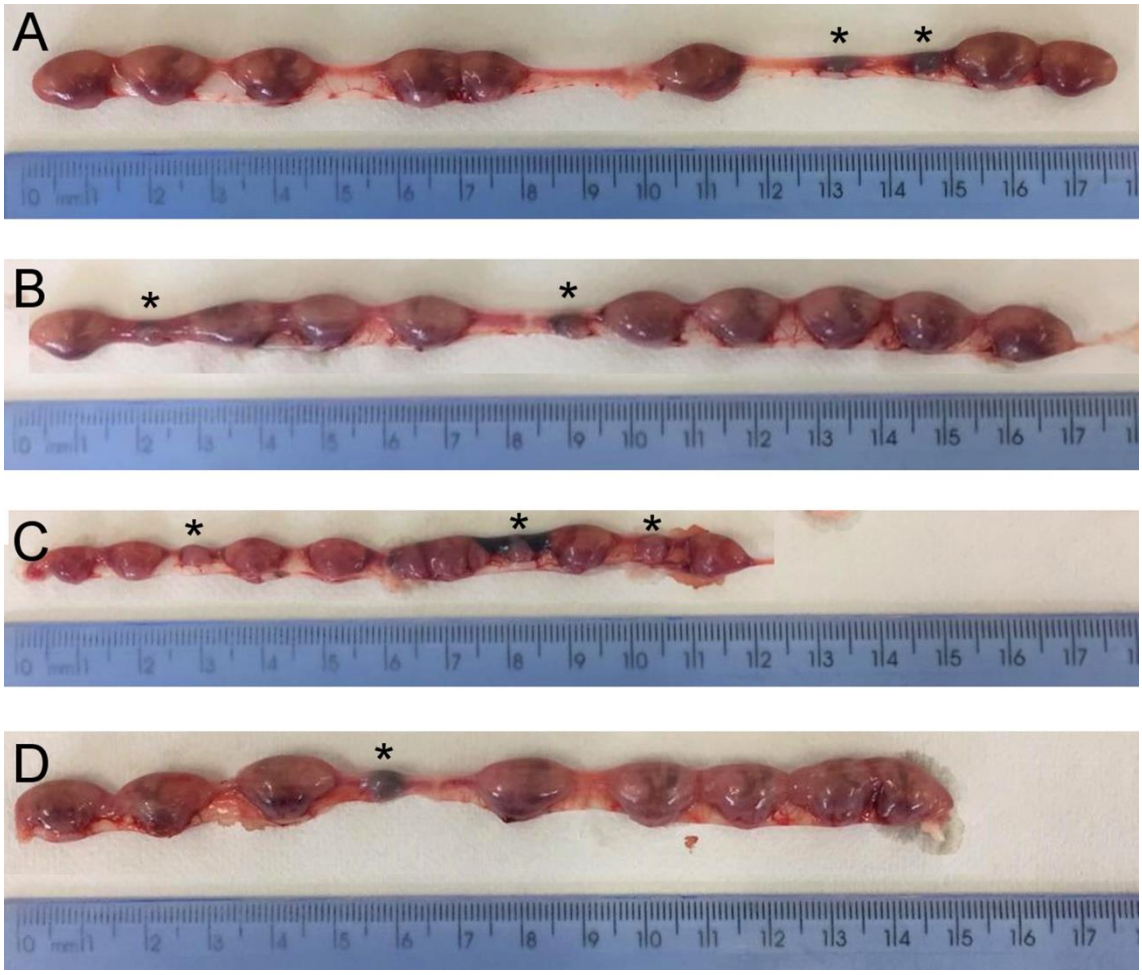

Figure 2 – Fetal reabsorptions in Swiss mice treated with paracetamol or ibuprofen. The amounts of fetal reabsorptions (*) were determined in untreated Control (A), PBS-treated (B), Paracetamol-treated (C), or Ibuprofen-treated (D) mouse groups.