Propranolol Treatment Increased Murine Follicular Activation After Ovarian Autotransplantation

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Ovarian follicles are classified as a functional unit of oogenesis, and it is well reported in the literature that ovarian transplantation is an alternative for maintaining female fertility. Furthermore, the mTOR signaling pathway has an effect on follicular activation and the drug propranolol has also been described as related to its activation. Thus, the objective of this study was to evaluate the effect of propranolol administration on follicular activation after ovarian autotransplantation in mice. 6-week-old female C57BL/6 mice were used (n=33), they were divided into six groups: control and propranolol-treated for 5 days; 14 days and 23 days. The body weight and estrous cycle of the animals were evaluated daily, and when in proestrus, they were subjected to anesthesia and bilateral ovariectomy. In the dorsal region of the forelimbs, two incisions were made for the ovarian grafts, followed by suturing and application of meloxicam for 3 days (5mg/kg). The control groups received daily *i.p.* injection according to the experimental period. of 150µl per animal, with PBS+DMSO, while the propranolol-treated received *i.p.* injection under the same conditions, of 150µl of propranolol (40mg/kg) + PBS. After treatment, the animals were euthanized, the ovaries removed and subjected to processing and staining in H&E (for quantification and percentage of each class of follicles: primordial, transitional, primary, secondary, antral and atretic; and morphometry of the diameter of the graft, ovary and capsule and subcapsular space area); immunohistochemistry for FOXO3a (D19A7 Rabbit mAb, Cell Signaling TECHNOLOGY) in the 14-day experimental groups (to evaluate follicular activation through the percentage of oocytes with FOXO3a translocated from the nucleus to the cytoplasm) and the TUNEL technique (DeadEnd^{TM} Colorimetric TUNEL System, Promega, G7132) at all experimental times (to analyze cells positive for apoptosis). For statistical analysis, GraphPad Prism 8.0.2 was used, with D'Agostino & Pearson and Shapiro-Wilk normality tests, followed by the t or Mann-Whitney test between groups, considering $p<0.05$. Body weight did not show significant differences between the groups. However, ovarian weight was lower in the 14-

day propranolol group compared to the control group (p=0.0077), and also lower in the 23-day control group compared to propranolol (p=0.0289). Regarding histological evaluation, at 5 days, there was a significant increase (p=0.0480) in the atretic class of the propranolol treated animals vs control. Regarding the immunohistochemical analysis for FOXO3a, no significant differences were observed between the groups. Capsular morphometry showed, at 5 days, a significant decrease in ovarian diameter between the control group (p=0.0289) and propranolol, and during this treatment period the majority of animals did not yet have a capsule around the ovary. At 14 days there were no significant differences and at 23 days there was a decrease in capsule diameter (p=0.0197) between the groups. The TUNEL method qualitatively demonstrated the presence of atresia in all groups, especially at 5 days, demonstrating that the initial days post-transplant lead to greater follicular apoptosis. Murine ovarian autotransplantation, added to the administration of propranolol daily at different experimental times, possibly helped with ovarian follicular activation, demonstrating the efficiency of the protocol and presenting itself as a possible viable alternative for preserving fertility.

Keywords: Ovary; transplant, propranolol, follicular activation; Fertility.

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