Summary

Evaluation of the effect of platelet-rich plasma on autologous ovarian transplantation in a fertility preservation procedure in a rat model. The study evaluates the effect of platelet-rich plasma on autologous ovarian transplantation in a rat model. The method of autologous transplantation of tissue harvested and frozen prior to anticancer therapy is an alternative to oocyte vitrification, serving not only as a fertility preservation technique but also as an opportunity to restore physiological hormonal balance and prevent premature menopause. The efficiency of this method is still relatively low, as the tissue transplanted into the body depends on the period of ischemia and hypoxia before the *de novo* formation of the blood vessels network. The network connects the tissue to the body resulting in a decrease in tissue viability, as well as a loss of primary follicle reserve. The study aimed to evaluate the physiological function of rat ovary after autologous transplantation and the usefulness of platelet-rich plasma as a catalyst for neovascularization in the process of graft acceptance, particularly in terms of degeneration of the follicle reserve. Eighteen female rats of the WAG strain were used in the study. The females were ovariectomized, and the ovaries were vitrified with the use of dimethyl sulfoxide (DMSO), ethylene glycol and sucrose. After 30 days of recovery, the rats underwent autologous transplantation. After thawing, the right ovaries were incubated for 15 minutes in Dulbecco-type saline (DPBS) with platelet-rich plasma (PRP), and the left ovaries were incubated in DPBS as a control. The ovaries were transplanted to the most vascularized edge of the uterine broad ligament in a right ovary+PRP, left ovary-control model. Approximately 250μl of PRP was also injected on the right side of the graft. Animals were sacrificed after 2 (D2), 7 (D7) and 30 (D30) days, and tissues were collected. Vesicle count and histopathological analysis were performed using Masson's trichrome staining. Assessment of apoptosis (immunofluorescent TUNEL staining) and tissue vascularization (immunohistochemical PECAM-1 staining) was performed. Relative mRNA expression analysis was performed for: ESRα, ESR β, AMH, AMHR2, PGR and TNFα using TaqMan probes by rtPCR technique. A higher number of primordial (D2, p=0.003; D7, p=0.0047), primary (D2, p=0.006; D7, p=0.004), secondary (D2, p=0.0016; D7, p=0.0002) and antral (D2, p=0.0048; D7, p=0.0031) vesicles in the study group relative to the control group was found. Apoptosis rates were found to be lower in the PRP-treated group than in the control group, in both short-term groups (D2, D7, $p<0.0001$). The results indicated the presence of higher number and density of vessels in the PRP group than in the control group at seven days after transplantation (D7, p=0.03), and there were statistically significant correlations between the number of vesicles and vessel density. There was a difference in mRNA expression of PGR (p=0.0098), ESRβ (p=0.038) and ESRα (p=0.0046) between the study and control groups in D7 group. The AMH expression was higher in the +PRP model at both time groups (D2, p=0.001; D7, p=0.02). The results can indicate that platelet-rich plasma administered immediately before and during the autologous ovarian transplantation procedure has a positive effect on maintaining tissue structure and function. The long-term impact of the method (D30 group) requires further study, as the group showed heterogeneous results. The data obtained allow a better understanding of the processes occurring in autologous transplanted ovarian tissue under the influence of platelet-rich plasma and provide a basis for further research into the procedures of fertility preservation.

Keywords: ovary, fertility preservation, rat model, oncofertility, vitrification