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The Effect of Rutin on Progesterone and Estrogen Receptor Expression in Uterine Endometrial Tissue in the Heterotopic Transplantation of Newborn Mouse Ovary

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Abstract

Background: Rutin (quercetin-3-rhamnosyl-glucoside), a flavonoid, is derived from plants and has antioxidant properties.
Objectives: This study aimed to evaluate the effect of different concentrations of rutin on mouse ovary heterotopic allotransplantation.
Methods: The present animal experimental study was conducted on 40 female adult Balb/c mice weighing 30 ± 5 g at the Jundishapur University of Medical Sciences, Ahvaz, Iran, during 2016 - 2018. The mice were divided by permuted block randomization into 8 groups (n = 5): OVX (ovariectomy), as the negative control; normal (positive control); OVX + OVA (ovariectomy and transplantation) (control), treated with 0.5 mL of normal saline; OVX + OVA + 10 mg/kg of rutin; OVX + OVA + 30 mg/kg of rutin; OVX + OVA + 60 mg/kg of rutin; OVX + OVA + 100 mg/kg of rutin; and the autograft. Groups were treated daily. Fourteen days after transplantation, ovarian grafts were collected and processed histologically for follicle number counting. Serum estrogen (E_2) and progesterone (P_4) levels were evaluated. Furthermore, the expression of Estrogen Receptor alpha (ERα), Estrogen Receptor beta (ERβ), and Progesterone Receptor (PR) in the uterine endometrial tissue was tested using qRT-PCR and western blotting.
Results: A decrease in the number of mature follicles and increase in the number of atretic follicles (mean ± SD: OVX + OVA + 30 = 19.00 ± 1.000, OVX + OVA + 60 = 25.00 ± 5.000, and OVX + OVA + 100 = 23.00 ± 2.646) were observed in all groups treated with rutin in comparison with the control group (mean ± SD: 12.33 ± 2.517) (P value < 0.05). The level of E_2 and P_4 (mean ± SD: OVX + OVA + 100 = 6.133 ± 1.026) increased in comparison with the OVX + OVA group (mean ± SD: 0.4667 ± 0.2517) (P value < 0.05). The protein expression of ERα (mean ± SD: OVX + OVA + 10 = 1.615 ± 0.1701 and OVX + OVA + 30 = 1.744 ± 0.1779) in comparison with the control group (mean ± SD: 0.7089 ± 0.1131), and ERβ (mean ± SD: OVX + OVA + 10 = 0.7747 ± 0.4365, OVX + OVA + 30 = 0.9220 ± 0.1245, OVX + OVA + 60 = 0.7701 ± 0.2150, and OVX + OVA + 100 = 0.6676 ± 0.1547) increased in a dose-dependent manner in all groups treated with rutin in comparison with the OVX + OVA group (mean ± SD: 0.1534 ± 0.06109) (P value < 0.05). No significant changes in PR were found in groups treated with rutin in comparison with the control group.
Conclusions: The results of the present study indicated that rutin increases E_2 and P_4 levels in ovarian hetero allograft mice. Rutin also upregulated the expression of ERα and ERβ but had no significant effect on PR.

Keywords: Allografts, Estrogen Receptor Alpha, Estrogen Receptor Beta, Follicle, Mice, Ovary, Polymerase Chain Reaction, Progesterone, Rutin, Transplantation, Up-Regulation, Western Blot

1. Background

The risk of sterility is very serious in young women with cancer. Upon the progress in treatments, there are now several options available for fertility preservation, and thus the use of transplantation in reproductive medicine has been considered. Despite being an attractive and helpful method in reproductive medicine in the early days, there are some problems with ovarian transplantation (1). The most important challenge in ovarian tissue transplantation is the survival rate and satisfaction of the functional longevity of the transplanted ovary (2).

During the engraftment and neovascularization of the

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transplanted ovarian tissue, an initial ischemia usually occurs, followed by a reperfusion period, which leads to the production of reactive oxygen species (ROS) with oxidative properties (3, 4). Superoxide and hydroxyl radicals are involved in tissue injury through the initiation of lipid peroxidation and damage the cell membrane in the transplanted tissue (5). ROS products are also directly responsible for the oxidative damage of cellular structures, such as DNA, RNA, proteins, and lipid ingredients in ischemic tissues (6). The mentioned events can lead to follicular loss and engraftment insufficiency in the transplanted ovarian tissue (7-9). Due to the critical effect of oxidative stress in transplantation, as described above, antioxidants can be applied to reduce the production of free radicals. Such drugs with antioxidant properties are able to counter the consequences of ischemia and reperfusion injury after ovarian tissue transplantation.

Rutin (quercetin-3-rhamnosyl-glucoside) is a flavonol, abundantly found in apples, tea, onions, and other plants (10, 11). It has multiple pharmacological activities, including antibacterial (12), antitumor, antiulcer (13), myocardial-protecting (14), vasoprotective, immunomodulatory, antioxidant, cytoprotective, and neuroprotective activities (15). Moreover, in the reproductive system, rutin has shown a possible protection of the testicular tissue and reproduction from oxidative stress in diabetes mellitus (16) and has led to the amelioration of cisplatin-induced reproductive toxicity (17). Considerable interest has recently been directed to the role and usage of natural antioxidants as a means of preventing oxidative damage in different conditions with a high oxidative stress (13).

Steroids play a prominent role in the acceptance of transplanted tissues. Extensive evidence suggests that estrogen directly modulates angiogenesis via effects on endothelial cells (18). Estrogen, 17β-estradiol (E_2), plays an important role in regulating the proliferation and maturation of ovarian follicles. It has a critical function in the female reproductive system (19, 20). Its effects are mediated through two types of receptor: Estrogen Receptor alpha (ERα) and Estrogen Receptor beta (ERβ). These nuclear receptors mediate the biological effects of estrogens and anti-estrogens and act mainly in the regulation of the Estrogen Receptor (ER) target-gene expression (21). Progesterone (P_4) is an endogenous steroid involved in the menstrual cycle and pregnancy (22). The physiological effects of P_4 are exerted by the progesterone receptor (PR), a member of the nuclear receptor superfamily of transcription factors (23).

2. Objectives

The above-mentioned facts form the basis for studying whether the antioxidant mechanisms involved in rutin-mediated protection from ischemic damage after ovarian transplantation can improve the conditions of fertility. Consequently, the present animal experimental study aimed to investigate the effects of different concentrations of rutin on the return of fertility after ovarian heterotopic allograft and the expression of ERα, ERβ, and PR in Balb/c mice.

3. Methods

3.1. Animals

The present animal experimental study was conducted on 40 adult female Balb/c mice, 5 - 6 weeks of age, weighing approximately 25 - 35 g. Animals were obtained from the Animal House of Jundishapur University of Medical Sciences, Ahvaz, Iran, and maintained at standard conditions (temperature 22 - 24°C and humidity 55% - 65% in a 12-hour light-dark cycle) during 2016 - 2018 in Jundishapur University of Medical Sciences, Ahvaz, Iran. Animals had free access to sufficient amounts of water and food during the study. This study was performed in accordance with the guidelines for Animal Research of the National Institutes of Health (NIH, Bethesda, MD, USA). The ethical code was approved by the Ethics Committee of Jundishapur University of Medical Sciences, Ahvaz, Iran, on 16 May, 2017 (Code: IR.AJUMS.REC.1396.40). For mating, a male and a female animal were housed in plastic cages. Ten days after birth, the newborn female mice were selected for the donation of the ovary to adult female mice. According to a similar study by Domitrovic et al. (24) in 2012, the mean relative weight of the liver in the control group was 5.0 $\pm$ 7.4 and, in the intervention group with CCl4, it was 4.0 $\pm$ 7.6. By considering the confidence level of 95% and a test power of 80%, five mice for each study group were estimated using the following formula.

$$n = \frac{\left(s_1^2 + s_2^2\right)\left(z_{1-\frac{\alpha}{2}} + z_{1-\beta}\right)^2}{\left(\bar{x}_1 - \bar{x}_2\right)^2}$$

The animals were divided into eight groups by permuted block randomization. The eight groups consisted of OVX (ovariectomy/negative control), undergoing ovariectomy; the normal group (positive control), normal and untreated mice; OVX + OVA (ovariectomy and transplantation) (control), undergoing ovariectomy and transplantation, treated with 0.5 mL of normal saline; OVX + OVA + Rut 10, OVX + OVA + Rut 30, OVX + OVA + Rut 60, and OVX + OVA + Rut 100, undergoing ovariectomy and transplantation, treated with 10, 30, 60, and 100 mg/kg of rutin, respectively; and autograft, without any treatment. All groups were treated once a day for two weeks with the intraperitoneal injection of normal saline for the control group

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and rutin for other groups. Rutin was purchased from the Sigma Aldrich Company (St. Louis, MO) and dissolved in DMSO. All mice were anesthetized using ketamine (60 mg/kg) and xylazine (6 mg/kg) and then ovariectomized in sterile conditions. One week after ovariectomy, two ovaries were transplanted under the muscles behind the neck.

3.2. Sample Collection

After transplantation, to ensure that the estrus phase is resumed in the OVX + OVA mice, the vaginal smear was prepared every day, 3 - 4 days after transplantation. For serum collection, 24h after the last dose of rutin, 1.5 mL of blood was collected from the heart. The blood sample was centrifuged at 3000 g for 5 min, and the serum was separated. Then, all samples were kept at -20 °C until use for the hormonal assay.

3.3. Hormonal Assay

Serum E_2 and P_4 levels were analyzed by the ELISA method in duplicate using a progesterone kit (Crystal Chem, USA) with the sensitivity of 0.04 ng/mL and assay range of 0.4 - 100 ng/mL; and an estrogen kit (Cusabio, USA) with the sensitivity of 40 pg/mL and assay range of 40 - 1500 pg/mL, according to the manufacturer's instructions.

3.4. Histology

Two weeks after transplantation, all grafted ovaries were collected and fixed in 10% formalin and then the tissues were embedded in paraffin, serially sectioned at 5 μm, and stained with the Haematoxylin and Eosin. Ovaries of age-matched 32-day-old mice were histologically processed as described above as the in-vivo development of normal control. Microphotography slides were obtained using a light microscope (Olympus CH-BI45-2) with the magnification of 20X and 40X and assessed by three experts. Primordial, primary, preantral, antral and atretic follicles were counted.

3.5. qRT-PCR

Total RNA was extracted from endometrial tissue using RiboEx (GeneAll Biotechnology Co, USA) according to the protocol of the manufacturer. Extracted RNA was kept at -80°C. For cDNA synthesis, 500 ng of total RNA was added to the cDNA synthesis tube (Thermo Scientific, USA). The program of cDNA synthesis was: 30°C for 5 min, 42°C for 1 h, 70°C for 5 min, heated at 95°C for 5 min, and stored at -20°C.

qRT-PCR primers were designed using AlleleID 6.0 software and ordered to the CinnaGen Company (Tehran, Iran) for commercial synthesis. The primer sequences of the gene of interest are fully described in Table 1. qRT-PCR reactions were performed in the ABI StepOne instrument (Applied Biosystems, USA) under the following conditions:

Table 1. List of the Primer Sequences of Interest Gene

Gene		Primers
GAPDH		
	FW	5'-AGCAAGGACACTGAGCAAGAG-3'
	RE	5'-GGATGGAAATTGTGAGGGAGATG-3'
ERα		
	FW	5'-TAGCGGCAACAGTGAAATCC-3'
	RE	5'-TGGCAAGGTAAGCAATGGC-3'
ERβ		
	FW	5'-ATGGACTGTAGAACGGTGTGG-3'
	RE	5'-GTGAGGTAGGAATGCGAAACG-3'
PR		
	FW	5'-GATTCAGAAGCCAGCCAGAG-3'
	RE	5'-CACAGGTAAGCACGCCATAG-3'

95°C for 15 s and 60°C for 1 min for up to 40 cycles. The data are presented as the relative quantity of normalized-target RNA using the $\Delta\Delta$CT method (25) (internal control gene GAPDH). Each sample was examined in duplicate.

3.6. Western Blotting

The expression of ERα, ERβ, and PR was assessed by the Western blot technique. It was prepared using the lysis buffer containing the RIPA buffer and a protease inhibitor cocktail. Total protein was measured by the Bradford method. Equal amounts of protein were resolved by 12% SDS-polyacrylamide gel electrophoresis using the Bio-Rad system (Mini-PROTEAN Tetra System, USA), and then transferred on the PVDF membrane (Immobilon-p transfer membranes) using the wet transfer method. The membrane was blocked overnight with casein 1% in Tris Buffered Saline (TBS) with Tween-20. The membrane was probed with primary antibody (Abcam, USA) and then with horseradish peroxidase-conjugated secondary antibody (Abcam, USA). Finally, ECL plus was used for the final detection of protein bands, and all data were analyzed using the ImageJ software.

3.7. Statistical Analysis

The data were analyzed using IBM SPSS Statistic Software for Windows, version 22.0 (IBM Corp., Armonk, N.Y., USA). All devices and equipment were calibrated to obtain unbiased data during the study. In this study, descriptive statistics, including mean, standard deviation, and error bar were used to plot the mean and standard deviation of each group. Descriptive data are presented as a mean ± standard deviation. The normality of variables was examined using the Shapiro-Wilk test (P value > 0.05). Since the data showed a normal distribution, one-way ANOVA with

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a post-hoc test was used. In this study, to determine the agreement between the two observers, the kappa coefficient was calculated which was 0.80, indicating that the agreement rate is reasonable. The P value < 0.05 was considered statistically significant.

4. Results

4.1. Level of E2 and P4

As illustrated in Figure 1, the serum level of E_2 and P_4 dramatically decreased in the OVX group in comparison with the control group. In OVX, OVX + OVA , OVX + OVA Rut 10, OVX + OVA Rut 30, OVX + OVA Rut 60, OVX + OVA Rut 100, autograft and normal groups, the level of E_2 was, 0, 25, 32.6, 31, 21.2, 23.3, 28, 26.3 (pg/mL) respectively and the level of P_4 was 0.4, 0.4, 2.9, 2.93, 1.26, 6.13,6.5, 8 (ng/mL), respectively. The level of E_2 increased in all OVA + rutin groups and the autograft group in comparison with the OVX group (P value < 0.01). Intra-group analysis of the relation between various concentrations of rutin showed the most increase in 0, 10, 30, 100, and the autograft groups. As expected, the level of P_4 was increased in all OVA + rutin groups, in addition to the autograft group, compared to the OVX + OVA group. Intra-group analysis indicated the less effect of OVX + rutin 60 compared to OVX + rutin 0, 10, 30, 100, and also the autograft group (P value < 0.0001) (Table 2).

Table 2. Changes in Sex Hormones in Groups Receiving Rutin[a]

Groups	Estrogen, pg/mL	Progestron, ng/mL
OVX	0.0 ± 0.0[b]	0.4667 ± 0.1155
Normal	26.33 ± 4.726	8.000 ± 1.600[b]
Treat 0	25.00 ± 10.15	0.4667 ± 0.2517
Treat 10	32.67 ± 4.163	2.900 ± 0.5196
Treat 30	31.00 ± 2.000	2.933 ± 0.6429
Treat 60	21.23 ± 14.03	1.267 ± 0.6658
Treat 100	23.33 ± 0.5774	6.133 ± 1.026[b]
Autograft	28.00 ± 6.245	6.500 ± 2.166[b]

[a]Values are expressed as mean ± SD.
[b]Significant difference compared to treat 0 (P < 0.05).

4.2. Follicle Development

The presence of numerous cysts in all groups under treatment with rutin was observed. The number of these cysts was decreased with a decrease in the dose of rutin. The number of primordial follicles in treated groups was in the following order: treat 60 > treat 0 > treat 30 > treat 10 > treat 100. Moreover, the number of primary follicles in treated groups was in the following order: treat 60 > treat 10 > treat 30 > treat 100 > treat 0. Also, the number of pre-antral follicles in the treated groups was in the following order: treat 10 > treat 60 > treat 30 > treat 0 > treat 100 (P value < 0.0001). In addition, the number of antral follicles in the treated groups was in the following order: treat 10 > treat 30 > treat 60 > treat 0 > treat 100 (P value < 0.0001). Finally, the number of atretic follicles in the treated groups was in the following order: treat 60 > treat 100 > treat 30 > treat 10 = treat 0 (P value < 0.001) (Figure 2 and Table 3).

4.3. PR, ERα, and ERβ mRNA Expression

Results indicated that, as expected, the PR, ERα, and ERβ mRNA expression in the OVX group was almost zero. OVX + rutin 10 and 30 groups had the highest levels of ERs mRNA (P value < 0.001). The mean mRNA expression level of ERα was 0.3513 ± 0.5687, 1.000 ± 0.0, 5.430 ± 1.000, 5.218 ± 1.000, 2.250 ± 1.000, 2.143 ± 1.100, 5.429 ± 1.000 and 5.760 ± 1.000 (Figure 3A); The mean mRNA expression level of ERβ was 0.3344 ± 0.1000, 1.000 ± 0.0, 3.608 ± 0.5774, 4.497 ± 1.000, 2.877 ± 1.528, 3.442 ± 1.000, 0.4454 ± 0.5774 and 2.998 ± 1.000 (Figure 3B) and The mean mRNA expression level of PR was 0.6776 ± 0.5774, 1.000 ± 0.0, 1.073 ± 1.000, 1.101 ± 1.000, 1.733 ± 1.000, 1.018 ± 1.000, 1.038 ± 1.000 and 3.528 ± 1.000 in OVX, OVX + OVA, OVX + OVA Rut 10, OVX + OVA Rut 30, OVX + OVA Rut 60, OVX + OVA Rut 100, autograft and normal, respectively. In all the treated groups, an increase in PR mRNA versus the OVX group was shown, but there was no statistically significant difference (Figure 3C).

4.4. Protein Expression Level of PR, ERα, and ERβ

The protein expression level of PR, ERα, and ERβ within the endometrial tissue of the mice had the same pattern across groups. In OVX, OVX + OVA, OVX + OVA Rut 10, OVX + OVA Rut 30, OVX + OVA Rut 60, OVX + OVA Rut 100, autograft and normal, the level of ERα was, 0.0044, 0.81, 1.88, 1.78, 1.46, 0.56, 1.76, 1.65, respectively. The level of ERβ was 0.025, 0.14, 1.25, 1.05, 0.56, 0.80, 0.19, 0.50, respectively, and the level of PR was 0.09, 0.21, 0.14, 0.88, 0.10, 0.28, 0.09, and 0.59 respectively. The rates in the OVX + rutin 100 and 0 groups had the lowest levels of ERs (P value < 0.05). The highest level of ERα and ERβ was seen in OVX + rutin 10 and 30. There were no detectable expressions in the OVX group (P value < 0.01). Moreover, the protein expression of PR in all OVX + OVA groups treated with rutin was increased in comparison with the OVX group, but there was no significant difference (Figures 3D - 3G and Table 4).

5. Discussion

Results of the present study demonstrate that fresh hetero allograft ovarian transplantation with different concentrations of rutin in mice can restore ovarian function, as evidenced by the production of E_2 and P_4 along

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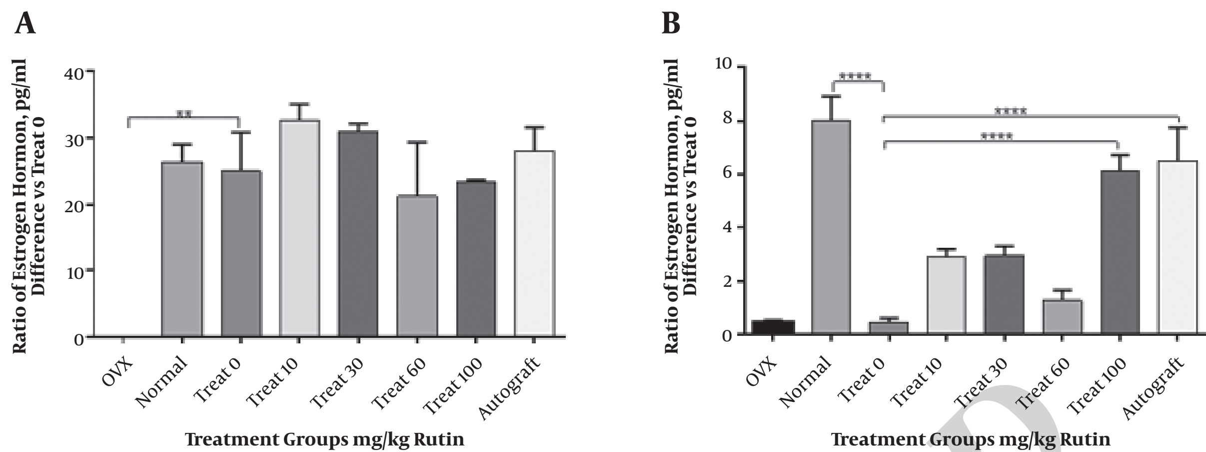

Figure 1. Serum ELISA results for estrogen and progesterone

Table 3. Follicular Density in Grafted Ovaries[a]

Groups	No. of Follicles				
	Primordial	**Primary**	**Preantral**	**Antral**	**Atretic**
Normal	19.67 ± 5.686	19.67 ± 5.686	21.00 ± 1.000[b]	23.00 ± 1.000[b]	5.000 ± 1.000[b]
Treat 0	17.67 ± 2.517	16.67 ± 2.082	0.3333 ± 0.5774	0.3333 ± 0.5774	12.33 ± 2.517
Treat 10	12.33 ± 2.517	17.67 ± 2.517	13.00 ± 1.000[b]	10.67 ± 3.055[b]	12.33 ± 2.517
Treat 30	13.67 ± 3.786	17.33 ± 2.082	1.000 ± 1.000	1.667 ± 1.528	19.00 ± 1.000[b]
Treat 60	19.67 ± 1.528	19.67 ± 1.528	3.000 ± 1.000b	1.000 ± 1.000	25.00 ± 5.000[b]
Treat 100	12.00 ± 3.000	17.00 ± 4.359	0.0 ± 0.0	0.0 ± 0.0	23.00 ± 2.646[b]

[a] Values are expressed as mean ± SD.
[b] Significant difference compared to treat 0 (P < 0.05).

Table 4. Protein Expression Level of Estrogen Receptor Alpha (ERα), Estrogen Receptor Beta (ERβ), and Progesterone Receptor (PR)[a]

Groups	ERa	ERb	PR
OVX	0.007864 ± 0.003837	0.05174 ± 0.03778	0.1156 ± 0.06584
Normal	1.495 ± 0.3662[b]	0.6670 ± 0.1752[b]	0.6010 ± 0.1626[b]
Treat 0	0.7089 ± 0.1131	0.1534 ± 0.06109	0.1423 ± 0.09290
Treat 10	1.615 ± 0.1701[b]	0.7747 ± 0.4365[b]	0.1593 ± 0.06674
Treat 30	1.744 ± 0.1779[b]	0.9220 ± 0.1245[b]	0.2064 ± 0.1529
Treat 60	0.9776 ± 0.7617	0.7701 ± 0.2150[b]	0.1591 ± 0.06259
Treat 100	0.4953 ± 0.2098	0.6676 ± 0.1547[b]	0.2763 ± 0.05559
Autograft	1.770 ± 0.1000[b]	0.1860 ± 0.02788	0.2537 ± 0.1264

[a] Values are expressed as mean ± SD.
[b] Significant difference compared to treat 0 (P < 0.05).

with the expression of ERα, ERβ, and PR in the transplanted mice. Also, results showed that the use of rutin in allograft for restoring the ovarian function could have an equal value to autograft transplantation in some concentrations.

Beazley and Nurminskaya explored the effects of flavonoid on fertility in female mice. They demonstrated that quercetin leads to a 60% reduction in the number of litters, but enhances folliculogenesis in the ovaries of female offspring (26). In this study, consistent with the study

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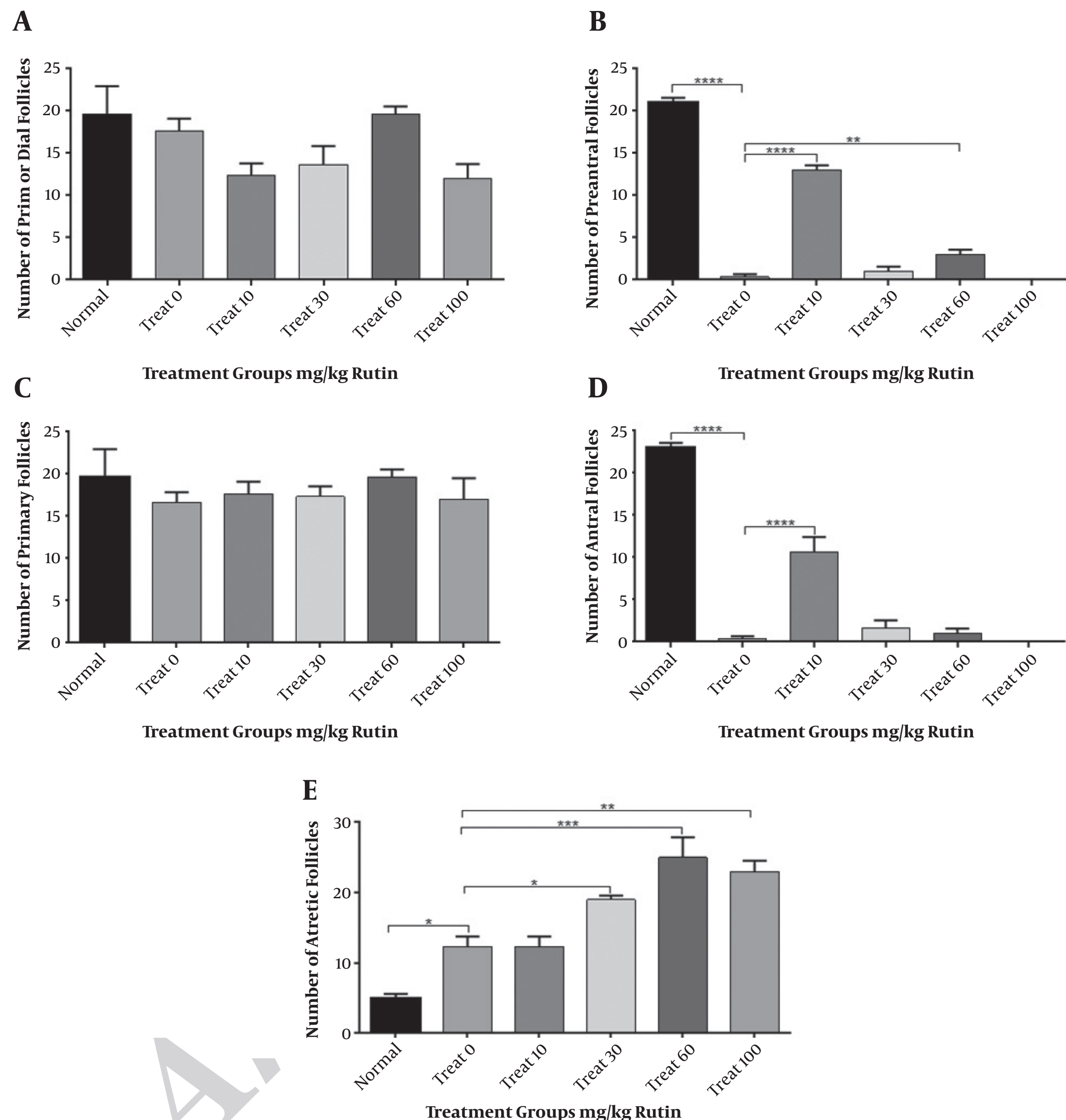

Figure 2. Number of follicles in examining the ovaries in normal, OVX + OVA, OVX + OVA + Rut 10, OVX + OVA + Rut 30, OVX + OVA + Rut 60, and OVX + OVA + Rut 100 groups; (A) number of primordial follicles, (B) number of primary follicles, (C) number of preantral follicles, (D) number of antral follicles, and (E) number of atretic follicles.

by Beazley and Nurminskaya, rutin as a flavonoid caused an increase in the maturation of follicles but was not able to increase fertility (increased atretic follicles).

Treatment of OVX + OVA mice with rutin possibly restored the estrous cyclicity. However, the rutin-treated groups at different concentrations showed different serum levels of E_2 and P_4.

In the present study, E_2 concentration in plasma was significantly increased. Guo et al. in a similar study reported that the administration of rutin has an effect on ovariectomized rats similar to the administration of E_2. They concluded that rutin is able to increase E_2 concentration in serum and mammary glands (27). In another study on flavonoids, Tan et al. demonstrated the same role of these molecules in increasing E_2 levels in pubertal female rats (28). In addition, Nynca et al. reported genistein action

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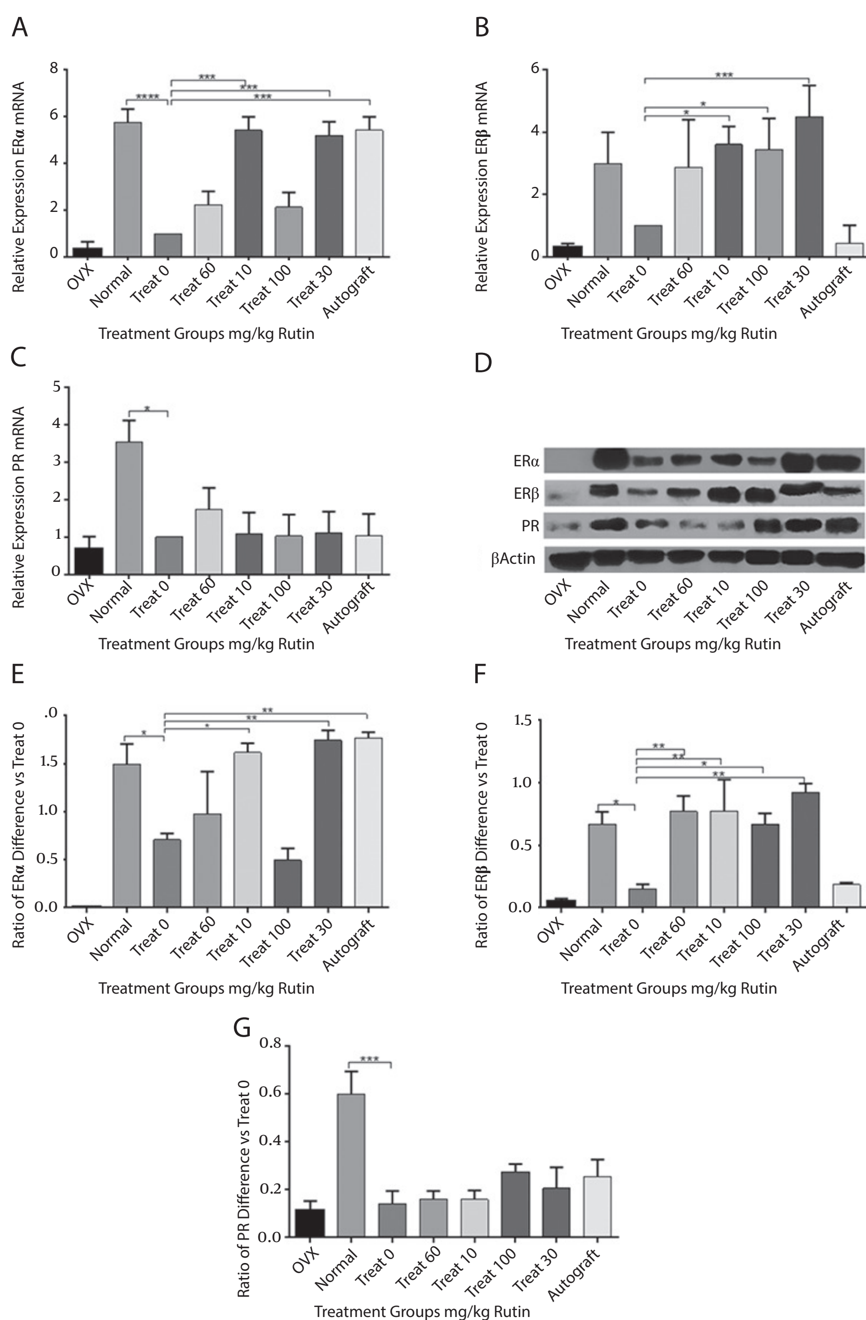

Figure 3. mRNA expression level of ERα, ERβ, and PR (A, B, C); protein expression level of ERα, ERβ, and PR (D, E, F, G)

(a flavonoid) on E_2 production by porcine granulosa cells of medium follicles. All doses of genistein increased basal E_2 secretion by the granulosa cells cultured for 48 h (29). In contrast, daidzein as another flavonoid did not alter the granulosal secretion of E_2 (29).

P_4 was also studied as a hormone with fluctuations in ovary activity and follicle growth. Interestingly, the level of P_4 was higher in the autograft group than rutin10, 30, and 60 groups, while there was no significant difference in comparison with rutin 100. Jahan et al. in a study on rats with polycystic ovary syndrome demonstrated an increase in P_4 due to the administration of rutin at two doses

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of 100 mg/kg and 150 mg/kg (30). In contrast, in other studies, genistein inhibited P_4 production by the porcine granulosa cells of medium follicles (29) and porcine luteinized granulosa cells isolated from large follicles (31).

Results showed that ERβ expression in the endometrial tissue was significantly increased in all OVX + OVA groups treated with rutin versus OVX and treat 0 groups. As demonstrated in the E_2 pattern, an increase in ERβ in the treated group had a significantly higher protein expression level than the autograft group, suggesting that rutin increased ERα expression in the endometrial tissue. The protein expression level of ERα has the same pattern as ERβ in all treated groups in comparison with control, OVX, and OVX + OVA groups. A prominent difference was found in the expression level of ERα which was considerably higher in the autograft group in comparison with all other groups. In their study on the possible pathway using which rutin can ameliorate oxidative injury, Hong et al. report that rutin can attenuate the ischemia/reperfusion injury in ovariectomized rats via ER-mediated signaling pathways (BDNF-TrkB and NGF-TrkA signaling pathway) (32). The structural similarity of rutin to endogenous estrogen and plant estrogen makes this molecule able to be absorbed by target cells, be bound to ER, and then exert estrogen-like effects (33). In addition to the direct effects of rutin on ER activation and signaling initiation, this agent is able to increase the production of estrogen directly, as mentioned by Guo (27). Similarly, Nynca et al. demonstrated that genistein caused a significant increase in the ERβ mRNA level in granulosa cells of large follicles (31) and granulosa cells of medium follicles (29). However, it did not alter ERα mRNA level in the culture porcine granulosa cells (29, 31). In contrast, the expression of ERβ protein was affected by genistein in the granulosa cells of medium follicles (29), but it was not detected in the granulosa cells of large follicles (31). Daidzein decreased mRNA expression of ERα in medium follicles, but the expression of ERβ mRNA was not affected by daidzein. ERα protein was not detected while ERβ protein was found in the nuclei of the cells. Daidzein upregulated the expression of ERβ protein in the cells (29).

The PR expression in the endometrial tissue had a decreased pattern in comparison with the control group. The maximum protein expression level of PR was observed in the treat rutin 100 group. In all transplanted groups, an increase of PR versus the OVX group was shown. However, in groups 0, 10, 30, 60, and the autograft group, there was no statistically significant difference. These results suggest that rutin is able to enhance PR. Parallel with this study; Rosenberg et al. demonstrated that flavonoids have a progesterone-like antagonist activity. In their study on PR in breast cancer cell lines, it was shown that flavonoids bind to the PR and act as a blocker. They demonstrated that flavonoids could modulate PR expression (34). It seems that rutin, as a flavonoid component, has a modulatory effect on PR beside its antioxidant and estrogen-like abilities. In agreement with Rosenberg, no statistically significant change in PR was observed in the present study.

5.1. Strengths and Limitations

5.1.1. Limitations

- The sample size was small (n = 5) for each group.

- No additional evaluation methods such as immunohistochemistry were used.

5.1.2. Strengths

- Evaluation methods such as qRT-PCR and Western blotting were employed.

- Four dosages of rutin were tested.

5.2. Conclusions

The present study demonstrates that the effects of rutin on restoring the estrous cyclicity after transplantation rely on its antioxidant effect on the inhibition of the increased oxidative stress. The results of the present study indicated that rutin increased the E_2 and P_4 levels in ovarian hetero allograft mice. Rutin also upregulated the expression of ERα and ERβ but had no significant effect on PR. ER upregulation led to an enhanced function of estrogen, improved the engraftment and function of the transplanted ovarian tissue, and restored estrous cyclicity.

Footnotes

Authors' Contribution: Ghasem Saki supervised the research. Aligholi Sobhani supervised the research and edited. Masoud Hemadi designed the project. Tayebeh Hadigol obtained the specimens, carried out the experiment, and writing the manuscript. Saeid Nekoonam analyzed the data. Maryam Dastoorpoor analyzed the statistics section. Alireza Shams carried out the experiment. Bahram Eslami Farsani writing the manuscript.

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