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Stress-associated ovarian damage, infertility, and delay in achieving pregnancy and treatment options.

Gulsah Aynaoglu Yildiz[1], Omer Erkan Yapca[2], Kemal Dinc[3], Cebrail Gursul[4], Betul Gundogdu[5], Mehmet Aktas[6], Zeynep Suleyman[7], Seval Bulut[7] and Halis Suleyman[8]

[1]Department of Perinatology, Etlik Zubeyde Hanim Maternity and Women's Health Teaching and Research Hospital, Ankara-Turkey.
[2]Department of Obstetrics and Gynaecology, Faculty of Medicine, Ataturk University, Erzurum-Turkey.
[3]Department of Obstetrics and Gynaecology, Faculty of Medicine, Erzincan Binali Yildirim University, Erzincan-Turkey.
[4]Department of Physiology, Faculty of Medicine, Erzincan Binali Yildirim University, Erzincan-Turkey.
[5]Department of Pathology, Faculty of Medicine, Ataturk University, Erzurum-Turkey.
[6]Department of Biochemistry, Faculty of Medicine, Erzincan Binali Yildirim University, Erzincan, Turkey.
[7]Department of Pharmacology, Health Sciences Institute, Erzincan Binali Yildirim University, Erzincan, Turkey.
[8]Department of Pharmacology, Faculty of Medicine, Erzincan Binali Yildirim University, Erzincan, Turkey.

Keywords: ovarian damage; sertraline; cerebrolysin; stress; rats.

Abstract. Many types of stress, including psychological stress, negatively affect reproductive health. This study aimed to investigate the effects of sertraline (a selective serotonin reuptake inhibitor), cerebrolysin (neuroprotective/neurotrophic), and a combination of both against stress-induced ovarian damage, infertility and pregnancy delay in female rats. The rats were divided into five groups (n=14/each group) as healthy (HG), stress control (StC), stress+sertraline (SS), stress+cerebrolysin (SC), and stress+sertraline+cerebrolysin (SSC). To induce stress, animals (except the HG) were kept in a supine position with their forelimbs and hindlimbs (FIM) tied for one hour. Then, sertraline (20mg/kg) was given orally to the SS. Cerebrolysin (2.5ml/kg) was injected into the SC subcutaneously. Sertraline+cerebrolysin was administered to SSC with the same methods and doses. FIM and drug administration continued for 30 days. Six rats from each

Corresponding Author. Halis Suleyman. Department of Pharmacology, Faculty of Medicine, Erzincan Binali Yildirim University, Erzincan, Turkey, Post code: 24100, Phone: +90 4462261818, Fax: +90 5309211909, E-mail: halis.suleyman@gmail.com

group were euthanized with high-dose anesthesia, right and left ovarian tissues were removed, and tissues were examined biochemically and histopathologically. The remaining rats were taken for breeding. Exposure to stress in rats caused an increase in malondialdehyde (MDA), tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) levels and a decrease in total glutathione (tGSH). Stress was related to histopathological damage, infertility, and delayed birth. The sertraline and cerebrolysin combination was the most effective in preventing these changes, with sertraline and cerebrolysin alone in second and third places, respectively. Regarding efficacy, selective serotonin reuptake inhibitors (SSRIs) and related drugs may be beneficial in treating stress-related ovarian damage, infertility, and delay in pregnancy.

Daño ovárico, infertilidad y retraso en la concepción relacionados con el estrés y opciones de tratamiento.

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Palabras clave: daño ovárico; sertralina; cerebrolisina; estrés; ratas.

Resumen. Muchos tipos de estrés, incluido el estrés psicológico, afectan negativamente a la salud reproductiva. El objetivo de este estudio fue investigar los efectos de la sertralina (un inhibidor selectivo de la recaptación de serotonina), la cerebrolisina (neuroprotector/neurotrófico) y una combinación de ambos contra el daño ovárico, la infertilidad y el retraso del embarazo inducido por el estrés en ratas hembra. Las ratas se dividieron en cinco grupos (n=14/cada grupo), como sanas (HG), control de estrés (StC), estrés+sertralina (SS), estrés+cerebrolisina (SC) y estrés+sertralina+cerebrolisina (SSC) . Para inducir el estrés, los animales (excepto el HG) se mantuvieron en posición supina con las extremidades anteriores y posteriores (FIM) atadas durante una hora. Luego, se administró sertralina (20 mg/kg) por vía oral al grupo SS. Cerebrolysin (2,5 mL/kg) se inyectó al grupo SC por vía subcutánea. Se administró sertralina+cerebrolisina al grupo SSC con los mismos métodos y dosis. La FIM y la administración de fármacos continuaron durante 30 días. Se sacrificaron seis ratas de cada grupo con anestesia de dosis alta, se extirparon los tejidos de los ovarios derecho e izquierdo y se examinaron bioquímica e histopatológicamente. Las ratas restantes se tomaron para reproducción. La exposición al estrés en ratas provocó un aumento de los niveles de malondialdehído (MDA), factor de necrosis tumoral alfa (TNF-α), interleucina-1β (IL-1β) e interleucina-6 (IL-6) y una disminución del glutatión total (tGSH). El estrés se relacionó con daño histopatológico, infertilidad y retraso en el parto. La combinación de sertralina y cerebrolisina fue la más efectiva para prevenir estos cambios, con sertralina y cerebrolisina solas en segundo y tercer lugar, respectivamente. Los inhibidores selectivos de la recaptación de serotonina (ISRS) y los medicamentos relacionados pueden ser beneficiosos en el tratamiento del daño ovárico relacionado con el estrés, la infertilidad y el retraso en el embarazo.

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INTRODUCTION

One of the fundamental reasons for psychological stress is socioeconomic factors[1]. It has been known that chronic stress is related to numerous diseases [2]. Abnormalities that cause infertility, such as ovulation, implantation disorders, and tube damage, have also been connected with psychological stress [3]. Many types of stress, including psychological stress, harm fertility and reproductive functions [4]. There is information in the literature that stress triggers depression and anxiety [5].

Furthermore, it is stated that depression and anxiety lead to infertility [6]. It has been observed that oxidative stress and pro-inflammatory cytokine production increase in depression and anxiety [7]. Degeneration, infiltration, and histopathological damage , such as atretic follicles, are seen in response to stress in rat ovaries [8]. Kadioglu *et al*. revealed that stress, which leads to infertility, increases total oxidant levels and decreases antioxidant levels in rat ovarian tissue [9]. This literature recommends that drugs with antioxidant, antidepressant, anti-inflammatory, and anxiolytic effects may be beneficial in treating stress-related ovarian damage and reproductive dysfunction.

Sertraline, is an antidepressant drug with selective serotonin re-uptake inhibitor antioxidant properties [10]. It has been experimentally revealed that sertraline exerts an anti-inflammatory effect by reducing tumor necrosis factor-alpha (TNF-α) levels [11]. It has been suggested that the antidepressant and anxiolytic effect of sertraline is based on the suppression of excessive production of TNF-α, interleukin-6 (IL-6), interleukin-1beta (IL-1β), and other pro-inflammatory cytokines [12,13]. Cerebrolysin is another drug we would like to see for its effects on stress-related ovarian damage, infertility, and preventing delay in maternity. Cerebrolysin is a porcine brain-derived drug with neuroprotective and neurotrophic effects and contains low molecular weight peptides and amino acids [9]. It has been defended that the antidepressant effect of cerebrolysin is based on its reducing oxidative stress and cytokine-related inflammation [14]. In addition, it has been argued that using an antidepressant drug with a drug with neuroprotective properties increases the effectiveness of antidepressant treatment [15]. All this information shows that sertraline, cerebrolysin, and a combination of both may be beneficial against increased stress, ovarian damage, infertility, and delay in achieving pregnancy in animals. This study aimed to investigate the effects of sertraline, cerebrolysin, and the combination of both on stress-induced ovarian injury, infertility, and pregnancy delay in rats.

MATERIALS AND METHODS

Animals

Seventy albino Wistar female rats provided by the Medical Experimental Application and Research Center of Ataturk University weighing between 272-288 grams at 6 months of age were utilized in our study. Rats were placed in a laboratory with a 12-hour light/12-hour dark cycle at appropriate humidity (45%) and temperature (22°C) and fed *ad libitum* before experimentation. Experimental applications were carried out considering the ARRIVE guidelines. The implementation of the experiment was started after the procedures were approved by the Atatürk University Animal Experiments Local Ethics Committee (date: 27.12.2018, meeting no: 13/253).

Chemicals

Sertraline was procured from PFIZER (Turkey), cerebrolysin from EVER Pharma (Austria), and sodium thiopental from IE Ulagay (Turkey).

Animal groups

The rats were randomly separated into healthy controls (HG), stress-applied control (StC), stress+sertraline (SS), stress + cerebrolysin (SC), and stress+ sertraline +

cerebrolysin (SSC) groups, with [14] animals in each group.

Experiment procedure

Stress was induced in rats by the forced immobilization method (FIM). For the implementation of this experiment, all animals except the HG group were placed in the supine position; their hindlimb and forelimbs were tied and kept in this position for one hour. After one hour, sertraline (20 mg/kg) was given to the SS rat group by oral gavage. SC group was injected with cerebrolysin (2.5 mL/kg) subcutaneously. Sertraline+cerebrolysin was administered to the SSC group at the indicated doses under the same method. The HG and StC groups were given the same volume of distilled water. FIM and drug applications were continued once a day for 30 days. Six rats from each group were euthanized (ip, 50 mg/kg thiopental sodium), and the right and left ovarian tissue were removed for biochemical and histopathological examination. The rest of the animals were kept in the same environment with mature male rats for two months for pregnancy.

The rats that were found to be pregnant were taken into separate cages and fed. Rats that did not become pregnant and give birth during this period were considered infertile. In addition, the time from the day the female rats were placed in the same cage with the male rats to the day they gave birth was determined. The delay in maternity was determined by subtracting the standard gestational period (21 days) from this period.

Biochemical analyses

Preparation of samples

Ovarian tissues were weighed. Tissues were made up to 2 mL with 1.15% potassium chloride solution for MDA determination and phosphate buffer with pH=7.5 for GSH determination and homogenized in an ice-cold medium, it was centrifuged for 15 minutes (10000 rpm, +4 °C). The supernatant portion was utilized as an analysis sample.

Malondialdehyde (MDA) and Total Glutathione (tGSH) Analysis

Malondialdehyde was measured to establish the oxidation level, tGSH was measured to determine the antioxidant level. MDA measurements were made according to the method defined by Ohkawa *et al.* [16]. tGSH measurement was performed in line with the method defined by Sedlak and Lindsay [17].

Tumor Necrosis Factor-α (TNF-α), Interleukin 1-β (IL-1β), and Interleukin-6 (IL-6) Analysis

Tumor Necrosis Factor-α, IL-1β, and IL-6 were measured to assess the pro-inflammatory status. The samples were weighed, and then all tissue was cut. These samples were then snap-frozen with liquid nitrogen and homogenized using a mortar and pestle. After the samples were melted, they were kept at 2-8 °C. PBS (pH 7.4), 1/10 (w/v) was added; after this, vortexed for 10 seconds, centrifuged at 10000 xg for 20 minutes, and supernatants were aliquoted. TNF-α, IL-1β, and IL-6 levels were measured using a commercial kit (Eastbiopharm Co Ltd ELISA kit, China).

Histopathological Examination

After the tissue samples were defined in 10% formaldehyde solution, they were washed in the cassettes under tap water for 24 hours. They were then treated with conventional-grade alcohol to remove water from the tissues, passed through xylol, and embedded in paraffin. Sections of 4-5 microns were taken from these paraffin blocks and subjected to hematoxylin-eosin staining. Tissues were examined under a light microscope, and then photographs were taken (Olympus® Inc. Tokyo, Japan, DP2-SAL firmware program). Ovarian tissues were evaluated regarding congestion, hemorrhage, degeneration in follicle cells, water accumulation in follicle cells, cystic changes in follicles, and edema. The severity of histopathological findings was graded from 0-3 (0-normal, 1-mild injury, 2-moder-

ate injury, and 3-severe injury). Histopathological evaluation was performed by a pathologist who was blinded to treatment and group allocations.

Statistical Analysis

This study used the "IBM SPSS 22® (Armonk, NY: IBM Corp.)" program for statistical analysis. Since the biochemical data were numeric, the analysis was done with one-way ANOVA, Tukey was used as a post hoc test, and the results were presented as Mean ± Standard error (X±SEM). Since the histopathological data were sequential, Kruskal-Wallis was preferred for analysis, and then the Dunn's test was used. Data were presented as Median (Minimum-Maximum), $p<0.05$ was accepted as statistical significance.

RESULTS

Biochemical Results

MDA and tGSH Analysis

The levels of MDA in the StC group (5.87±0.02) were significantly higher than in the HG (1.34±0.06), SS (2.85±0.02), SC (3.45±0.08) and SSC (1.52±0.06) groups as can be seen in Fig. 1A ($p<0.001$). The increase in MDA levels in the SSC group was observed to be significantly lower than in the SS and SC groups ($p<0.001$). For MDA, the HG and SSC groups were similar ($p=0.123$). The amount of tGSH measured in the ovarian tissue of the StC (1.30±0.05) group was observed to be significantly lower than the values measured in the HG (6.00±0.19), SS (3.64±0.08), SC (2.37±0.05) and SSC (5.42±0.18) groups ($p<0.001$). In the treatment combination group, inhibition in the decrease of tGSH was more significant than in the sertraline and cerebrolysin groups (Fig. 1B).

TNF-α, IL-1β and IL-6 Analysis

As seen in Fig. 2, TNF-α (Fig. 2A), IL-1β (Fig. 2B) and IL-6 (Fig. 2C) levels in the StC group (6.73±0.05, 8.05±0.17, 8.52±0.23, respectively) were significantly increased compared to HG (1.73±0.17, 2.25±0.04, 2.87±0.12, respectively), SS (3.27±0.04, 4.28±0.11, 4.89±0.03, respectively), SC (4.66±0.05, 5.81±0.04, 6.82±0.03, respectively) and SSC (2.04±0.10, 2.39±0.05, 3.14±0.03, respectively) groups ($p<0.001$). The increase in TNF-α, IL-1β, and IL-6 levels was significantly lower in the combination group when compared with the SS and SC groups alone ($p<0.001$). Cytokine levels were similar in SSC and HG groups ($p>0.05$).

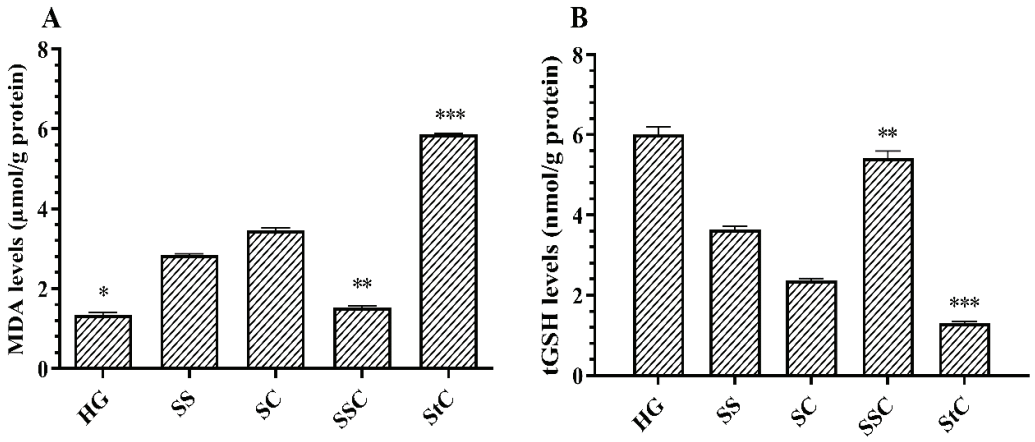

Fig. 1. MDA (A) and tGSH (B) levels in the ovarian tissue of study groups.
*p=0.123 vs SSC group; **p<0.001 vs SS and SC groups; ***p<0.001 vs HG, SS, SC and SSC groups. Statistical analysis was done with one-way ANOVA, followed by the Tukey test. HG, healthy group; StC, stress-treated control group; SS, stress+sertraline group; SC, stress+cerebrolysin group; SSC, stress+sertraline+cerebrolysin group.

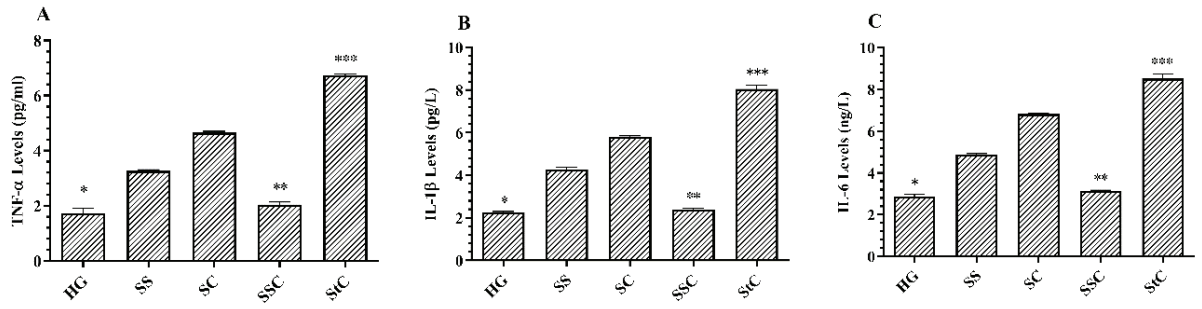

Fig. 2. TNF-α (A), IL-1β (B), and IL-6 (C) levels in the ovarian tissue of study groups. *p>0.05 vs SSC group; **p<0.001 vs. SS and SC groups; ***p<0.001 vs. HG, SS, SC and SSC groups. Statistical analysis was done with one-way ANOVA, followed by the Tukey test. HG, healthy group; StC, stress-treated control group; SS, stress+sertraline group; SC, stress+cerebrolysin group; SSC, stress+sertraline+cerebrolysin group.

Reproduction Test Results

Animals in the HG group gave birth within 23-26 days, as observed in Table 1. Six of the eight rats in the SS group gave birth within 27-36 days, while two did not give birth within two months. Three of the eight rats in the SC group gave birth within 33-38 days, but three did not give birth within two months. In the SSC group, all eight female rats gave birth on days 24-28. One of eight female rats in the StC group gave birth on day 49, but the remaining seven did not give birth during this time.

Histopathological findings

As seen in Fig. 3A and Table 2, no pathological findings were found in the ovarian tissue of the HG group; corpus luteum and follicle structure were observed within normal limits. Grade-3 degenerated secondary follicle and congestion were observed in the ovarian tissue of the StC group (Fig. 3E, Table 2). Moreover, grade-3 dilated congested vessels, hemorrhage, and edema were seen in the ovarian tissue of the StC group (Fig. 3F, Table 2). Vascular congestion (grade-1) and relatively normal follicle and corpus luteum structure (grade-0) were seen in the ovarian tissue of the SS group treated with sertraline (Fig. 3B, Table 2). In the SC group, mild fluid accumulation in the lumen, cystic changes (grade-1), minimal vascular congestion (grade-1), and corpus luteum damage (grade-1) were detected (Fig. 3C, Table 2). There were no histopathological signs other than mild vascular congestion (grade-1) and relatively normal corpus luteum (grade-0) in the SSC group (Fig. 3D, Table 2).

DISCUSSION

The effects of sertraline, cerebrolysin, and their combination against FIM-related stress-associated ovarian damage, infertility, and delay in achieving pregnancy in female rats were investigated in this study. Various stress factors lead to damage to all organs and tissues of the body, as can be understood from the literature [18]. Previous studies have revealed that psychological or physiological stress is related to oxidative stress [19]. Stress increased the levels of MDA, known as the toxic product of lipid peroxidation (LPO), and decreased the antioxidant tGSH levels in the ovarian tissue of animals, as can be observed in our results. These biochemical findings show that stress changes the oxidant-antioxidant balance in favor of oxidants in the ovarian tissue. In the literature, it has been shown that stress increases oxidants in ovarian tissue while decreasing antioxidants [20]. As it is known, membrane lipids are oxidized by reactive oxygen species (ROS), and a toxic product, MDA, is formed. MDA resulting from LPO greatly disrupts the structure and functions of the cell membrane

Table 1
Infertility and reproductive process in rats preproductiveprocess in rats.

Groups	Non-infertile rats n %		İnfertile rats n %		Reproductive process (RP) (day)	Delay in maternity (RP-21 days)
HG (n=8)	8	100	-	-	24.63 ± 0.42*	3.63 ± 0.42*
SS (n=8)	6	75	2	25	30.17 ± 1.45**	9.17 ± 1.45**
SC (n=8)	3	37.7	5	62	36.00 ± 1.53***	15.00 ± 1.53***
SSC(n=8)	8	100	-	-	25.75 ± 0.59	4.75 ± 0.59*
StC (n=8)	1	12	7	88	49.00	28.00

*, p>0.05 vs SSC; **, p<0.05 vs SC and SSC, ***; p<0.05 vs SS and SSC. Statistical analysis was done with one-way ANOVA, followed by the Tukey test. Results are expressed as mean±standard error of the mean. HG, healthy group; StC, stress-treated control group; SS, stress+sertraline group; SC, stress+cerebrolysin group; SSC, stress+sertraline+cerebrolysin group; n, number of animals.

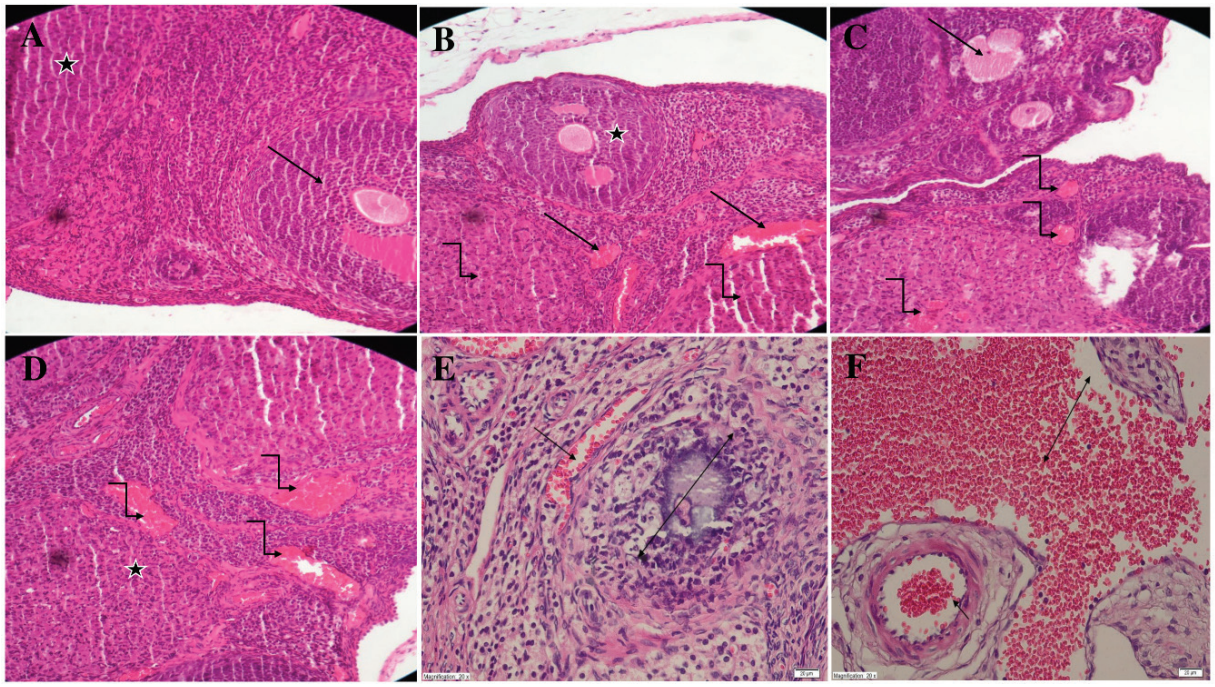

Fig. 3 (A-F). Histopathological examination of ovarian tissues in study groups. **A.** Ovarian tissue of the SG group; view of healthy corpus luteum (star) and follicle structure (arrow) within normal limits. **B.** Ovarian tissue of the StC group; Section showing degenerated secondary follicle (bilateral arrow), dilated congested blood vessel (straight arrow). **C.** Ovarian tissue of the StC group; section showing dilated congested blood vessel (straight arrow), hemorrhage, and edema (bilateral arrow). **D.** Ovarian gland of the SS group; Section showing congested blood vessels (arrows), the follicle (star), and corpus luteum (zigzag arrow). **E.** Ovarian tissue of the SC group; Follicle structure with fluid accumulation in the lumen and cystic change (arrow), section showing mild congestion (zigzag arrow). **F.** Ovarian tissue of the SSC group; Section, showing mildly congested blood vessel (zigzag arrow), corpus luteum (star). H&E x 200. HG, healthy group; SS, stress+sertraline group; SC, stress+cerebrolysin group; SSC, stress+sertraline+cerebrolysin group; StC, stress-treated control group. H&E x 200.

Table 2
Histopathological examination of ovarian tissues.

Groups	Congestion	Hemorrhage	Follicle cell degeneration	Water accumulation in follicle cells	Cystic change in follicles	Edema
HG (n=6)	0(0-0)*	0(0-0)*	0(0-0)*	0(0-0)*	0(0-0)*	0(0-0)*
SS (n=6)	1(0-2)*	0(0-0)*	0(0-1)*	0(0-0)*	0(0-0)*	0(0-0)*
SC (n=6)	1(0-2)*	0(0-0)*	0(0-0)*	1(0-2)**	1(1-1)**	0(0-0)*
SSC (n=6)	1(0-1)*	0(0-0)*	0(0-0)*	0(0-0)*	0(0-0)*	0(0-0)*
StC (n=6)	3(2-3)**	3(3-3)**	3(2-3)**-	0(0-0)*	0(0-0)*	3(2-3)**

Histopatological grading; 0-normal, 1- mild injury, 2-moderate injury, and 3-severe injury. *, $p>0.05$ vs other groups with the same sign; **, $p<0.05$ vs other groups. Kruskal Wallis test was used. Results are expressed as median (minimum -maximum). HG, healthy group; SS, stress+sertraline group; SC, stress+cerebrolysin group; SSC, stress+sertraline+cerebrolysin group; StC, stress-treated control group.

and leads to further destruction [21]. As such, MDA level is known as a marker of oxidative stress and antioxidant status in patients [22]. Our results, which align with these previous findings, showed that the amount of tGSH decreased significantly. At the same time, MDA increased in the ovarian tissues of animals exposed to stress. GSH is a tripeptide that can be found in most cells. GSH protects cells from the toxic effect of ROS by detoxifying hydrogen peroxide and organic oxides [23].

It is known that pro-inflammatory cytokine production plays a role in parallel with excessive oxidant production in the pathogenesis of ovarian damage, infertility, and delay in achieving pregnancy that develops due to stress and other factors [9,24]. Our results, accordingly, showed that TNF-α, L-1β and IL-6 levels increased in the ovarian tissue of animals with infertility and delay in achieving pregnancy. In studies with patients, it has been reported that TNF-α, IL-6, and other pro-inflammatory cytokines are among the factors that lead to infertility in ovarian pathologies [25]. Oxidative stress and pro-inflammatory cytokines increase in psychological disorders such as depression and anxiety, as mentioned above [7].

It has been reported that stress is associated with depression, and depression may lead to ovarian dysfunction [26]. Sertraline was more effective than cerebrolysis against stress-related ovarian damage, infertility, and pregnancy delay. The fact that sertraline is more effective than cerebrolysin may be due to its more significant inhibition of overproduction of oxidant and pro-inflammatory cytokines than cerebrolysin. Sertraline has antioxidant properties as mentioned hereinabove [10]. Moreover, it is argued that the antidepressant and anxiolytic effect of sertraline is based on suppressing excessive production of TNF-α, L-1β, IL-6, and other pro-inflammatory cytokines [12,13]. It has been documented that the antidepressant effect of cerebrolysin is due to the reduction of oxidative stress and cytokine-related inflammation [14]. Cerebrolysin is also a neuroprotective and neurotrophic drug [27]. The fact that the use of an antidepressant drug together with a drug with neuroprotective properties increases the effectiveness of the treatment has been reported in the literature [15]. The administration of sertraline and cerebrolysin in combination suppressed oxidant and inflammatory markers in ovarian tissue better than sertraline and cerebrolysin administered alone. It also better prevented stress-related infertility and delay in achieving pregnancy in our study.

Severe histopathological injury was seen in the ovarian tissue of the stress group. In addition, mild cystic changes in

the follicles and water accumulation in the follicle cells were observed in the stress and cerebrolysin group, while adding sertraline to the treatment prevented these changes. Combination therapy, sertraline, and cerebrolysin were the best suppressors of histopathological damage, respectively. It is known that oxidant and pro-inflammatory cytokines lead to hemorrhage, congestion, follicle degeneration, inflammatory cell infiltration, and necrosis in the ovarian tissue [24]. Infertility and delay in achieving pregnancy developed in the stress-treated control group in which severe histopathological damage was detected in the ovarian tissue, as can be understood from our results. Ince et al. reported severe follicle degeneration in ovaries with high MDA and low tGSH levels [28]. In another study by Ince et al. stated that severe degeneration was found in the ovarian follicles of animals in which sterility and delay in achieving pregnancy developed [29]. It was revealed in the study of Kadioglu et al. that the stress induced by the forced immobilization method causes widespread congestion, hemorrhage, accumulation of fluid, and inflammatory infiltration in the subcapsular area in the ovaries. Infertility, delay in achieving pregnancy, decrease in the number of offspring, and intrauterine physical developmental retardation were found in the animal group with these histopathological signs [9]. The stress induced by the FIM has led to oxidative and inflammatory damage in the ovarian tissue of animals, sterility, and delay in achieving pregnancy. The combination therapy of sertraline and cerebrolysin were the drugs that best prevented ovarian damage, infertility, and delay in achieving pregnancy, respectively. This information has revealed the fact that antidepressant drugs with antioxidant and anti-inflammatory effects might be useful in the treatment of stress-related ovarian damage, infertility and delay in achieving pregnancy. It has been revealed particularly that the combination of antidepressant (sertraline) and neuroprotective / neurotrophic (cerebrolysin) drug combination may be more beneficial. In line with this information sertraline, cerebrolysin and their combination may be preferred in treating stress-associated ovarian damage, infertility, and delay in achieving pregnancy. However, it is required to investigate antidepressant and neuroprotective/neurotrophic drug combinations from different groups in the future to confirm these findings.

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Conflict of interest

There is no conflict of interest among the authors.

ORCID number of authors

- Gulsah Aynaoglu Yildiz (GAY): 0000-0002-3283-7783
- Omer Erkan Yapca (OEY): 0000-0002-5578-0126
- Kemal Dinc (KD): 0000-0003-4955-455X
- Cebrail Gursul CG): 0000-0001-6521-6169
- Betul Gundogdu (BG): 0000-0002-3786-3286
- Mehmet Aktas (MA): 0000-0003-1931-8353
- Zeynep Suleyman (ZS): 0000-0003-0128-7990
- Seval Bulut (SB): 0000-0003-4992-1241
- Halis Suleyman (HS): 0000-0002-9239-4099

Participation of authors

Substantial contribution to conception and design: GAY, HS; Acquisition of data: MA, HS; Analysis and interpretation of data: KD, ZS, SB; Drafting of the manuscript: GAY, OEY, HS; Critical revision of the manuscript for important intellectual content: GAY, HS; Statistical analysis: ZS, SB; Research group leadership: GAY, HS; Have given final approval of the submitted manuscript: GAY, OEY, KD, CG, BG, MA, ZS, SB, HS.

REFERENCES

1. **Nargund VH.** Effects of psychological stress on male fertility. Nat Rev Urol 2015;12:373-382.
2. **Sominsky L, Hodgson DM, McLaughlin EA, Smith R, Wall HM, Spencer SJ.** Linking Stress and Infertility: A Novel Role for Ghrelin. Endocr Rev 2017;38:432-467.
3. **Abdel Hafez SMN, Allam F, Elbassuoni E.** Sex differences impact the pancreatic response to chronic immobilization stress in rats. Cell Stress Chaperones 2021;26:199-215.
4. **Mamgain A, Sawyer IL, Timajo DAM, Rizwan MZ, Evans MC, Ancel CM, Inglis MA, Anderson GM.** RFamide-related peptide neurons modulate reproductive function and stress responses. J Neurosci 2021;41:474-488.
5. **Damone AL, Joham AE, Loxton D, Earnest A, Teede HJ, Moran LJ.** Depression, anxiety and perceived stress in women with and without PCOS: a community-based study. Psychol Med 2019;49:1510-1520.
6. **Lakatos E, Szigeti JF, Ujma PP, Sexty R, Balog P.** Anxiety and depression among infertile women: a cross-sectional survey from Hungary. BMC Womens Health 2017;17:48.
7. **Li M, Li C, Yu H, Cai X, Shen X, Sun X, Wang J, Zhang Y, Wang C.** Lentivirus-mediated interleukin-1β (IL-1β) knockdown in the hippocampus alleviates lipopolysaccharide (LPS)-induced memory deficits and anxiety- and depression-like behaviors in mice. J Neuroinflammation 2017;14:190.
8. **Chukwuebuka NB, Emeka OA, Irukefe OS, Iju WJ, Godsday OU, Temitope OG, Nneamaka EC, Peter AC.** Stress-Induced Morphological Changes of Ovarian Histology in Female Wistar Rats. Biomedical & Pharmacology Journal. 2020;13:1625-1643.
9. **Kadioglu B, Gundogdu B, Kurt N, Bilgin AO, Suleyman H, Suleyman Z.** The effect of rhodiola rosea root extract on stress-induced ovarian damage, infertility and reproductive disorders in female rats. Clinical and Experimental Obstetrics & Gynecology. 2020;47:530-536.
10. **Abdel Salam OM, Mohammed NA, Sleem AA, Farrag AR.** The effect of antidepressant drugs on thioacetamide-induced oxidative stress. Eur Rev Med Pharmacol Sci 2013;17:735-744.
11. **Baharav E, Bar M, Taler M, Gil-Ad I, Karp L, Weinberger A, Weizman A.** Immunomodulatory effect of sertraline in a rat model of rheumatoid arthritis. Neuroimmunomodulation 2012;19:309-318.
12. **Shulyak A, Gorpynchenko I, Drannik G, Poroshina T, Savchenko V, Nurimanov K.** The effectiveness of the combination of rectal electrostimulation and an antidepressant in the treatment of chronic abacterial prostatitis. Cent European J Urol 2019;72:66-70.
13. **Hou R, Ye G, Liu Y, Chen X, Pan M, Zhu F, Fu J, Fu T, Liu Q, Gao Z, Baldwin DS, Tang Z.** Effects of SSRIs on peripheral inflammatory cytokines in patients with Generalized Anxiety Disorder. Brain Behav Immun 2019;81:105-110.
14. **El-Marasy SA, El Awdan SA, Hassan A, Ahmed-Farid OA, Ogaly HA.** Anti-depressant effect of cerebrolysin in reserpine-induced depression in rats: Behavioral, biochemical, molecular and immunohistochemical evidence. Chem Biol Interact 2021;334:109329.
15. **Safarova T, Gavrilova S.** The use of neuroprotectors in the treatment of late depression. Zhurnal Nevrologii i Psikhiatrii Imeni SS Korsakova 2020;120:46-53.

16. **Ohkawa H, Ohishi N, Yagi K**. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351-358.
17. **Sedlak J, Lindsay RH.** Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem 1968;25:192-205.
18. **Cakir B, Kasımay O, Kolgazi M, Ersoy Y, Ercan F, Yegen BC.** Stress-induced multiple organ damage in rats is ameliorated by the antioxidant and anxiolytic effects of regular exercise. Cell Biochem Funct 2010;28:469-479.
19. **Islam MT.** Oxidative stress and mitochondrial dysfunction-linked neurodegenerative disorders. Neurol Res 2017;39:73-82.
20. **Aynaoglu Yildiz G, Yildiz D, Yapca OE, Suleyman B, Arslan YK, Kurt N, Suleyman H**. Effect of diazepam, sertraline and melatonin on the stress-induced reproductive disorders and intrauterine growth restriction in female rats. J Matern Fetal Neonatal Med 2021;34:4103-4109.
21. **Yuceli S, Suleyman B, Yazici GN, Mammadov R, Cankaya M, Kunak CS, Bulut S, Suleyman H, Altuner D.** Effect of taxifolin on ischemia/reperfusion-induced oxidative injury of sciatic nerve in rats. Transplant Proc 2021;53:3087-3092.
22. **Gaweł S, Wardas M, Niedworok E, Wardas P.** Malondialdehyde (MDA) as a lipid peroxidation marker. Wiadomosci lekarskie (Warsaw, Poland: 1960) 2004;57:453-455.
23. **Forman HJ, Zhang H, Rinna A.** Glutathione: overview of its protective roles, measurement, and biosynthesis. Mol Aspects Med 2009;30:1-12.
24. **Unlubilgin E, Suleyman B, Balci G, Atakan Al R, Cankaya M, Arslan Nayki U, Suleyman H.** Prevention of infertility induced by ovarian ischemia reperfusion injury by benidipine in rats: Biochemical, gene expression, histopathological and immunohistochemical evaluation. J Gynecol Obstet Hum Reprod 2017;46:267-273.
25. **Wang XM, Ma ZY, Song N.** Inflammatory cytokines IL-6, IL-10, IL-13, TNF-α and peritoneal fluid flora were associated with infertility in patients with endometriosis. Eur Rev Med Pharmacol Sci 2018;22:2513-2518.
26. **Senashova O, Reddy AP, Cameron JL, Bethea CL.** The effect of citalopram on midbrain CRF receptors 1 and 2 in a primate model of stress-induced amenorrhea. Reprod Sc. 2012;19:623-632.
27. **Berent D, Zboralskı K, Macander M.** Antioxidant properties of cerebrolysin–an old drug with newly discovered capabilities. The Polish Journal of Aviation Medicine and Psychology. 2014;20:25.
28. **Ince S, Ozer M, Goktug Kadioğlu B, Kuzucu M, Karahan Yilmaz S, Özkaraca M, Gezer A, Suleyman H.** The effect of adenosine triphosphate on bevacizumab-induced ovarian damage and reproductive dysfunction in rats. Gen Physiol Biophys. 2021;40:71-78.
29. **Ince S, Ozer M, Kadioglu BG, Kuzucu M, Ozkaraca M, Gezer A, Suleyman H, Cetin N.** The effect of taxifolin on oxidative ovarian damage and reproductive dysfunctions induced by antipsychotic drugs in female rats. J Obstet Gynaecol Res. 2021;47:2140-2148.