

The effect of maternal separation stress-induced depression on ovarian reserve in Sprague Dawley Rats: The possible role of imipramine and agmatine through a mTOR signal pathway

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ABSTRACT

Purpose: To examine the possible role of imipramine and agmatine through a mTOR signal pathway on rat ovary after maternal separation stress-induced depression.

Methods: Sprague Dawley neonatal female rats were divided into control, maternal separation (MS), MS+imipramine, and MS+agmatine groups. Rats were subjected to MS for 4 hours daily from postnatal day (PND) 2 to PND 21 and pups were exposed to social isolation (SI) on PND23 for 37 days for model establishment treated with imipramine (30 mg/kg; ip) or agmatine (40 mg/kg; ip) for 15 days. In order to examine behavioral changes rats were all subjected to locomotor activity and forced swimming tests (FST). Ovaries were isolated for morphological evaluation, follicle counting and mTOR signal pathway protein expression levels were detected. **Results:** Increased number of primordial follicles and diminished ovarian reserve in the MS groups were detected. Imipramine treatment caused diminished ovarian reserve and atretic follicle; however, agmatine treatment provided the maintenance of ovarian follicular reserve after MS. mTOR signal pathway may have an important role during rat ovarian follicular development in model of MS.

Conclusions: Our findings suggest that agmatine may help to protect ovarian reserve during follicular development by controlling cell growth.

1. Introduction

Major depressive disorder (MDD) is a common type of depression, diagnosed in almost 20% of the population and its prevalence in adolescents has risen up by 47% from 12% between 2013 and 2016 [41]. A major cause of depression is early life stress, maternal separation as low maternal care increases depressive-like behavior with repeated MS [26]. Maternal separation (MS), one of the common types of depression, is a widely used paradigm in rodents to examine how early life adversities can alter susceptibility to later life challenges and contribute to individual vulnerability or resistance to psychopathologies such as anxiety, depression, or addiction [13].

Recent models of depression suggest that major depressive disorder (MDD) accelerates biological aging by making connections between depression and cellular aging markers. However, it is not yet known whether depression is one of the underlying causes of ovarian aging in the reproductive system. One of the epidemiological studies

emphasizing the link between depression and ovarian aging, the Harvard Study of Moods and Cycles has shown that women with MDD have a 20% increase in perimenopause [18]. Also, according to the Hamilton Rating Scale for Depression, the risk of perimenopause in those with more severe depression symptoms and antidepressant drug use is increased [8].

Agmatine, decarboxylated arginine, is an endogenous cationic polyamine that is widely but unevenly distributed in mammalian tissues and has been recognized as a novel neurotransmitter and/or neuromodulator exerting various biological actions in the brain [32]. It has been reported by several experimental studies that agmatine produces anti-stress and antidepressant-like effects [15]. Besides, in a recent pilot clinical study, the treatment with agmatine was shown to produce antidepressant effects in patients with depression [37]. However, the exact mechanisms underlying this effect remain unclear [15]. Imipramine, a widely used antidepressant is known to cause cell death of malignant tumor cells by apoptosis and inhibit norepinephrine and

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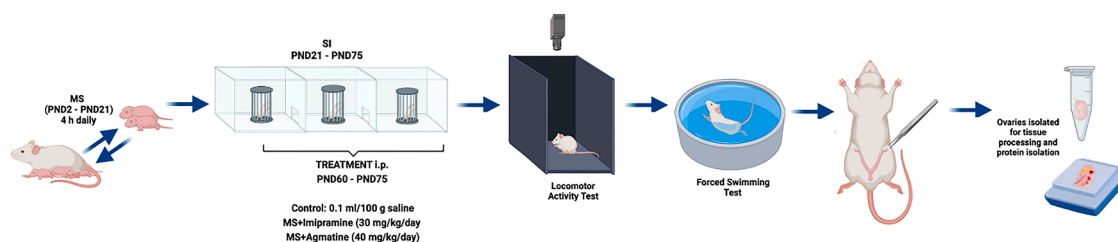


Fig. 1. Experimental design of the study.

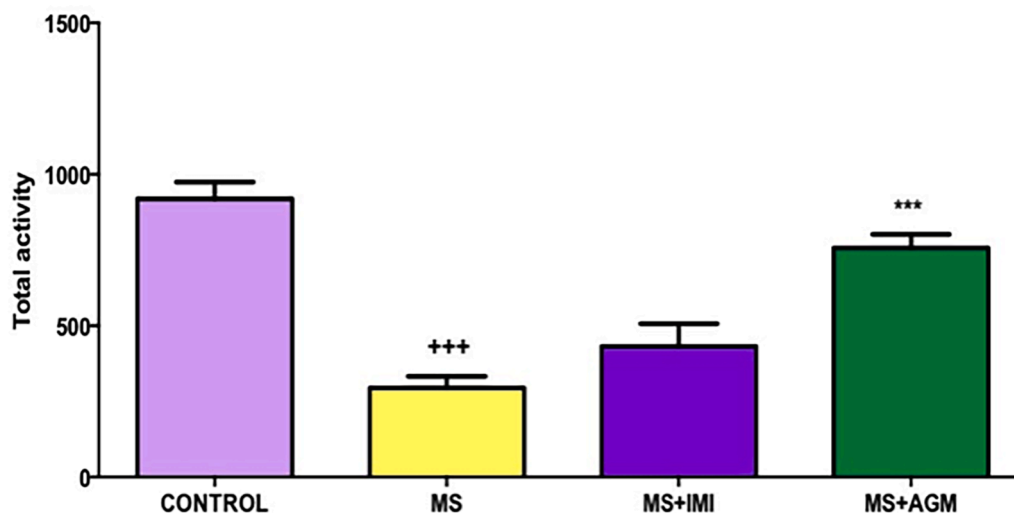


Fig. 2. Effect of MS, imipramine and agmatine treatments on locomotor activity. The locomotor activity test was performed for 5 min, and data is represented as mean $\pm$ standard error ($n = 6$ /group). +++ $p < 0.001$; compared with the Control group, *** $p < 0.001$; compared with the MS group. Maternal separation: MS; Imipramine: IMI; agmatine: AGM.

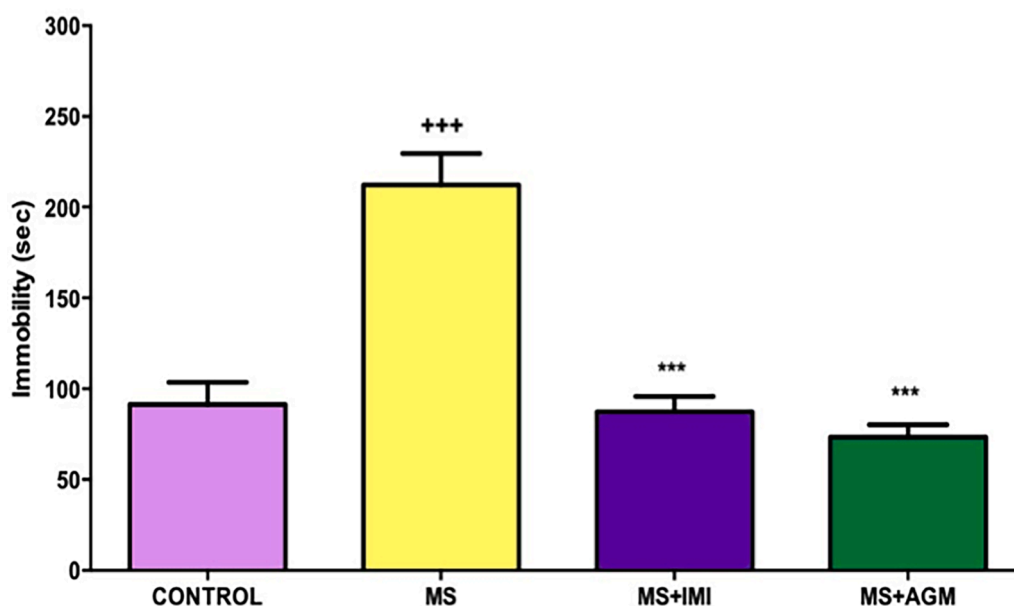


Fig. 3. Effect of MS, imipramine and agmatine treatments in FST. Data is represented as mean $\pm$ standard error ($n = 6$ /group). +++ $p < 0.001$; compared with the Control group, *** $p < 0.001$; compared with the MS group. Maternal separation: MS; Imipramine: IMI; agmatine: AGM.

5-hydroxytryptamine (serotonin) reuptake and it has been stated that imipramine treatment reduced mTOR signaling [19].

The mammalian target of rapamycin (mTOR) signal pathway regulates cell proliferation, growth, survival, apoptosis and autophagy by

participating in multiple signaling pathways in the cell [21]. mTOR signal pathway is essential during folliculogenesis, such as mitotic division of granulosa cells [44], follicle survival, development, and activation [12]. It has been shown that by inhibiting mTOR in ovarian

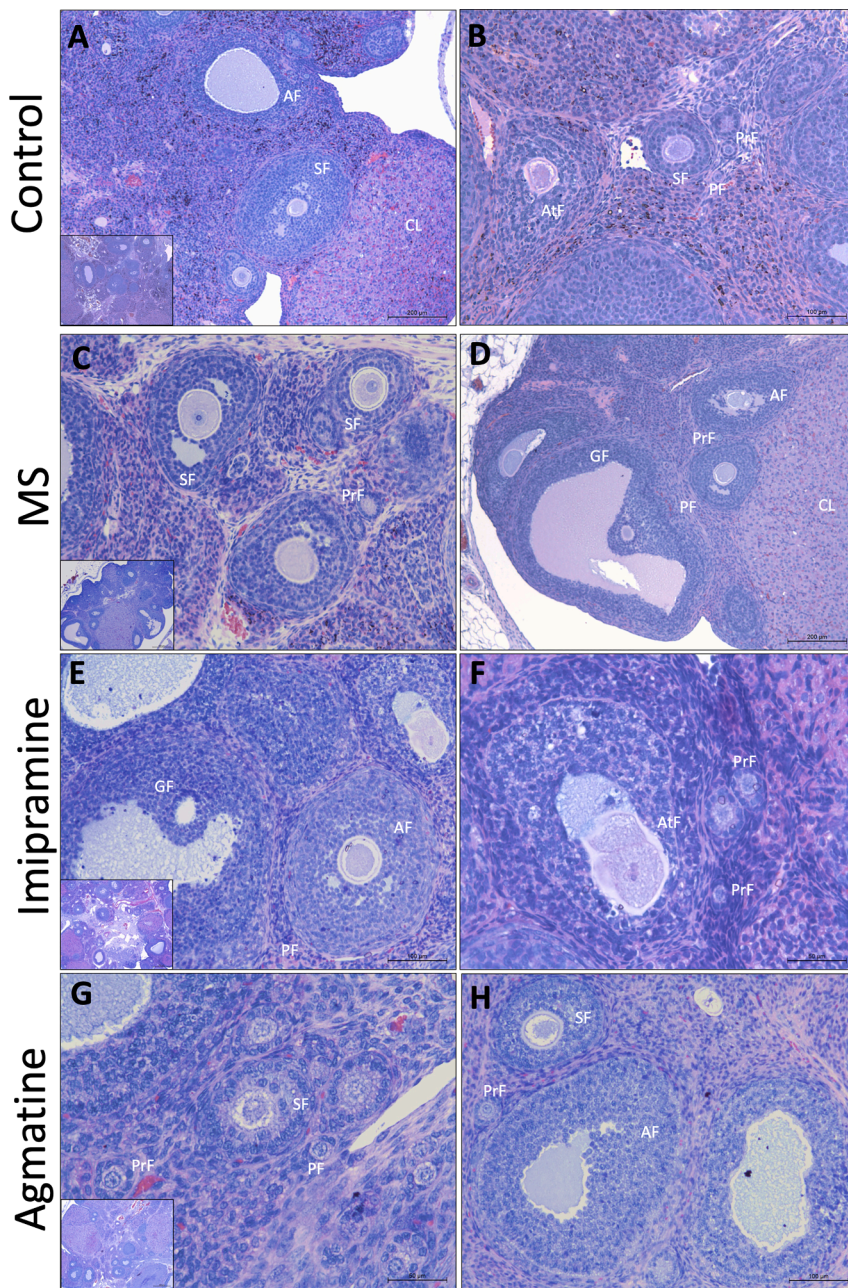


Fig. 4. Morphological evaluation of hematoxylin and eosin stained ovaries. A-B. Follicular structures of the control group showing normal ovarian morphology C-D. Maternal separation group E-F. imipramine-treated group G-H. agmatine treated group. Primordial follicle (P), and primer follicle (PrF), secondary follicle (SF), antral follicle (AF), Graafian follicle (GF), atretic follicle (AtF) and corpus luteums (CL), MS:maternal separation. Original magnification $\times 100$ for panel D; $\times 200$ for panels A-C, F, H; $\times 400$ for panels E, G and all inserts are $\times 40$.

follicles and granulosa cells, follicle growth was reduced, and granulosa proliferation was compromised [45]. During folliculogenesis, as a result of mTORC1 stimulation, the primordial follicles might be over-activated and might cause premature depletion of ovarian reserve [12]. A protein kinase found in the downstream of mTOR pathway is P70S6K, which is activated by phosphorylation at numerous sites and is responsible for cell cycle control, cell motility during tissue repair, immune response, and tumor metastases [7].

In this study, we hypothesized that early life stress may play a role in the reduction of ovarian reserve in female rats and that antidepressant drugs used for the treatment of stress may also have an effect on the ovarian reserve. Therefore, we aimed to evaluate the effect of early life stress on ovarian follicle pool size after the administration of agmatine and imipramine, and to perform this evaluation through mTOR and its downstream effector p70S6K.

2. Materials & methods

2.1. Animals and study design

All animals used in this study were obtained from Yeditepe University Faculty of Medicine Experimental Research Center (YÜDETAM) and all the experimental procedures have been approved by Yeditepe University Ethical Committee. This manuscript complies with the ARRIVE guidelines [30]. All procedures were carried out under the supervision of veterinarians. All experiments were performed in accordance with guidelines and regulations of Faculty of Medicine of Yeditepe University. Pregnant females were individually housed with sawdust until delivery. Rat pups were separated from their mothers for 4 h daily from PND 2 to PND 21 [11,16]. The pups were then returned to their home cage and cared for by their mothers. On day 21 mothers and pups were placed in separate cages and socially isolated until the last day of the experiment. On day 60, maternal separated animals were treated with

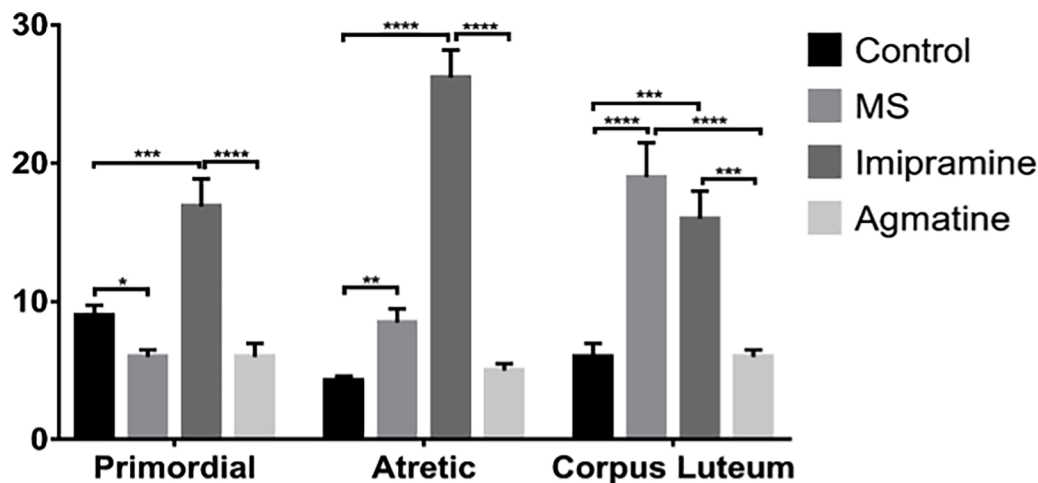


Fig. 5. Follicle counting results. Comparison of the number of primordial follicle, atretic follicle and corpus luteum with control, MS, imipramine and agmatine treated groups. The number of primordial follicles and atretic follicles increased significantly in the imipramine group. Corpus luteum increased significantly in the imipramine and MS group (**** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.1$).

agmatine or imipramine for 15 days. Female pups were divided into four groups (6 rats per group) as follows: (i) the control group (non-separated rats; received only saline), (ii) the MS group (maternal separation (MS) rats; received only saline), (iii) the MS+AGM: MS+agmatine group (MS rats received 40 mg/kg/day i.p) [42], and (iv) the MS+IMI: MS+imipramine group (MS rats received 30 mg/kg/day i.p) [46] (Fig. 1).

2.2. Drugs and reagents

Imipramine hydrochloride (I7379) was purchased from Sigma-Aldrich® Co Ltd (St. Luis, Missouri, USA) and given at 30 mg/kg/day doses [4]. Agmatine sulfate (A7127) was purchased from Sigma-Aldrich® Co Ltd (St. Luis, Missouri, USA) and given at 40 mg/kg/day doses [1]. Imipramine and agmatine were dissolved in saline (0.9%) and injected intraperitoneally [28]. All drug solutions were freshly prepared each day before the administrations.

2.3. Locomotor activity test

The locomotor activity test is one of the most traditional and simplest test used in drug treatments and experimental model studies affecting the central nervous system. The locomotor activity test and the many behaviors that can be evaluated during this test can be used for different purposes. There are two reasons for using the locomotor activity test and evaluating the total activity in experimental animals in this study: i) To see if there is a change in activity compared to control animals in the depression model created by the MS model, and ii) To evaluate whether the applied treatments reverse the changes caused by MS depression model on locomotor activity. For this purpose, in 1×1 meter dimension box with walls of 0.5 m height to assess general locomotor activity of rats. The floor of the chamber was divided into 25 equal square quadrants. Locomotor activity was measured for 20 mins which was recorded in 5 mins intervals by video tracking.

2.4. Forced swimming test (FST)

FST which is one of the most commonly used assays for the study of depressive-like behavior in rodents described by Porsolt [5]. The test is widely used in experimental models of depression as it involves the exposure of animals to stress, which has been shown to play a role in the tendency to major depression, and is a screening test to determine whether drugs have the potential for antidepressant effects. The FST is based on the assumption that when placing an animal in a container

filled with water, it will first make efforts to escape but eventually will exhibit immobility that may be considered to reflect a measure of behavioral despair. Therefore, in this study, the effect of MS (Maternal Separation) and the treatments on despair-like behaviors was assessed by FST. In FST, the main assessment parameter is the time of immobility as an indicator of despair-like behavior which is defined as the small movements only necessary for keeping the head above the water surface or in other terms, the moments in which active swimming is not presented. The prolonged time of immobility is associated with despair-like behavior whereas reduced time is an indicator of antidepressant action. When applied to rats, FST consisted of training and test sessions performed at a 24-h interval.

In this study, rats were gently placed into cylinder plexiglass (40 cm high, 14 cm diameter) filled with constant and neutral heated water ($25 \pm 1^\circ\text{C}$) and allowed to swim for 15 min for the training session. The height of the filled water was adjusted to prevent escaping and clinging by the tail (approx. 30 cm). At the end of the swimming session, rats were removed from the cylinder, gently dried with a soft towel, and put in a warm cage before being returned to their housing conditions. The water was replaced after each session for the next rat. 24 h following the training, rats were allowed to swim under the same conditions for 5 min and their movements were video-recorded. An experienced experimenter blind to the treatment conditions video-scored the time of immobility during the 5-min test session. The time of immobility was defined as the total moments lacking motion of the whole body except for the small movements necessary to keep the animal's head above the water.

2.5. Morphologic evaluation

Ovarian tissue samples from each group were fixed in 10% formaldehyde for 6 h. Afterwards samples dehydrated in each alcohol series (70%, 80%, 90%, and 100%) for 24 h, cleared in xylene and embedded in paraffin. 5 μm sections were taken from paraffin blocks and deparaffinized in xylene and rehydration in a graded series of ethanol. Afterwards sections were stained with Hematoxylin (Bio-Optica, Via San Faustino, Milano, Italy) and Eosin (Bio-Optica, Via San Faustino, Milano, Italy) staining and examined under a light microscope (Nikon Eclipse i5, Tokyo, Japan).

2.6. Ovarian follicle counting

The follicular organization in the mouse ovary begins after birth and the classification of the follicles is based on the number of granulosa cell

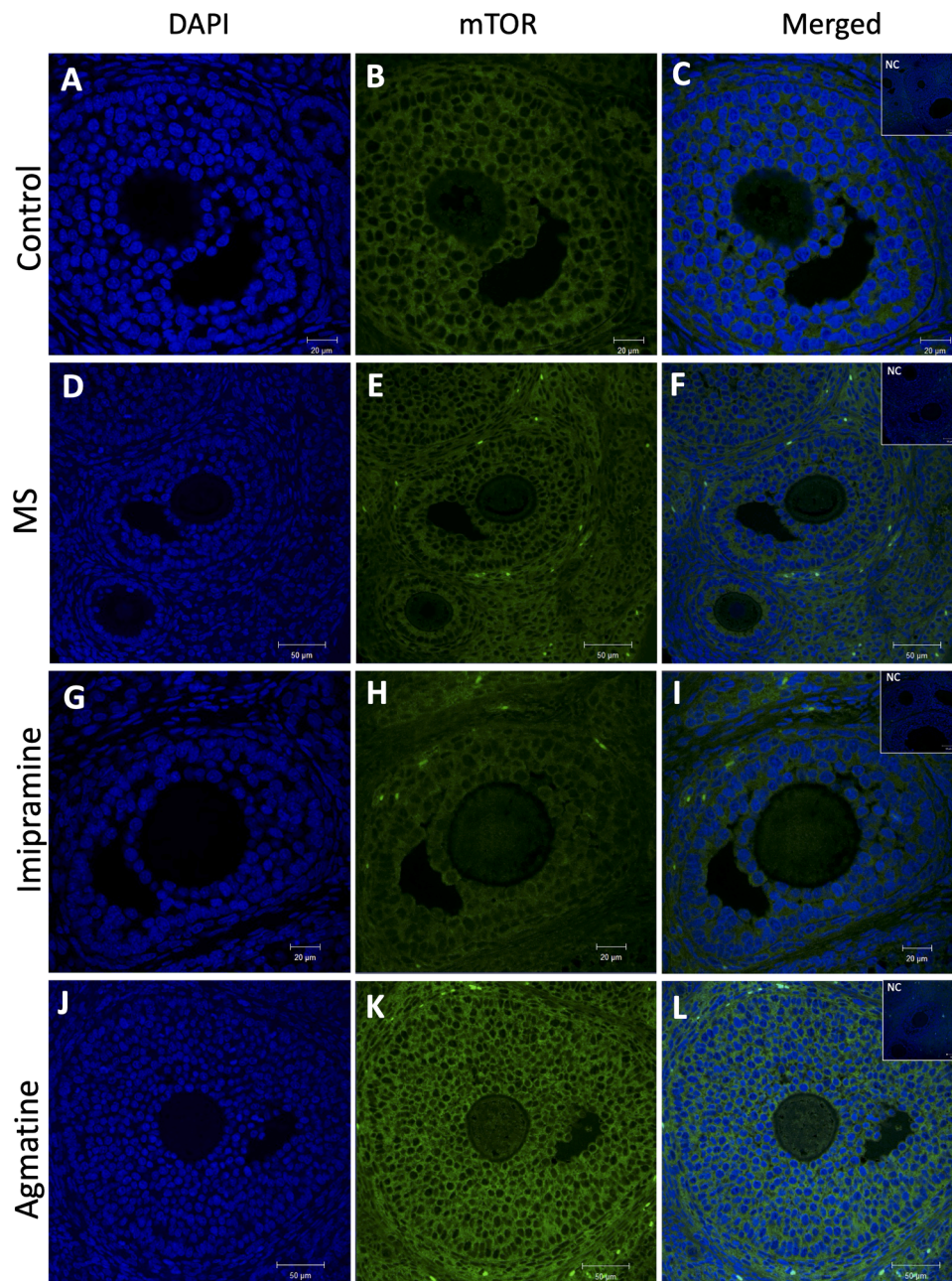


Fig. 6. Immunofluorescence staining for mTOR in control, MS, imipramine and agmatine group ovaries. mTOR localization in control ovaries (A-C), MS ovaries (D-F), imipramine-treated ovaries (G-I) and agmatine treated ovaries (J-L). DAPI was used for nucleus staining. mTOR: Green, DAPI: Blue.

layers surrounding the oocyte. Primordial follicle is defined as surrounded by single-layer, flat follicular cells surrounding the primary oocyte [45]. Follicles in which the connection between the granulosa layers were lost, pycnotic granulosa cells were seen, the antrum and theca layers were occupied by cells with abnormal morphology, and degenerated oocyte were considered as atretic follicles [43]. The corpus luteum structure, in which large granulosa luteal cells and small theca-luteal cells were formed, was determined [38]. Only follicles containing an oocyte with a visible nucleus were counted to avoid double counting. The total numbers of primordial and atretic follicles and corpus luteum were calculated for analysis. The ratio was obtained by dividing the total counted follicles by the number of sections of the counted group.

2.7. Immunofluorescence analysis

5 μ m ovarian sections were incubated at 37 °C overnight and then at 60 °C for 1 hour. Following incubation, the slides were passed from xylene and decreasing alcohol series. After wash in distilled water and PBS incubation, slides were boiled in EDTA for antigen retrieval and were washed in PBS then incubated in 3% of hydrogen peroxide endogenous peroxidase enzyme blockage. Afterwards slides were incubated in 5% Normal Goat Serum (NGS) Blocking Solution (Vector Labs, S-1000) in order to block non-specific antigen binding. Next the slides were incubated at 4 °C for overnight in mTOR (1:100 Cell Signaling, #2983) and P-mTOR primary antibodies (1:50, Cell Signaling, #5536). After washing with TBS-T, slides were incubated with Goat anti-Rabbit IgG (H + L) secondary antibody, Alexa Fluor 488 (1:250, ThermoFisher, #A-11,008) and washed again with TBS-T. Sections were mounted with a DAPI mounting medium and visualized with a Confocal microscope

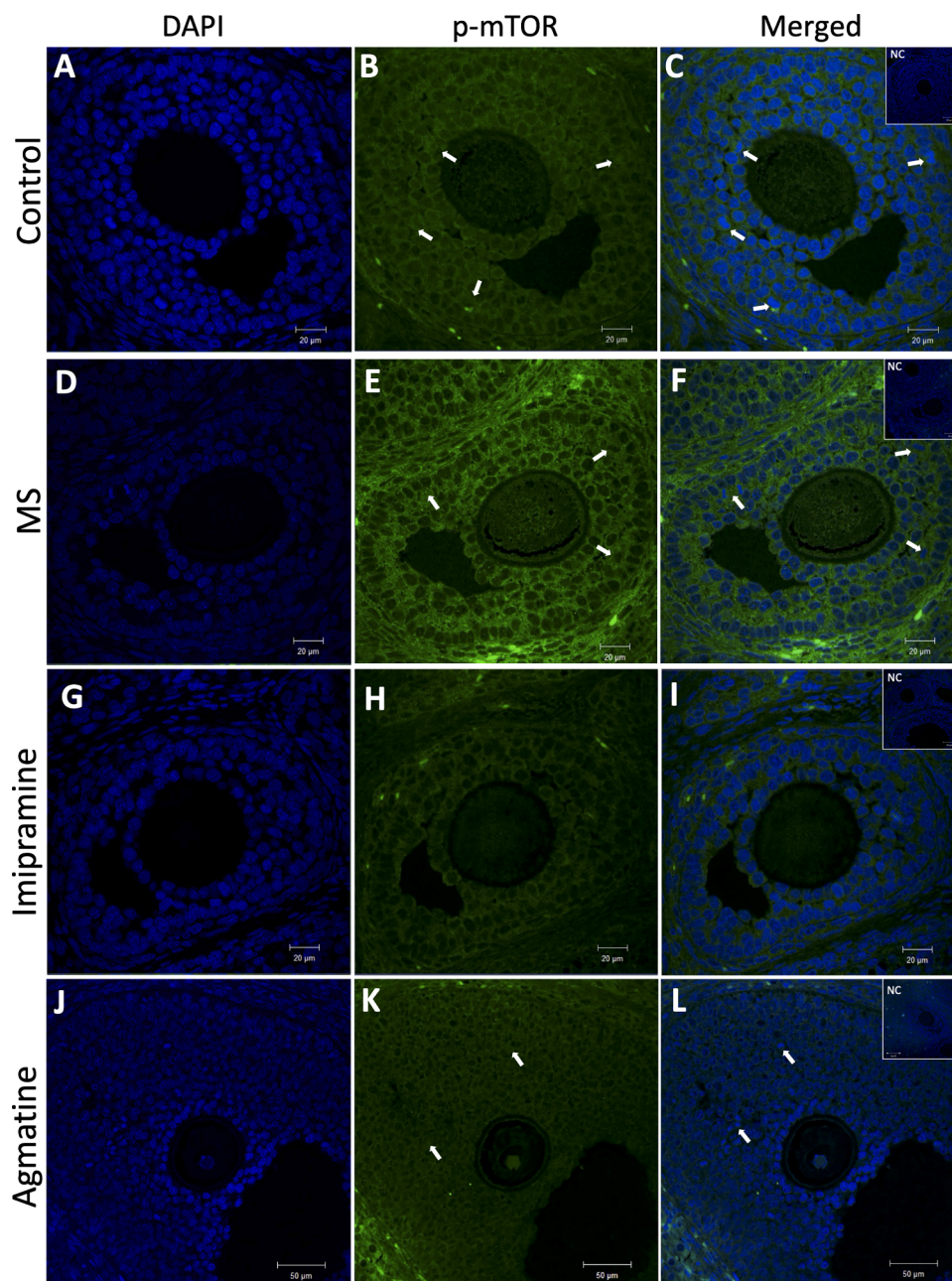


Fig. 7. Immunofluorescence staining for P-mTOR localization in control, MS, imipramine and agmatine group ovaries. P-mTOR localization in control ovaries (A-C), MS ovaries (D-F), imipramine-treated ovaries (G-I) and agmatine treated ovaries (J-L). Arrows indicate the P-mTOR expressing dividing granulosa cells.

(Zeiss, LSM780).

2.8. Western blot

Total protein from the ovary tissues from each group were extracted using RIPA lysis buffer, supplemented with protease inhibitor cocktail. The protein concentration was determined by BioTek SynergyHT microplate reader (using Gen5 software). Briefly, 20-µg of protein was loaded into each lane, separated electrophoretically by NuPAGE 4–12% Bis-Tris gel, and electroblotted onto nitrocellulose membrane. The membrane was blocked with 5% nonfat dry milk in TBS-T buffer (0.1% Tween-20 in Tris-buffered saline) for 1 h to reduce the non-specific binding. The membrane was then incubated with rabbit polyclonal mTOR antibody (1:1000 dilution; Cell Signaling Technology, Danvers, MA, USA) overnight at 4 °C, and washed three times with TBS-T for 20

min. Then, the membrane was incubated for 1 h with peroxidase-labeled goat anti-rabbit IgG (Pierce; USA) and subsequently washed with TBS-T three times for 15 min. mTOR protein expression was detected using chemiluminescence detecting reagents (Perkin Elmer Life Sciences, Boston, MA, USA) and visualized with CCD camera and software (Bio-Rad). We performed the procedure explained above for mTOR Rabbit mAb (1:1000, Cell Signaling #2983), P-mTOR Rabbit mAb (1:1000, Cell Signaling #5536), p70S6K (1:1000, Cell Signaling #2708), P-p70S6K (1:1000, Cell Signaling, #9234), and β-actin (8H10D10) mouse monoclonal antibody (1:5000, Cell Signaling #3700) primary antibodies. β-actin is used as internal control. All experiments were repeated at least three times, and the intensity of the signals was analyzed using NIH ImageJ.

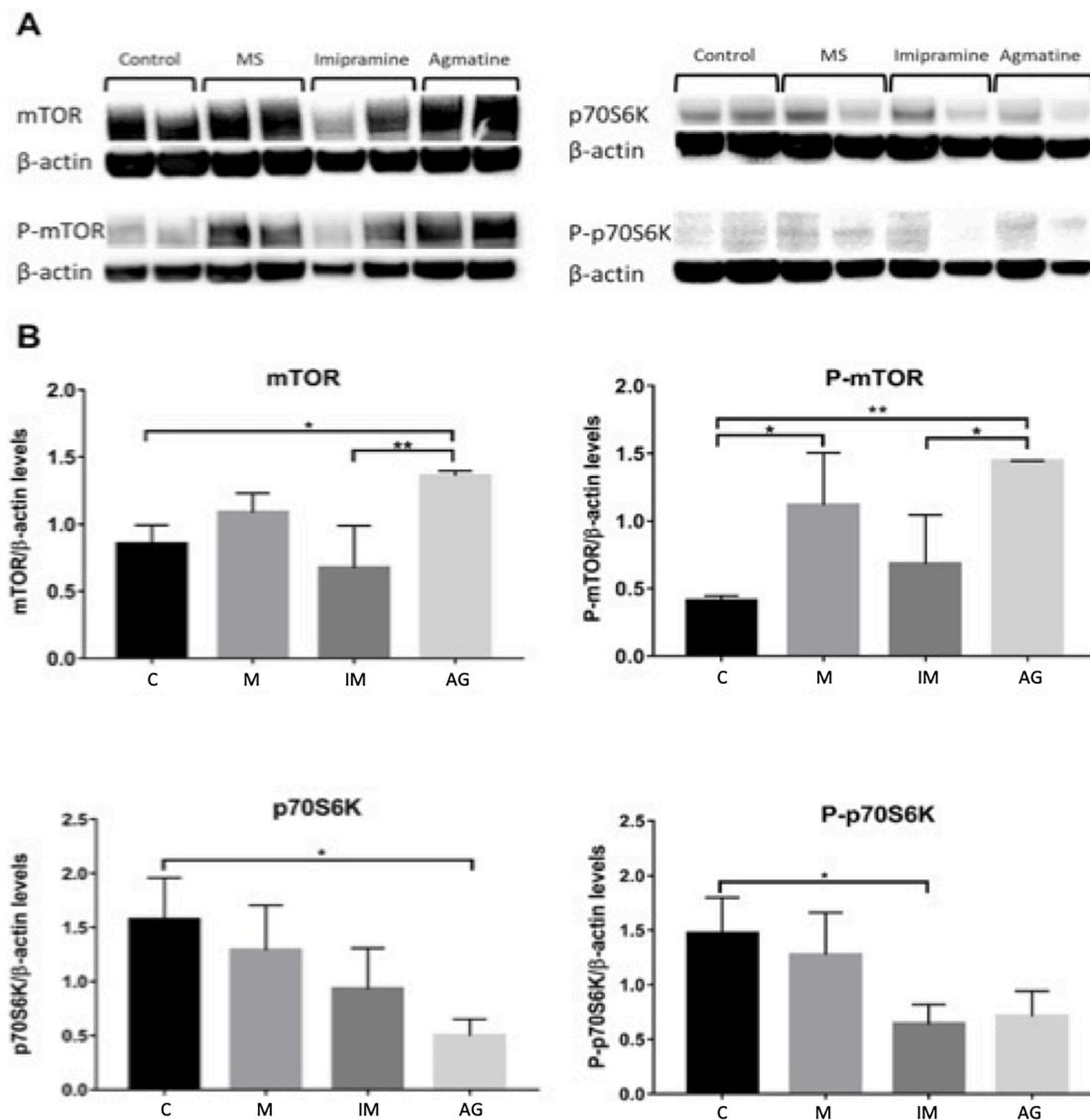


Fig. 8. Expression of mTOR signal pathway. **A.** Western blot analysis results of mTOR, P-mTOR, p70S6K and P-p70S6K in control, MS, agmatine and imipramine groups rat ovaries **B.** Statistical analysis of mTOR, P-mTOR, p70S6K and P-p70S6K western blot results. Protein levels were normalized to β-actin. Data is presented as means ± standard deviation (* $p < 0.01$, * $p < 0.1$).

2.9. Statistical analysis

Data were analyzed by using GraphPad Prism 7 (GraphPad Software, USA) were expressed as mean ± standard deviation. A one-way analysis of variance (ANOVA) was used to determine the main effects of MS and compared with imipramine and agmatine treatment groups. Normally distributed data was analysed by Student's *t*-test to compare two groups and ANOVA with Tukey's post-hoc test to compare multiple groups. A *p* value lower than 0.05 was considered statistically significant.

3. Results

3.1. Results of locomotor activity tests

In the locomotor activity test, the maternal separation (MS) group had a significantly lower activity compared to the control group ($p < 0.005$). Treatment with agmatine significantly increased this activity ($p < 0.005$). The chronic administration of imipramine had only very limited effects on their behavior and did not demonstrate any significant effects of the drug treatment, and the activity of rats was only slightly increased. The imipramine treatment was found to be ineffective

compare to MS group (Fig. 2).

In FST, the time of immobility was significantly increased in the MS group ($p < 0.005$) compared to the non-stressed control group. On the other hand, chronic treatment with imipramine ($p < 0.005$) and agmatine ($p < 0.05$) resulted in a reduction in the immobility time compared to the MS group (Fig. 3). The significant reduction in the duration of immobility was observed in imipramine and agmatine groups to compare MS group. These results indicate that immobility is effected after chronic treatment with imipramine and agmatine.

3.2. Morphological evaluation

Ovaries from each experimental group were compared morphologically. Serial sections were taken from the ovaries for follicle counting and stained with hematoxylin-eosin. While making follicular comparison, primary follicles were identified by flat follicular cells surrounding the oocyte. Atretic follicles were defined according to the degenerative oocyte, irregular volume and distorted shapes it contains [43]. In addition, corpus luteum with signs of ovulation in each ovary was compared morphologically for the four experimental groups (control, MS, imipramine and agmatine).

Primordial, primary, secondary, and antral follicles were detected in the control group morphology. However, Graafian follicles were not observed in some of the ovaries. In addition to these, atretic follicles were also existent in all ovaries, and their corpus luteum numbers were consistent with the average of 7. Apoptosis and hemorrhaging were also detected as normal appearance in the control group (Fig. 4. A-B). It was detected that the number of primordial follicles were decreased the MS group ovaries. In addition, the morphology of developing follicles (primary, secondary and antral follicle) were normal. Graafian follicles were observed in all the ovaries, atretic follicles were existent in four out of five ovaries and the number of corpus luteum were the average of 7. Hemorrhage was observed in the middle of the two ovaries (Fig. 4. C-D). We detected increased number of primordial and atretic follicles in imipramine treated group, and the number of corpus luteum were varied with the average of 9.8. Hemorrhage occurred in the middle of CL (Fig. 4. E-F). We detected almost same number of primordial follicles, developing follicles, atretic follicles and corpus luteum (average of 6.8) (Fig. 4. G-H).

3.3. Follicle counting

Follicle counting showed that primordial follicles decreased in the MS group ($*p<0,5$) while atretic follicles increased ($**p<0,05$) compared to the control group (Fig. 5). The number of primordial follicles in imipramine treatment group was determined higher than the agmatine treated group ($****p<0,0005$) (Fig. 5).

When the maternal separation group was compared with the control group, increased CL numbers were detected, which is a sign of ovulation in the maternal separation group ($****p<0,0005$) (Fig. 5). Afterwards, when agmatine treatment was compared with imipramine treatment, an increased number of CL in the imipramine treated rat ovary was observed ($***p<0,005$) (Fig. 5). In summary, according to morphological analysis results, it can be said that maternal separation depression is very effective on follicular development and causes rapid decrease of ovarian follicle reserve in rat ovary.

3.4. m-TOR and P-mTOR, protein localization during folliculogenesis

Immunofluorescence staining was performed in order to detect the localization of mTOR and P-mTOR in oocyte and granulosa cells for each ovary from each experimental group. Cytoplasmic mTOR expression was detected in oocyte and granulosa cells in rat ovary from each experimental group (Fig. 6). When we evaluate mTOR immunofluorescence staining from control group (Fig. 6A-C), MS group (Fig. 6D-F), imipramine group (Fig. 6G-I) and agmatine group (Fig. 6F-L), we did not detect any staining difference or intensity between experimental groups in both granulosa cells and oocyte. P-mTOR expression was observed specifically in the dividing granulosa cells (Fig. 7). We also did not observe staining difference or intensity for P-mTOR in control group (Fig. 7A-C), MS group (Fig. 7D-F), imipramine group (Fig. 7G-I) and agmatine group (Fig. 7J-L) for granulosa cells. P-mTOR expressed granulosa cells were marked with white arrows.

3.5. m-TOR, P-mTOR, p70S6K and P-p70S6K protein expression in experimental groups

P-mTOR protein expression levels increased in the MS group compared to the control group ($*p<0,1$) (Fig. 8). mTOR and P-mTOR protein expression levels were significantly higher in the agmatin group compared to the control group ($**p<0,05$, $*p<0,5$) (Fig. 8); however, when the agmatin and imipramin groups were compared between them, mTOR and P-mTOR expressions were higher in the agmatin group ($**p<0,05$, $*p<0,5$) (Fig. 8). p70S6K protein expression level was significantly decreased in the agmatin group compared to the control group ($*p<0,5$) (Fig. 8). It was shown that P-p70S6K protein expression level was significantly lower in the imipramin group compared to the

control group ($*p<0,5$) (Fig. 8).

4. Discussion

The female reproductive system is very sensitive to stressors and MS stress-induced depression models can cause dysfunctions of the reproductive system such as decreased follicle reserve, disruption of the estrus cycle, and infertility. In this study, we aimed to compare imipramine, which has been one of the most widely used antidepressant drugs in the clinic for many years, with agmatine, whose antidepressant potential has been investigated in recent years on ovarian reserve after MS stress-induced depression. The findings of our study showed that MS stress led to decreased follicle reserve and agmatine may help to protect ovarian reserve during follicular development by controlling cell growth through the mTOR signaling pathway.

Studies have shown that the mother's MS stress has significant effects on the female reproductive system and reduces the number of primordial, primary, and secondary follicles, oocyte number, and diameter [3]. Female rodents present estrous cycle disorders which can be induced in response to repeated stress situations [2,9,24]. A study showed that hypoxic-ischemic-maternal separation (HI-MS) rats showed a more frequent absence of estrus during the estrus cycle but the length of the estrus cycle of the repeated maternal separation did not change [14]. Another study suggested that chronic unpredictable mild stress lengthens the estrous cycle to 6–7 days in female rats due to the prolonged diestrus phase [24]. However, Lau et al. showed that repeated maternal deprivation stress does not affect either on estrous cycles or vaginal opening [33]. The stress of separation from the mother caused significant morphological changes in ovarian tissue, and the decrease in the number of primordial follicles increased the number of secondary and Graafian follicles [3]. In addition to reducing the number of primary follicles, stress likely accelerates the transition of primary follicles to secondary and Graafian follicles [20]. Parental separation stress from the mother can reduce ovarian reserve and shorten female reproductive life [17]. The studies showed that agmatine and imipramine present effective role in rodent estrus cycle [25,27,47,40]. In our study, we showed that rats separated from their mothers as offspring have a decreased number of primordial follicles, an increased number of atretic follicles and corpus luteum in the MS group. Imipramine treatment caused increased primordial follicle activation and the number of atretic follicles. Therefore, we suggested that imipramine treatment may shorten reproductive life with increased follicle activation and early ovarian aging. In light of all of this information, agmatine treatment can slow down ovarian aging and prevent rapid diminish of ovarian follicle reserve and thence it may maintain follicle reserve in rats after MS stress.

In MS studies, offspring experience recurrent maternal absence in the early PND [6]. This separation can be performed for short or long periods ranging from a few minutes (e.g. less than 30 min, usually less than 15 min) to hours (e.g. 3 h or 4 h) [22]. One of the depression-like findings is a decrease in locomotor activity. We observed a significant decrease in the motor activity of rats in the MS model in this study is one of the key findings confirming depression and thus showing the validity of the model. On the other hand, the finding that decreased locomotor activity was normalized with agmatine treatment while imipramine failed to be effective, showed that agmatine treatment could suppress depression symptoms more effectively compared to imipramine. In terms of behavior, another main finding in our study was that the duration of inactivity increased significantly in the MS group compared to the control group without stress as a result of the FS test. The chronic treatment with imipramine and agmatine resulted in a significant reduction in the immobility time compared to the MS group. The depressive-like responses in MS rats are in agreement with literature data that show behavioral despair with increased despair immobility time in the FS test. Our study showed that imipramine reverses depressive-like behavior in the MS model. We have previously shown that agmatine when administered acutely at a dose of 40 mg/kg as,

elicited antidepressant and anxiolytic-like effects; but to our knowledge, this is the first study to show that agmatine also reverses despair immobility time in MS rats. We showed before for the first time that acute agmatine administration in rats reversed sub-chronic restraint stress [34]. The present findings therefore could provide novel insights for future translational studies addressing the role of agmatine in early life stress/depression and other stress-related disorders. mTOR signal pathway presents extracellular and intracellular signals and serves as a central regulator of cell metabolism, growth, and proliferation [21] and it is essential during folliculogenesis [44], follicle survival, activation, and development [12]. We showed before that by inhibiting mTOR in ovarian follicles and granulosa cells, follicle growth was reduced, and the proliferation of granulosa cells was compromised [45]. In rats that were induced to develop insulin resistance by High Fat Diet (HFD), mTOR expression was elevated; but in agmatine-treated rats, agmatine reduced HFD-induced elevation in the expression of mTOR significantly but made no difference in mTOR levels of control animals [35,39]. Additionally, agmatine produces antidepressant-like effects by activating AMPA receptors and mTOR signaling since decreased immobility times of animals in tail suspension tests increase with rapamycin treatment that inhibits mTOR. In addition, TST immobility time also decreases with DNQX (AMPA antagonist), BDNF antibody, K-252a (TrkB antagonist), and Ly294002 (PI3K inhibitor). These results showed that the antidepressant effect of agmatine in the tail suspension test might depend on the activation of TrkB and AMPA receptors, mTOR and PI3K signaling along with the increase in synaptic proteins and inhibition of GSK-3 β . Additionally, BDNF treatment activates TrkB and cyclinD1-creb signaling to stimulate cell proliferation in the ovary after 10 consecutive days of daily ip injection of rhBDNF rescued ovarian function in aged mice. Targeting BDNF-TrkB signaling may help to reverse ovarian aging [23]. The Ab4B19, an agonistic antibody for the BDNF receptor TrkB, activates TrkB signaling restores the number and quality of oocytes, and ameliorates fertility in aged and cyclophosphamide-induced premature ovarian failure mouse models [31]. The antidepressant-like effect of agmatine was completely prevented by the treatment of animals with rapamycin and a lack of mTOR signaling in major depression patients occurs [29].

In a previous study, imipramine presented in obstruction of cholesterol trafficking through lysosome by imipramine in endothelial cells leads to decreased activity of mTOR. Imipramine has been shown to cause accumulation of cholesterol in the late endosome and lysosome. The epithelial cells show a dose-dependent inhibition of P70S6K phosphorylation while mTOR activity inhibition by imipramine indicates that to block the mTOR pathway, inhibition of intracellular cholesterol trafficking might be enough on its own [36]. As a tricyclic antidepressant, imipramine induces autophagic cell death in U-87MG glioma cells. Imipramine treatment to U-87MG cells resulted in the induction of cell death and inhibition of PI3K/Akt/mTOR signaling reduction of clonogenicity. Imipramine, as well as dephosphorylated p70S6K, is a downstream target of mTOR. Therefore, imipramine induces cell death through induces autophagy [19]. Also in another study, it had been shown that pretreatment of luteal steroidogenic cells with imipramine, which is an acid sphingomyelinase activity inhibitor, weakens sFasL-induced ceramide production [10]. Additionally, ceramide is associated with the downregulation of AKT signaling and this leads to the downregulation of mTOR activity, which in turn leads to the induction of apoptosis and the arresting of cell growth.

In conclusion, we suggested that rats that were separated from their mother as pups present increased follicle activation and diminished ovarian reserve. Diminished ovarian reserve is defined as a reduction in the number and/or quality of follicles in the ovary. The ovaries lose their normal reproductive and hormonal function, cause impairment of fertility. Imipramine treatment as an antidepressant caused diminished ovarian reserve with an increased number of developing follicles and atretic follicles. In contrast, agmatine treatment provided the maintenance of ovarian follicular reserve after MS. Furthermore, we showed

that mTOR and P-mTOR (Serine 2448) may play an important role during ovarian follicular development in a model of maternal separation. We detected increased mTOR and P-mTOR expression with decreased p70S6K and P-p70S6K expression in the agmatine-treated group. Our findings suggest that agmatine may help protect the ovarian reserve in the process of follicular development by slowing down cell growth. With further investigations, these findings may have important implications for an improved understanding of how the ovarian reserve is influenced by depression and antidepressant usage.

Data availability

The data that support this study will be shared upon reasonable request to the corresponding author.

Ethics and consent

All animal handling and experimental procedures were approved by the Animal Care and Usage Committee of Yeditepe University.

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There is no funding information.

Declaration of Competing Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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