# Modulation of Melatonin Receptors Regulates Reproductive Physiology: The Impact of Agomelatine on the Estrus Cycle, Gestation, Offspring, and Uterine Contractions in Rats

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**Summary**

Agomelatine is a pharmaceutical compound that functions as an agonist for melatonin receptors, with a particular affinity for the MT1 and MT2 receptor subtypes. Its mode of action is integral to the regulation of diverse physiological processes, encompassing the orchestration of circadian rhythms, sleep-wake cycles, and mood modulation. In the present study, we delve into the intricate interplay between agomelatine and the modulation of estrus cycles, gestation periods, offspring numbers, and uterine contractions, shedding light on their collective impact on reproductive physiology. Both *in vivo* and *in vitro* experiments were performed. Wistar Albino rats, divided into four groups: two non-pregnant groups (D1 and D2) and two pregnant groups (G1 and G2). The D1 and G1 groups served as control groups, while the D2 and G2 groups received chronic agomelatine administration (10 mg/kg). Uterine contractions were assessed in vitro using myometrial strips. Luzindole, a melatonin receptor antagonist, was employed to investigate the pathway mediating agomelatine's effects on uterine contractions. In *in vivo* studies, chronic agomelatine administration extended the diestrus phase ($p<0.05$) in non-pregnant rats, prolonged the gestational period ($p<0.01$), and increased the fetal count ($p<0.01$) in pregnant rats. Additionally, agomelatine reduced plasma oxytocin and prostoglandin-E levels ($p<0.01$) during pregnancy. *In vitro* experiments showed that agomelatine dose-dependently inhibited spontaneous and oxytocin-induced myometrial contractions. Luzindole (2 μM) reverse the agomelatine-induced inhibition of myometrial contractions. These findings suggest that agomelatine holds the potential to modulate diverse reproductive parameters during the gestational period, influencing estrus cycling, gestational progression, offspring development, and the orchestration of uterine contractions.

**Key words**

Agomelatine • Myometrial contractions • Gestational age • Number of offspring • Rat pregnancy

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## Introduction

Agomelatine (S20098), marketed as Valdoxan, is a novel antidepressant approved by the European Union Medicines Agency in 2009 [1]. Agomelatine primarily exerts its antidepressant properties by indirectly increasing dopamine and norepinephrine release through its antagonistic effect on the serotonin 5-HT2C receptor, alongside exhibiting a melatonergic effect as a high-affinity MT1 and MT2 receptor agonist [2,3]. Notably, the affinity of agomelatine on melatonine receptors, MT1 and MT2, surpasses even that of melatonin [4]. Owing to its ability to regulate circadian rhythms, agomelatine has been



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classified as a 'rhythm regulating antidepressant' [5].

Previous studies have demonstrated that melatonin receptors are expressed in many central nerves and numerous peripheral tissues, including testis and ovary [6]. In particular, melatonin plays a crucial role in the modulation of the hypothalamic-pituitary-gonadal (HPG) axis, which is a very important regulatory center for animal reproduction in both seasonally breeding animals and non-seasonally breeding animals including humans [7]. It is known that the circadian clock system plays an important role in the biological activities of the gonads, including the ovaries and testicles, and is involved in the regulation of steroid hormone synthesis, oocyte maturation, ovulation, and seasonal oestrus [8,9]. This information raised the question of how agomelatine, a melatonin receptor agonist, would affect the hormones oxytocin and PGE, which are very effective in determining the gestational age. Some studies report that the MT1 receptor is widely distributed in endocrine tissues and brain regions, which are major response sites to melatonin-induced physiological and circadian effects. However, the MT2 receptor is generally absent in the mammalian hypothalamus and pituitary gland [10]. These data suggest that MT1 is a more important receptor for melatonin-modulated reproductive regulation in mammals, and therefore agomelatine may also have an effect on reproductive functions.

It is a matter of curiosity how agomelatine, which is widely used today, has an effect on other systems as well as an antidepressant effect. One of these systems is the reproductive system and there are not enough studies on its effectiveness on reproductive functions. In a study we conducted on male and female rats, we found that agomelatine administered from the 21st day after birth accelerated the entrance to puberty by advancing the vaginal opening in female rats, and delayed puberty by causing a decrease in sperm count and motility in male rats [11]. Again, in this study, we found that agomelatine facilitated sexual behavior by decreasing the frequency of intramission in male sexual behavior tests, while it increased uterine and ovarian weights in female rats [11]. In addition, in another study we conducted with female rats, we found that rats detected in diestrus caused an inhibitory effect on myometrial contractions [12]. In another study in the literature, agomelatine was found to prevent ovarian ischemia/reperfusion injury in rats with overtorsion [13]. This study is another proof that agomelatine may have an effect on the female reproductive system. Based on the findings of our research on the effectiveness of agomelatine on the reproductive system, it is seen that agomelatine has an effect on reproductive functions. However, the fact that no studies have been conducted in the literature on the efficacy of agomelatine on estrus cycle, fetal number and gestation period makes our research subject unique. Within the findings in the literature, we aimed to investigate the effectiveness of agomelatine on some reproductive parameters, especially gestational period and fetal number, since it is in category B in terms of use in pregnancy. To our knowledge, this study provides the first evidence of the effects of chronic agomelatine administration on gestational duration and fetal number in pregnant rats.

Considering this information, our main aim in our study is to reveal the effect of agomelatine on gestational duration, fetal number and myometrial contractions in pregnant rats and its possible effects on cycle cycle parameters in non-pregnant rats. We believe that the data obtained will fill an important gap in the literature by revealing the potential efficacy of agomelatine and provide useful information about its efficacy in clinical use.

## Materials and methods

*Chemicals and reagents*

The antidepressant known by the trade name Valdoxan, whose active ingredient is agomelatine (Valdoxan; produced by Servier Industries in Arklow, Co. Wiclow, Ireland; licensed by Les Laboratoires Servier Industries in Gidy, France) was utilized in the study. Commercially available tablets containing 25 mg of agomelatine were ground to a homogeneous powder using a mortar and a pestle. The powder was mixed with 0.2 mL salin solution (%0,09 NaCl) to make a 10 mg/kg agomelatine suspension (0.5 ml/tablet). The volume administered to the rats was calculated to yield a final agomelatine dose of 10 mg/kg of body weight and administered regularly using the oral gavage method between 10:00 and 12:00 every day during the experiment period. This dosage was selected based on prior research indicating the effectiveness of agomelatine at a dose of 10 mg/kg [14,15]. After a 30-minute equilibration period allowing for the stabilization of myometrial strips with 1 g stretch tension and the development of spontaneous contractile activity, a single dose of 800 mU/L extracellular oxytocin was applied to obtain a contractile response to oxytocin [16]. This dose was chosen based on previous studies demonstrating the efficacy of oxytocin at a dose of 800 mU/L [16]. Oxytocin (O3251) was sourced

from Sigma-Aldrich (St Louis, MO, USA). The majority of the strips developed spontaneous contractions within the 30-minute equilibration period, and strips without spontaneous activity within this time were discarded. Luzindole (2 uM) [17], a melatonin receptor antagonist, was subsequently administered prior to the introduction of agomelatine, in order to investigate the mechanism of action of agomelatine on myometrial smooth muscles. Luzindole (L2407) was also obtained from Sigma-Aldrich (St Louis, MO, USA).

*Animals and procedures*

All animal procedures were approved by the Firat University Animal Experiments Local Ethics Committee, and performed according to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (approval no: FUHADEK 02.03.2017/18; Elazig, Turkey). Utilized in this study were 28 female Wistar Albino rats, obtained from Firat University Experimental Research Center (FUDAM), exhibiting regular cycles and an average weight of 200-250 g. The rats were housed in plastic cages with metal covers, at room temperature of 21±1 °C, with a 12-hour light-dark cycle. Special rat pellet feeds, procured from the Elazig Feed Factory, were provided for the rats, with water available ad libitum. The animals included in the study were monitored for their cycles every day at the same time (09:00-10:00) for at least 14 days by the vaginal smear method to examine the regularity of their cycles. Rats exhibiting regular behavior were then selected and incorporated into the study. Depending on the experimental groups formed, agomelatine was administered regularly via the oral gavage method or an equivalent volume of saline was applied using the same method as the oral gavage procedure might induce stress in different experimental groups.

While parameters were examined in normal female rats, assessments were also conducted on pregnant rats. Consequently, the experimental groups were segregated based on two different protocols: non-pregnant (the first experimental protocol) and pregnant (the second experimental protocol). Groups D1 and G1 were vehicle groups created to control for potential effects arising from the use of the oral gavage method. The data acquired were analyzed in two separate sections: in vivo and in vitro. In this study, the experimental groups designed to examine the effects of agomelatine on the female cycle are denoted with D1 and D2 (non-pregnant=diestrus=D), while the groups formed to investigate the effects on pregnancy are marked as G1 and G2 (pregnancy=gestation=G).

*The first experimental protocol (non-pregnant experimental group):* The first experimental protocol comprised the D1 and D2 experimental groups. The influence of agomelatine administration on the cycle phases was assessed in these groups. The cycle stages of the first protocol's experimental animals were monitored for 14 days, with the duration of their stay at each phase being recorded. Following 14 days of cycle monitoring, the animals were decapitated during the diestrus phase, and the variations in their blood estrogen and progesterone levels were assessed.

*D1 group (n=7) (Control):* These rats received 14 days of saline administration.

*D2 group (n=7):* These rats received 14 days of agomelatine administration.

*The second experimental protocol (experimental groups exposed to pregnancy):* The second experimental protocol focused on the experimental groups exposed to pregnancy. During the cycle follow-up, rats in the oestrus phase were placed in the same cage as the male rat for mating. Vaginal smears were performed the following day, and rats with detectable sperm in the smear sample were considered pregnant, assuming they were on the 0th day of pregnancy. After pregnancy detection, the pregnant rats were administered 10 mg/kg of agomelatine orally once daily from two weeks before conception until the day of delivery. Immediately after the birth of the first offspring, the animals were decapitated, and blood samples were collected to examine the variations in oxytocin and prostaglandin E (PGE) levels. Additionally, the changes in the number of fetuses and uterine contractions were assessed during the gestation period. The pregnant rats were divided into two groups for the second experimental protocol:

*G1 group (n=7) (Control):* Saline treatment (vehicle) was administered for 14 days during the pre-pregnancy period and after pregnancy detection, saline treatment was continued until delivery.

*G2 group (n=7):* Agomelatine treatment was administered for 14 days in the pre-pregnancy period and after pregnancy detection, agomelatine treatment was continued until delivery.

*Tissue preparation and evaluation of contractile activities*

Following the conclusion of the study, the uteruses of the decapitated rats were promptly removed to assess uterine contractions, and 1-2 cm in length, 2 mm in width and 1 mm in thickness were obtained. These sections

were then placed in an isolated tissue bathallowing a tension of 1 g to be applied, and the resulting contractions were recorded. After a period of ninety minutes, the spontaneous contractions became regular. Subsequently, agomelatine was administered at different concentrations (50 μM, 100 μM, and 200 μM), and the contractions were recorded for a duration of twenty minutes (10 min before and after agomelatine application).

The study utilized an isolated tissue bath system consisting of four chambers, with each chamber containing uterine strips obtained from different experimental animals. This heat-jacketed double-walled device creates an in vitro environment for examining smooth muscle contractions. The electrical forces generated by the contraction of the tissues were transmitted from the transducer to the amplifier, where the signals were amplified. The system employed BIOPAC acknowledge software, MP data analysis and acquisition hardware platforms, transducers, stimulation modules, amplifiers, and other components to form a comprehensive data acquisition and analysis system.

A waiting period of 90 minutes was observed to allow spontaneous contractions to become regular before the administration of drugs in the isolated tissue bath. The study results were displayed on a monitor in the form of peaks, and key interpretable data regarding the study were obtained by analyzing the frequency, amplitude (P-P), and integrated areas of the peaks. Krebs solution (NaCl: 11.8 mM, KCl: 4.7 mM, $MgSO_4$: 1.2 mM, Glucose: 11.5 mM, $CaCl_2$: 2.4 mM, $KH_2PO_4$: 1.18 mM, $NaHCO_3$: 15.8 mM, EDTA: 0.016 mM) was used as the solution in the isolated tissue bath system. To explain the physio-pathological mechanism underlying the effect on uterine muscles, the MT1 and MT2 receptor antagonist luzindole was applied (Luzindole was administered 10 minutes before agomelatine application). The effects of agomelatine on the contraction curves of spontaneous and oxytocin (Oxytocin 800 mU/L)-induced contractions in pregnant rat myometrial strips were examined. Consequently, the dose-dependent effects of agomelatine on uterine contractions were examined and recorded. Frequency, P-P, and area values were normalized for comparison. The values before agomelatine application were normalized and counted as 100 %, which were then compared with the values after agomelatine application.

*The ELISA method*

Approximately 4-5 ml of blood was collected from each rat into blood tubes containing aprotinin and centrifuged at 5000 rpm for 10 minutes. After centrifugation, the upper part of the serum was separated using a 200 μL micropipette and stored in a refrigerator at -20 °C until the ELISA test was performed. in a refrigerator at -20 °C until the ELISA was performed. The optical densities were measured at a wavelength of 450 nm in the ELISA device. The indirect micro-ELISA method was conducted using SunRed brand kits (Oxytocin; Catalogue No: 201-11-1725, sensitivity: 2-600 ng/L, PGE; Catalogue No: SRB-T-86655, sensitivity: 0.05-15 ng/ml, corelation coefficient R is over 0.95, Estrogen; Catalogue No: 201-11-0175, sensitivity: 5-900 ng/L, Progesterone; Catalogue No: 201-11-0742, sensitivity: 2-300 ng/ml, Shanghai/China).

*Statistical analysis*

The statistical analyses were performed using IBM SPSS Statistics (Version 22). One-Way Analysis of Variance and Paired Samples T-Test were utilized for the statistical evaluations. The sample size for the experiments was determined based on a power analysis, with consideration of an 8 % deviation, a type 1 error (α) of 0.05, and a type 2 error (β) (Power=0.80). A minimum of 7 animals were included in the study. The relative changes in drug-induced (agomelatine and luzindole) contractile responses were calculated as a percentage of the maximum levels induced with KCl. Statistical significance was defined at $p<0.01$ and $p<0.05$. The study findings were presented as Mean ± Standard Error (SE) of the measured values.

## Results

*In vivo results*

*In vivo results of the first experimental protocol*

*- The menstrual cycle*

The duration of stay in the diestrus phase was found to be significantly prolonged in the D2 group of experimental animals compared to the D1 group of experimental animals. The D2 group experimental animals remained in the diestrus phase for an average of 8.07±0.31 days, while the D1 group experimental animals remained in the diestrus phase for an average of 7.42±0.22 days ($p<0.05$).

*- The estrogen and progesterone hormones*

The estrogen level was 173.97±7.63 ng/L for the D1 group and 184.64±7.33 ng/L for the D2 group. The progesterone level was 59.28±1.93 ng/ml for the D1 group

and 61.80±3.91 ng/ml for the D2 group. Both estrogen (p=0.982) and progesterone (p=1.000) levels increased in the D2 group compared to the D1 group, but the difference was not statistically significant.

*In vivo results of the second experimental protocol*

*- The gestational period*

The gestational period of the G2 group was significantly prolonged compared to the control group G1 (p<0.01) (Fig. 2A). Additionally, the mean gestation periods of the groups were 21.69±0.13696 days (520.6±3.28701 hours) in the G1 group and 22.48±0.07292 days (539.62±1.30845 hours) in the G2 group, indicating a 0.79-day difference between the groups.

*- The numbers of offspring*

The mean number of offspring in the G1 group, one of the pregnant experimental groups, was 9.1±0.65, the mean number of offspring in the G2 experimental group was 12.75±0.23. It was determined that the number of offspring in the G2 group increased compared to the control pregnant group G1 and this increase was statistically significant (p<0.01) (Fig. 2B).

*- The oxytocin and PGE hormones*

The mean oxytocin level in the G1 group, one of the pregnant experimental groups, was 109.59±3.97 ng/L, the mean oxytocin level in the G2 experimental group was 87.69±8.14 ng/L. This decrease in oxytocin release was statistically significant (p<0.01) (Fig. 2C). The PGE level was 3.77±0.14 ng/ml in the G1 group, and it decreased to 2.98±0.207 ng/ml in the G2 group with agomelatine administration. This decrease in PGE level was statistically significant (p<0.01) (Fig. 2D).

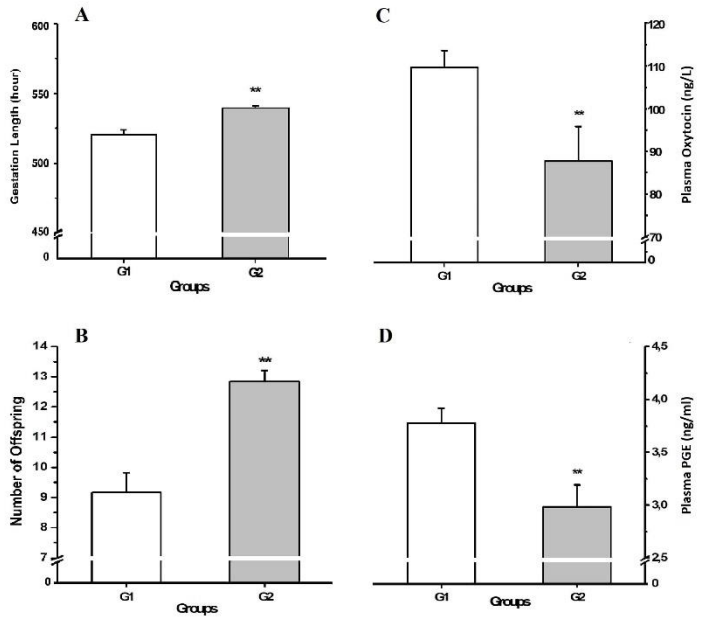


**Fig. 1.** (**A**) The change in the gestational periods of the G1 (saline treatment (vehicle)) applied for 14 days in the pre-pregnancy period and after pregnancy detection, saline treatment was continued until delivery) and G2 (agomelatine treatment was applied for 14 days in the pre-pregnancy period and after pregnancy detection, agomelatine treatment was continued until delivery) groups (*: p<0.05, **: p<0.01). (**B**) The change in the number of offspring born in a litter belonging to groups G1 and G2 (*:p<0.05, **:p<0.01). (**C**) Change in oxytocin values of G1 and G2 groups (*:p<0.05, **:p<0.01). (**D**) Change in prostaglandin E (PGE) values of G1 and G2 groups (*:p<0.05, **:p<0.01).

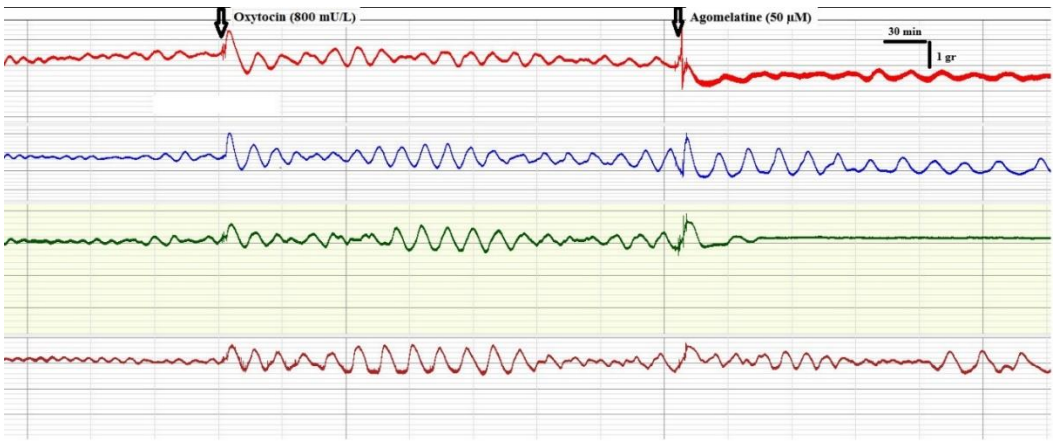


**Fig. 2.** Contractile diagram of uterine contractions induced by oxytocin (1 mIU/ml) after administration of 50 µM agomelatine.

**Table 1.** Frequency, amplitude (P-P) and area values of G1 and G2 groups (*:p<0.05, **:p<0.01)

| G1 Group | Before Ago. | After Ago. | G2 Group | Before Ago. | After Ago. |
|---|---|---|---|---|---|
| *Frequency 50 μM* | 100±0.0 | 74.85±11.12 | Frequency 50 μM | 100±0.0 | 40.26±15.36 ** |
| *Frequency 100 μM* | 100±0.0 | 31.65±15.46 | Frequency 100 μM | 100±0.0 | 0.0±0.0 ** |
| *Frequency 200 μM* | 100±0.0 | 0.0±0.0 | Frequency 200 μM | 100±0.0 | 0.0±0.0 |
| *Amplitude 50 μM* | 100±0.0 | 91.84±3.69 | Amplitude 50 μM | 100±0.0 | 23.18±10.14 ** |
| *Amplitude 100 μM* | 100±0.0 | 14.84±8.77 | Amplitude 100 μM | 100±0.0 | 0.0±0.0 ** |
| *Amplitude 200 μM* | 100±0.0 | 0.0±0.0 | Amplitude 200 μM | 100±0.0 | 0.0±0.0 |
| *Area 50 μM* | 100±0.0 | 70.85±6.23 | Area 50 μM | 100±0.0 | 20.29±8.20 ** |
| *Area 100 μM* | 100±0.0 | 10.17±5.90 | Area 100 μM | 100±0.0 | 0.0±0.0 ** |
| *Area 200 μM* | 100±0.0 | 0.0±0.0 | Area 200 μM | 100±0.0 | 0.0±0.0 |

*In vitro results*

*The contraction*

The results of contraction demonstrated that agomelatine inhibited spontaneous and oxytocin-induced myometrial contractions in a dose-dependent manner (as shown in Fig. 2). In the non-pregnant rat uterus experiment performed, contraction values (frequency, P-P and area) increased with oxytocin inhibited by agomelatine (as shown in Fig. 2). Agomelatine at concentrations of 50 μM, 100 μM and 200 μM was administered and the results of contraction were analyzed in the second experimental protocol (Groups G1 and G2) formed from pregnant experimental animals of groups G1 and G2, respectively. The frequency, P-P and area values of the G1 and G2 groups are shown in the Table 1.

After the lowest dose of 50 μM agomelatine was administered, a decrease in frequency, P-P and area values were found. The results of contraction were analyzed in the second experimental protocol (Groups G1 and G2) formed from pregnant experimental animals in both the G1 and G2 groups. After the administration of an agomelatine dose of 50 μM, the decrease in all frequency, P-P and area values, except for the G1 group, was found to be statistically significant ($p<0.01$) (Table 1). It was found that the frequency values in the G2 group decreased more than in the G1 group and that this difference between the two groups was statistically significant ($p<0.05$). In addition, it was found that the P-P and area values in the G2 group decreased more than in the G1 group and that this difference between the two groups was statistically significant ($p<0.01$) (Table 1, Fig. 3).

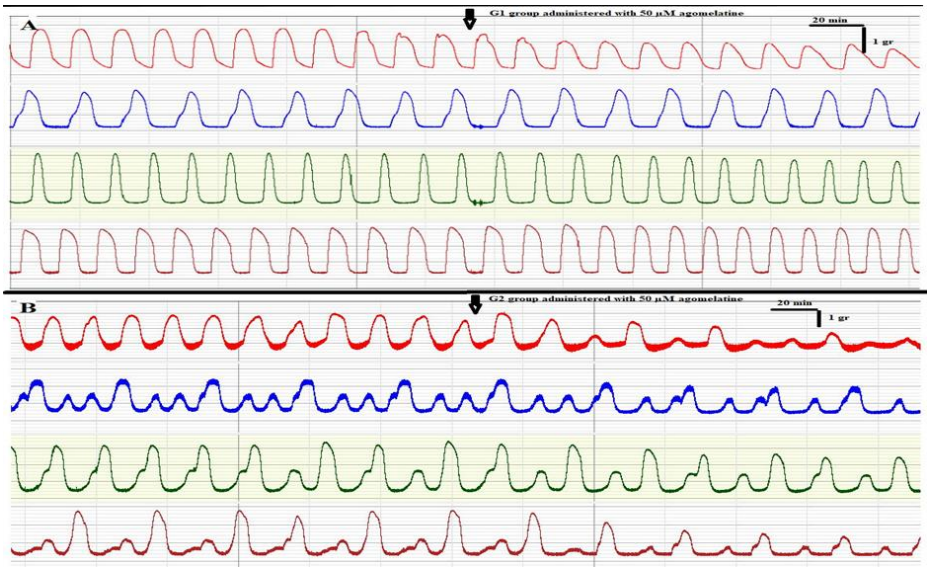


**Fig. 3.** (**A**) Baseline uterine contractions in the G1 group administered with 50 μM agomelatine. (**B**) Baseline uterine contractions in the G2 group administered with 50 μM agomelatine.

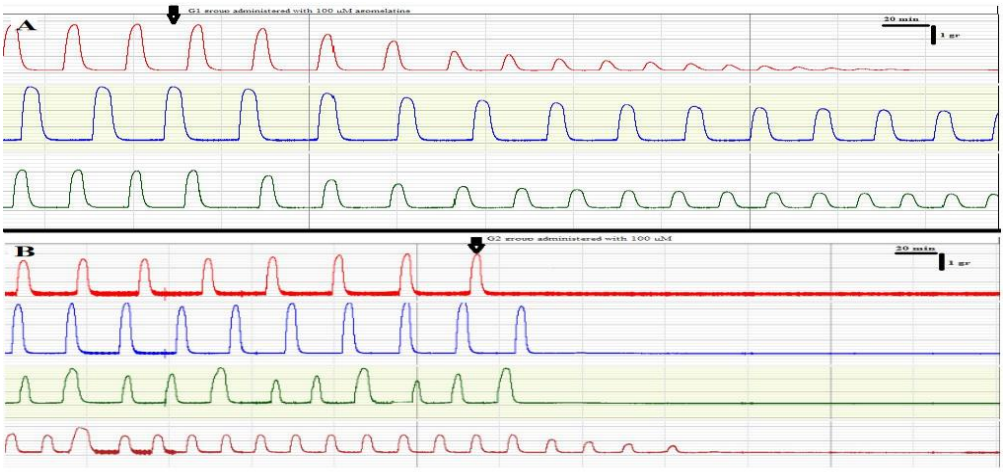


**Fig. 4.** (**A**) Baseline uterine contractions in the G1 group administered with 100 µM agomelatine. (**B**) Baseline uterine contractions in the G2 group administered with 100 µM agomelatine.

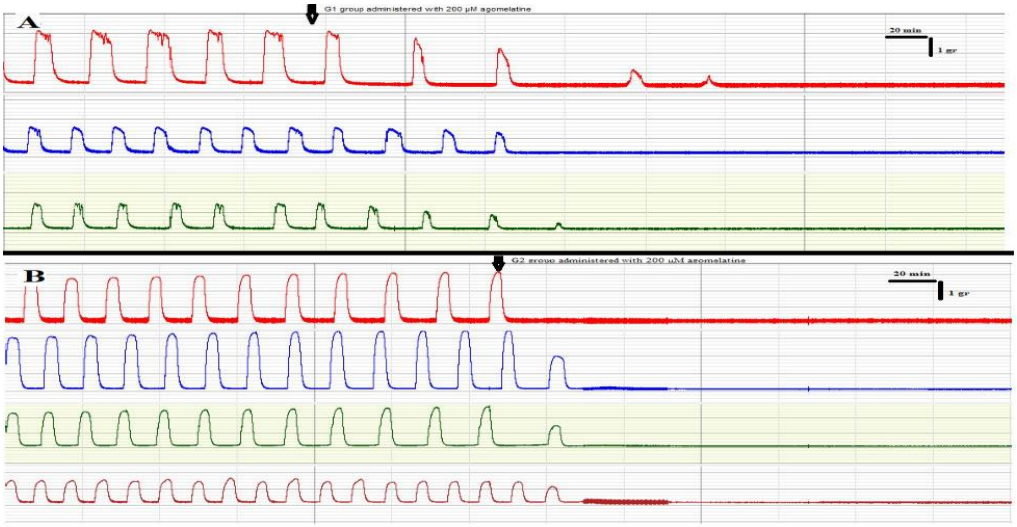


**Fig. 5.** (**A**) Baseline uterine contractions in the G1 group administered with 200 µM agomelatine. (**B**) Baseline uterine contractions in the G2 group administered with 200 µM agomelatine.

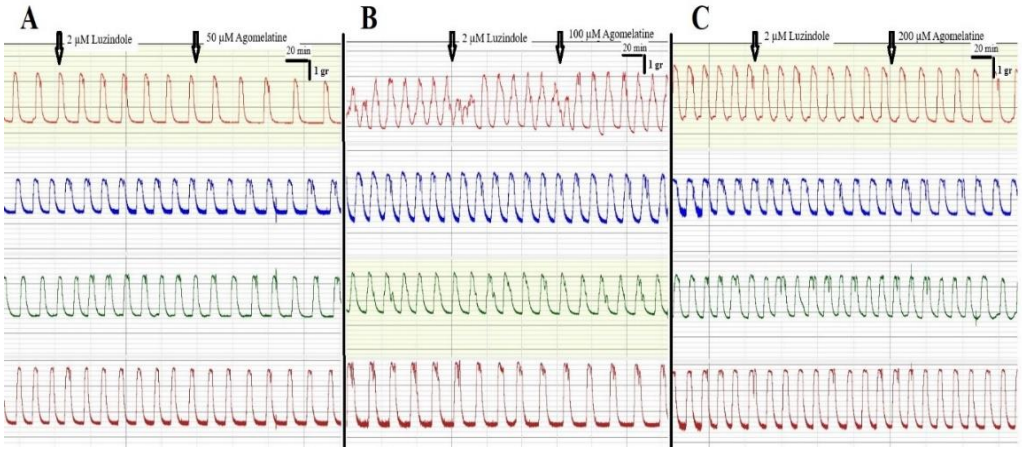


**Fig. 6.** Effect of 50, 100, and 200 µM doses of agomelatine on uterine contractions after 2 µM luzindole administration.

As a result of the application of 100 μM agomelatine, a statistically significant decrease was detected in the frequency, P-P and area values of the G1 group. The results of contraction were analyzed in the second experimental protocol (Groups G1 and G2) formed from pregnant experimental animals ($p<0.01$), while 100 % inhibition occurred in the G2 group. The results of contraction were analyzed in the second experimental protocol (Groups G1 and G2) formed from pregnant experimental animals ($p <0.01$) (Table 1, Fig. 4). After the highest dose of 200 μM agomelatine was administered, 100 % inhibition was observed in the G1 and G2 groups ($p<0.01$) (Table 1, Fig. 5). It was determined that agomelatine administration did not inhibit contractions after luzindole administration (Fig. 6).

## Discussion

In this study, we address an understudied aspect: the impact of agomelatine on various reproductive parameters, specifically its effects on gestation periods, offspring numbers, and myometrial contractions in pregnant rats. The context is crucial, as the melatonergic system is known to play a role in regulating the female reproductive system and its overall function. Notably, previous researchs in mice and pigs have highlighted the significance of the melatonergic system in corpus luteum function, which is pivotal for mammalian reproduction [18]. Our findings signify a substantial modification of the reproductive cycle due to chronic agomelatine administration. Remarkably, the effects are distinct in different reproductive states. In non-pregnant rats, agomelatine extends the diestrus phase's duration, suggesting a role in cyclic regulation. Intriguingly, in pregnant rats, agomelatine not only substantially prolongs the gestation period but also augments fetal count. Most notable is its potent inhibition of myometrial contractions in pregnant rats compared to non-pregnant rat myometria. These effects collectively suggest a potential role for agomelatine in enhancing fertility and supporting pregnancy maintenance.

Our findings propel the need for further exploration into the intricate mechanisms underpinning these observations. This pursuit has the potential to illuminate novel insights into reproductive health and pregnancy management. By virtue of the limited research into agomelatine's impact on the female reproductive system, our study emerges as a pioneering endeavor in this arena. The insights garnered here offer a significant stepping stone towards unraveling uncharted territories in the realm of reproductive physiology

*Agomelatine and cycle change*

Our findings show that chronic agomelatine administration prolongs the estrous cycle by prolonging the diestrus phase. Normally, diestrus occurs at the end of the estrus phase, characterized by the continuity of the corpus luteum and the dominance of progesterone. In the absence of pregnancy, decreased progesterone levels lead to the regression of the corpus luteum. The maintenance of the menstrual cycle relies on high levels of estrogen and progesterone [19]. Previous studies have shown a correlation between melatonin levels and estrogen-progesterone levels, with melatonin potentially influencing the increase in estrogen and progesterone levels [20]. Similarly, concentrations of melatonin in fluid collected from human ovarian follicles were significantly higher than in blood samples collected simultaneously, suggesting that agomelatine may also have positive effects on cycle completion [21,22]. In a study on mice, the MT1 receptor expression in the ovarian tissue of a rat decapitated during oestrus was found to be significantly higher than that of a mouse decapitated during estrus [23]. Agomelatine exerts its biological effects on the reproductive system by combining with specific and high-affinity melatonin receptors [24]. When previous studies were examined, it was determined that melatonin receptors were expressed in many central nerves and many peripheral tissues, including testes and ovaries [6]. Some researchers have determined that melatonin plays a role in the modulation of the hypothalamic-pituitary-gonadal (HPG) axis, which is an important regulatory center for reproductive functions in animals and humans [7]. In these studies, it was revealed that the physiological and circadian effects caused by the MT1 receptor are widely distributed in endocrine tissues and brain regions, which are the main response areas. In a study conducted on female animals, it was determined that the MT1 receptor is widely distributed in the ovaries and plays very important roles in reproductive activities [25]. Mechanistic studies have shown that MT1 and MT2 receptors are detectable in oocytes and granulosa cells and respond to estrogen levels during follicle development [25].

Our study corroborates existing literature by indicating that chronic agomelatine administration extends the estrous cycle primarily by prolonging the diestrus phase. This alignment with established knowledge suggests agomelatine's potential influence through

melatonin receptors. The precise mechanisms governing agomelatine's impact on diestrus in rats warrant further exploration. In essence, while our findings align with previous information, our study prompts further investigation to elucidate the intricate mechanisms underlying agomelatine's effects on diestrus in rats. This nuanced exploration holds the potential to unravel novel insights into the interactions between agomelatine and the reproductive cycle.

*Agomelatine and change in number of fetuses*

The number of fetuses was significantly higher in the G2 group, consisting of pregnant rats in the second experimental group, compared to the G1 group. It was quite remarkable that chronic agomelatine administration increased fetal number in pregnant rats. Although there is no study in the literature on the effect of agomelatine on fetal number, there is a study on the prevention of hypertension by agomelatine in the offspring of pregnant rats exposed to continuous light [27]. Although there are few studies examining the effect of agomelatine, a melatonin receptor agonist, on pregnancy and fetus, there are some studies investigating the effectiveness of melatonin on pregnancy and fetus. Some of these studies have demonstrated that melatonin can increase the number of blastocysts in mice and cattle [23, 28]. Additionally, melatonin has been shown to elevate progesterone levels before fetal implantation, promoting a more suitable uterine environment for implantation [29, 30]. However, our findings regarding the effect of chronic agomelatine administration on progesterone level during pregnancy do not support the idea that agomelatine increases fetal number by changing plasma progesterone level.

It is not known exactly by which physiological mechanisms melatonin or agomelatine exerts this effect, but previous studies give some ideas. As an antioxidant, it would not be wrong to say that melatonin can eliminate ROS and reduce oxidative stress, protect oocytes and granulosa cells, and thus increase the fertilization rate and pregnancy rate. Agomelatine, a melatonin receptor agonist, is likely to improve reproductive functions by the same physiological mechanism. Some studies have shown that MT1 and MT2 receptors are detected in oocytes and granulosa cells and play a role in the change of estrogen level during follicle development [31]. There are studies showing that melatonin has a positive effect on in vitro embryo production (IVEP) and improves blastocyst quality in some mammals [32,33]. Mechanistic studies suggest that melatonin has a positive effect on oocyte sufficiency, not only with its antioxidant ability by scavenging ROS, but also with increased mitochondrial activity and ATP levels [32]. In addition, it was found that this melatonin-induced improvement in embryo quality was partially blocked by the melatonin receptor antagonist luzindole [34]. Another possible physiological mechanism between melatonergic receptors and embryo development and thus fetal number may be its activity on apoptotic genes. During maternal pregnancy, melatonin can suppress the expression of proapoptotic genes including Bax and Caspase-3 and activate the expression of the antiapoptotic gene Bcl-2, thereby reducing the apoptosis rates of blastocysts and improving embryo quality and subsequent embryo implantation rate [35]. Some studies have confirmed that MT1 and MT2 exert an antiapoptotic effect [36]. One of the possible physiological mechanisms between melatonergic receptors and embryo development and thus fetal number may be the development of the uterine glands. Prior to implantation, the endometrium undergoes a rapid and widespread proliferation of uterine epithelial and stromal cells that prepare the uterus for implantation [37,38], and it is an acceptable fact that the uterine glands are the nutrient source for the embryo. It would not be wrong to say that this development of the uterine glands is directly proportional to the success of implantation and maintenance of pregnancy [39,40]. Also, recent research has shown that melatonin can prevent experimental preterm labor and significantly increase live birth rates [41]. In a study on mice, it was determined that MT2 expression increased in the early stages of pregnancy, that is, during the implantation period [23]. These findings support the findings of our study. We believe that one of the effective mechanisms of chronic agomelatine administration to increase fetal number may be through melatonergic effect. In conclusion, agomelatine, a melatonin receptor agonist, may increase implantation success in humans, facilitate conception, and support reproductive functions for female infertility, possibly by increasing MT2 receptor activity or by a different physiology mechanism. More research is still needed to reveal the mechanism linking agomelatine and therefore melatonin receptors and animal reproduction.

*Agomelatine and change in gestational period*

One of the notable findings of this study was the significantly prolonged gestational period observed in the G2 group compared to the G1 group. While there are very few studies investigating the effects of agomelatine on pregnancy, there is no study investigating its effectiveness

on the duration of pregnancy. Hence, this study contributes novel data to the literature in this regard. The results demonstrate that agomelatine effectively inhibits spontaneous and oxytocin-induced myometrial contractions in both control pregnant (G1) and experimental pregnant rats (G2) in a dose-dependent manner. Notably, the inhibition of myometrial contractions was more pronounced in the experimental group (G2) treated with chronic agomelatine administration. The findings of our study, unlike our previous study, are that the inhibition of agomelatine on the pregnant myometrium is much stronger than the inhibition it caused in the myometrium of a non-pregnant rat at the same dose. This shows that the melatonergic or serotonergic effect increases even more in myometrial contractions during pregnancy. Previous studies have attributed the inhibitory actions of agomelatine on myometrial contractions to its effect on suppressing prostaglandin synthesis in the uterus. Analysis of hormonal parameters at the time of birth revealed a statistically significant decrease in oxytocin and prostaglandin E (PGE) levels in the G2 group compared to the G1 group. The reduction in oxytocin and PGE levels likely contributes to the delay in delivery and prolongation of pregnancy.

The wide distribution of melatonin receptors constitutes the first step of its extensive biological effects, apart from acting as an antioxidant to prevent oxidative stress damage [42]. Also, in addition to genes associated with melatonin receptors, rhythmic genes (Clock, Bmal1, Per2 and Cry1) are quite common in the hypothalamic-pituitary-gonadal (HPG) axis. Therefore, agomelatine, which is a melatonin receptor agonist, can regulate the expression of rhythmic genes in different developmental stages of the follicles and can regulate the reproductive system by acting on gonadal function and endocrine functions [43].

There are findings in the literature that agomelatine increases the level of oxytocin in rats with a social isolation model, and it has been stated that this increase can be achieved through the 5HT-2C receptor [44]. Existing literature indicates that oxytocin is a crucial hormone for successful delivery, as its levels increase near term and play a pivotal role in initiating uterine contractions [45,46]. Similarly, PGE is involved in the initiation and maintenance of delivery [47]. Hence, delivery typically occurs before reaching a certain threshold of oxytocin and PGE plasma levels. Although our findings that chronic agomelatine administration reduces plasma oxytocin level during pregnancy are different from the agomelatine-oxytocin relationship in the literature, the change in reproductive functions during pregnancy has made it a matter of curiosity by which physiological mechanisms this result occurs. Considering the lower plasma hormone values observed in the G2 group compared to the G1 group, it is plausible that this contributes to the prolongation of gestational period in the G2 group. The findings of our study are not sufficient to definitively state the physiological mechanisms by which agomelatine reduces oxytocin and prostaglandin levels during pregnancy. However, the fact that luzindole, a melatonin receptor antagonist, reverses oxytocin-induced myometrial contractions inhibited by agomelatine, suggesting that agomelatine exerts its effect through melatonergic receptors. Additionally, despite the potential increase in intra-uterine pressure associated with a higher number of fetuses, agomelatine's ability to reduce oxytocin and PGE levels suggests the possibility of alternative clinical uses beyond its antidepressant effectiveness, particularly in preventing premature births [48]. Currently, the fact that there is no alternative method other than bed rest and exogenous progesterone therapy to prevent preterm labor makes agomelatine a potential alternative treatment candidate.

*Agomelatine and in vitro results*

Another important aspect of this study was the investigation of the effects of agomelatine on myometrial contractions using isolated myometrial strips obtained from rats during birth. Importantly, this inhibitory effect was more pronounced in the G2 group, suggesting that chronic agomelatine administration sensitized the myometrium to its inhibitory effects, possibly influenced by estradiol levels near birth.

Given the prevalence and increased intensity of exposure to artificial light at night, it is a fact that must be acknowledged that exposure of pregnant women to light or to common pharmacological agents that suppress melatonin secretion may be inadvertently affecting the progression of labor and reproductive functions. Our study may offer a new treatment option to therapeutically influence labor and delivery timing, as endogenous melatonin levels can be suppressed by both mild and pharmacological agents, and melatonin receptors can be activated by agomelatine. In clinical studies, it was noted that more than 12 % of all pregnancies in western societies resulted in preterm delivery [49]. Premature births continue to be the main cause of perinatal morbidity and

are known to be associated with 70 % of neonatal mortality [49]. In addition, most of the preterm births occur in women without significant known risk factors, and all preterm etiologies cause premature contractions [50]. In this respect, the inhibitory effect of chronic agomelatine administration on uterine contractions during pregnancy is very important. Chronic administration of agomelatine, a melatonin receptor agonist, may cause uterine melatonin receptors to be expressed or activated during pregnancy.

The data obtained from our study are not sufficient to show by which physiological mechanisms the inhibitory effect of chronic agomelatine administration on uterine muscles occurs. However, previous studies suggest that changes on the activity of voltage-dependent $Ca^{+2}$ channels cause inhibition in uterine muscles [51]. To further confirm the involvement of voltage-dependent $Ca^{+2}$ channels in the agomelatine-induced inhibition of myometrial contractility, additional electrophysiological evidence using isolated myometrial cells would be necessary. These findings indicate that agomelatine may play a significant physiological role in pregnancy control and the prevention of preterm labor the pharmacological inhibition of myometrial contractions by agomelatine may provide an effective means to control the onset of labor.

*Agomelatine and in vitro Luzindole administration*

To investigate the physiological and pathological mechanism underlying the inhibitory effect of agomelatine on myometrial contractions, luzindole, an antagonist of MT1 and MT2 receptors, was administered to the isolated tissue bath chamber at a dose of 2 μM [16], 10 minutes before the administration of agomelatine. Interestingly, when agomelatine was administered after luzindole, it was observed that agomelatine had no inhibitory effect on myometrial contractions. This indicates that the inhibitory effect of agomelatine on the myometrium is mediated through the MT1 and MT2 receptors. It is known that the 5-HT2C receptor stimulates IP3 and intracellular calcium release through inositol phosphate metabolism, which in turn leads to muscle contraction. Therefore, agomelatine's inhibition of 5-HT2C receptor-mediated intracellular calcium increase, leading to inhibition of muscle contraction [52], may be one of the possible underlying physiological mechanisms. However, further studies are needed to fully understand the precise mechanism involved in the inhibition of contractions by agomelatine.

When considering all these findings together, it appears that there are two factors contributing to the prolongation of the gestational period in the G2 group. Firstly, the plasma levels of oxytocin and PGE, which are necessary for the initiation and continuation of delivery, were lower in the G2 group compared to the G1 group. Secondly, the prolongation of the gestational period can be attributed to the inhibitory effect of agomelatine on myometrial contractions. These findings are supported by previous studies that have demonstrated the role of prostaglandins in childbirth. Inhibition of prostaglandin synthesis has been shown to reduce myometrial contractility, although its clinical use is limited due to the important roles of prostaglandins in various physiological functions [53]. Studies have also indicated that melatonin inhibits oxytocin secretion via the MT1 receptor in rats [54], and suppresses the secretion of prostaglandin F2 alpha (PGF2 alpha) and PGE in the rat uterus and hypothalamus [55, 56]. Considering the existing literature, we propose that the melatonergic effect of agomelatine is responsible for the decrease in oxytocin and PGE levels in the G2 group compared to the G1 group.

Based on the data obtained in this study, agomelatine appears to prolong the duration of diestrus in non-pregnant rats. In pregnant rats, agomelatine was found to increase the number of fetuses, delay the onset of delivery by prolonging the gestational period, and decrease plasma oxytocin and PGE levels. Although an increase in the number of fetuses typically triggers early birth by increasing intrauterine pressure, we believe that the prolonged gestational period in the G2 group is primarily due to the lower levels of oxytocin and PGE, which are necessary for the initiation of delivery. Additionally, it was observed that agomelatine, when applied to an external isolated tissue bath, exerted an inhibitory effect on uterine contractions in uterine tissues taken from pregnant rats, with a stronger effect observed in the pregnant group treated with chronic agomelatine. The disappearance of the inhibitory effect of agomelatine on uterine contractions when luzindole, a melatonin receptor antagonist, was applied to the isolated tissue bath, indicates that the effect of agomelatine is achieved through the MT1 and MT2 receptors. We believe that uterine inhibition is another contributing factor to the prolongation of pregnancy. This study is the first in the literature to investigate the effectiveness of agomelatine, a drug that can be used in young reproductive-age women and during pregnancy, on the female reproductive system. Therefore, we believe that it will pave the way for the use of agomelatine by considering its effects on the female reproductive system.

In conclusion, based on the findings obtained, it can be concluded that agomelatine, an antidepressant that

regulates the circadian rhythm, exerts significant effects on the female reproductive system in rats. Considering its effects during both non-pregnancy and pregnancy, agomelatine could have clinical applications beyond its antidepressant properties. Specifically, due to its impact on the gestational period and uterine contractions, agomelatine may be considered a potential treatment option for patients at risk of preterm birth or abortion.

**Author Contribution Statement**

EK, FT and HK designed and supervised the study. FT, SS and GZ conducted all animal experiments. IS, OB and ZE performed hormone measurements. EK and HK contributed to the data analysis. EK and FT wrote the manuscript, and all authors approved the final manuscript.

**Ethics Approval and Consent to Participate**

Ethical approval for all animal procedures was obtained from the Firat University Animal Experiments Local Ethics Committee. The study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (approval no: FUHADEK 02.03.2017/18; Elazig, Turkey).

## Conflict of Interest

There is no conflict of interest.

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