



The histological investigation of the effects of electromagnetic radiation on rat ovaries

Bahar Kartal¹ · Ebru Alimoğulları¹ · Gökhan Akkurt² · Mustafa Alimoğulları³ · Sevil Çaylı¹

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Abstract

People are now exposed to higher levels of electromagnetic radiation (EMR) due to the widespread use of mobile phones in recent years. The possible effects of this exposure on human health are related to EMR. It has been suggested that exposure to EMR has serious effects on reproduction. The study aimed to investigate the impact of exposure to EMR (4.5 GB; 2600 MHz) emitted by mobile phones on rat ovaries. 18 adult female Wistar albino rats were used in the study, and the animals were divided into three groups ($n=6$): control, stand-by, and dialing. For 8 weeks, the experimental groups were subjected to 4.5 GB EMR at 2600 MHz while on standby and making 10-min calls every hour. The rats in the control group received no exposure. Hematoxylin–eosin (H&E) staining of ovarian tissues was performed for histomorphological examinations. Additionally, immunoexpression of autophagy-related protein Beclin-1, apoptosis marker Caspase-3, ovarian reserve marker FSH, and oxidative stress marker iNOS were investigated in the rat ovaries. Microscopic examinations showed follicular degeneration in the ovaries of the rats in the stand-by and dialing groups. The immunoexpression of Beclin-1, Caspase-3, FSH, and iNOS was detected in granulosa cells and the corpus luteum in ovarian tissues obtained from the two EMR-exposed groups. There was a significant increase in the immunoexpression of Beclin-1 and Caspase-3 in the dialing group compared to the other two groups. Additionally, the iNOS and FSH expressions were increased in both EMR exposure groups compared to the control. Our results suggest that EMR exposure harms the ovaries, and autophagy and apoptosis are involved in this process.

Keywords Electromagnetic radiation (EMR) · Mobile phones · Beclin-1 · Caspase3 · FSH · iNOS · Rat · Ovary

Introduction

Nowadays, radiofrequency electromagnetic fields (RF-EMF) are used in many different technologies globally, and people are frequently exposed to them in both daily life and at work. Human exposure limits to RF-EMF have been established and updated regularly (ICNIRP 2020). Mobile phones receive microwave signals from base stations and use low-frequency radiation in the range of 900/1800 MHz for 2G, 2100 MHz for 3G, and 2600 MHz for 4.5 G-type communication (Postaci et al. 2018).

The specific absorption rate (SAR) is the rate of energy absorbed by a unit mass of the person and expressed as W/kg (SAR VALUES website 2006). Cell cycle, growth rates, enzyme activity, membrane structure, and cellular conversion are all altered by SAR values even 10 W/kg (Devrim et al. 2008).

Studies conducted to date are very diverse, reporting different specific outcomes. According to a study, trigeminal nerve injury and neuronal degeneration may result from

✉ Bahar Kartal
bahar.kartal@outlook.com

Ebru Alimoğulları
ebrualimogullari@gmail.com

Gökhan Akkurt
drakkurt06@gmail.com

Mustafa Alimoğulları
alimogullari49@gmail.com

Sevil Çaylı
sevilcayli@yahoo.com

¹ Department of Histology and Embryology, Medical Faculty, Ankara Yıldırım Beyazıt University, Ankara, Turkey

² Department of General Surgery, Ankara City Hospital, Cankaya/Ankara, Turkey

³ University of Health Sciences Ankara Atatürk Sanatorium Training and Research Hospital/General Surgery Clinic, Ankara, Turkey

exposure to 1800 MHz EMR ($\text{SAR} = 1.79 \text{ W/kg}$) from mobile phones during the prenatal and early postnatal periods (Akakin et al. 2021). Another study provided evidence that the human brain was sensitive to the effects of RF-EMR from mobile phones and recommended using hands-free devices or texting to reduce exposure. Although placing the mobile phone away from the head region, e.g., in trouser pockets, when using the talk mode can lower the exposure of the brain to these waves, this can increase the RF-EMR exposure of other parts of the body, namely the gonads (Kuhn et al. 2009).

Reproductive health and the impact of cell phone radiation have emerged as important research areas, and it is indicated that microwave radiation generated by mobile phones may negatively affect the quality of the sperm, egg, and embryo (Batellier et al. 2008). According to one study, pregnant rats exposed to mobile phone radiation had fewer ovarian follicles, which resulted in endometrial apoptosis (Gul et al. 2009).

Apoptosis is morphologically detected by cytoplasmic shrinking, nuclear compaction, membrane blebbing, and apoptotic body formation, which are engulfed by macrophages. (Cohen 1997). Although autophagy is a cytoprotective mechanism, it may lead to cell death under certain conditions. Proteins and organelles can be degraded by lysosomes through the autophagic process (Clarke and Puyal 2012).

Weimei Zhou and colleagues have examined the connection between autophagy and apoptosis in ovarian disorders. One of the main causes of infertility in women worldwide is premature ovarian failure (POF). POF is primarily caused by abnormal autophagy and excessive apoptosis in ovarian granulosa cells (Weimei Zhou et al. 2023a, b).

Diem et al. assessed the impact of continuous and intermittent RF-EMR from mobile phones, on DNA strand breaks on in vitro cultured granulosa cells of rats. After 16 h of exposure (either continuous wave or intermittent 5 min on/10 min off) and following various mobile phone modulations, RF-EMR exposure caused DNA single- and double-strand breaks (Diem et al. 2005).

Additionally, the impact of cell phone RF-EMR on rat ovaries was examined. Eighty-two female pups (21-day-old rats) were utilized in one study. Throughout their whole pregnancy, the study group's pregnant rats ($n = 43$) were exposed to cell phones that were positioned underneath their cages. Every 12 h, a mobile phone that had been in standby mode for 11 h and 45 min was switched to speech mode for 15 min. To determine the number of follicles, the female rat pups were killed and their right ovaries removed on the twenty-first day following delivery. They discovered that there were fewer follicles than in the control group that was not exposed (n039), which may indicate that RF-EMR has a harmful effect during pregnancy (Gul et al. 2009).

We hypothesize to determine whether apoptosis or autophagy involves the potentially harmful effects of RF-EMR. Therefore, the study aimed to investigate the cellular mechanism under the possible damage of the ovaries of rats exposed to RF-EMR (4.5 GB; 2600 MHz) by using immunohistochemistry methods.

Material and methods

Animals experimental design

In this study, we used 18 (4 weeks old) Wistar albino female rats. Animals were obtained from the Kobay Animal Breeding and Experimental Research Laboratory in Ankara, Turkey. The animal ethics committee approved the experimental protocol (Approved number: 338) of Kobay Company (Turkey).

The rats were kept in a laboratory with regulated lighting and temperature, and they had unlimited access to water and a regular pellet diet.

Animals were housed in a 12-h light–dark cycle. The study was started with preadult female rats (at 35 days and 220 g). The animals were assigned to two experimental groups and one control group.

Group I control: The rats received no exposure;

Group II (stand-by): The rats were subjected to 4.5 GB EMR at 2600 MHz while they were on standby for 8 weeks.

Group III (dialing): The rats were subjected to 4.5 GB EMR at 2600 MHz and made 10-min calls every hour for 8 weeks.

Mobile phone exposure set-up

The exposure set-up was designed as described previously (Devrim et al. 2008). Both experimental groups were caged, and a mobile phone (LG p705; Digital Communication System; 4.5 GB EMR; 2600 MHz and SAR value 0.762 W/kg) (www.gsmaarena.com) was placed immediately above the cages (approximately 10 cm away from the rats). The control group was kept in another room, in which there was no EMR signal. Following exposure for 8 weeks, all rats reached (65–110 days old) and were sexually mature (Soylu 2012), approximately on the same day of the estrus cycle. All rats were sacrificed by administering ketamine hydrochloride (40 mg/kg) and xylazine hydrochloride (5 mg/kg) under anesthesia (Ketalar, Eczacibasi, Istanbul, Turkey; Rompun, Bayer, Istanbul, Turkey). The ovarian tissues were then extracted and promptly placed in the formalin solution.

Histological examinations

The ovarian tissues were preserved for 24 to 48 h in a 10% formalin solution. Then, the samples were rinsed for 6 h under running water. The samples underwent a 10-min increasing ethanol series (70%, 80%, 96%, and 100%) and cleaned in xylene for 15 min, infiltrated with two paraffin changes at 60 °C, and then embedded in paraffin. Serial ovarian sections in 5 µm were cut from every paraffin block using a microtome. The sections were stained hematoxylin and eosin (H&E), followed by dehydration with increasing alcohol concentrations, xylene clearing, and Entellan glass mounting. The slides were examined under the light microscope (Yarar Demir et al. 2018).

Classification and counting of follicles

We categorized and counted healthy follicles using accepted morphological criteria (Duncan et al. 2017). Atretic follicles with damaged oocyte and pyknotic granulosa cells were not included. Briefly, an oocyte surrounded by a layer of squamous granulosa cells was called a primordial follicle; an oocyte surrounded by a single of cuboidal granulosa cells was called a primary follicle; an oocyte surrounded by two or more layers of granulosa cells was called a secondary follicle; and an oocyte surrounded by multiple layers of granulosa cells with a cavity filled with clear fluid was referred to as an antral follicle.

Secondary and antral follicles were only counted to prevent double counting when the oocyte nucleus was visible. Two kinds of follicles were distinguished: primordial and growing. Any primary, secondary, or antral follicle was considered to be growing (Grover et al. 2018). A quantitative evaluation was performed by counting the number of follicles in each ovarian section ($n=6$). Three counts and an average were made of the follicles with visible oocytes in the sections (Tayefi et al. 2015).

Immunohistochemical examinations

5µ tissue sections were placed on polylysine slides and subjected to immunohistochemistry for the proteins involved in autophagy and apoptosis. In short, a graded series of ethanol was used to rehydrate the paraffin sections after they had been dewaxed using an alternative to xylene. The samples were heated to 95 °C in 10 mM sodium citrate (pH 6.0) to retrieve the antigen. 0.3% hydrogen peroxide in methanol was used to quench endogenous peroxidases for five minutes at room temperature. The sections were blocked in blocking serum (Ultra V Block, ScyTek Laboratories, Utah, USA) for 5 min. After that, the primary antibodies were incubated with them Beclin-1 (FNab00860, 1:30, Fine Test, Wuhan, China), Caspase-3

(ab13847, 1: 50; Abcam, Cambridge, UK), anti-FSH (sc-374452; 1:100; Santa Cruz) and Anti-iNOS (Anti-iNOS antibody (SP126; 1:100; ab115819) overnight at 4 °C.

Following the application of primary antibodies, tissues were rinsed three times with PBS and then incubated for 10 min with biotinylated anti-mouse (BA-9200; 1:400 Dilution; Vector Laboratories, Burlingame, CA) secondary antibodies. After the secondary antibody was applied, the tissues were rinsed three times in PBS, incubated for 10 min with streptavidin peroxidase, and then transferred back into PBS.

For the reaction, the DAB Substrate Kit solution was used. Before applying entellan, Mayer's hematoxylin was used as a counterstain on the slides. Under a light microscope, the slides were viewed and captured on camera (Kartal et al. 2023).

Analysis and evaluation of immunohistochemistry

A semi-quantitative assessment of positive antibody expression was conducted using HSCORE analysis, as previously reported (Hirsch et al. 2003; John et al. 2009). First, each cell in a fixed field has its staining intensity (0 = no expression, 1 = mild, 2 = moderate, or 3 = strong) established. The HSCORE may be more sophisticated than the total of the HSCORES for each intensity level observed, or it may be based on a prevailing staining intensity. After calculating the proportion of cells at each staining intensity level, an HSCORE is ultimately assigned using the method below:

$$\text{HSCORE} = [1 \times (\% \text{ cells } 1+) + 2 \times (\% \text{ cells } 2+) + 3 \times (\% \text{ cells } 3+)]$$

Higher-intensity staining in a sample is given more relative weight by the final score, which ranges from 0 to 300. Five randomly chosen regions from each Sect. (36 slides for each group) were examined under a light microscope with a 40X objective. Two researchers, who were blinded to the study, independently calculated the proportion of cells at each intensity score intensity level throughout the five locations. Both observers' average scores were used.

Statistical analyses

All continuous data were reported as mean $\pm$ standard deviation. Windows version of SPSS 20.0 (SPSS Inc., Chicago, USA) was used for all statistical analyses. The Kolmogorov–Smirnov one-sample test was used to compare normality of sample distribution. Statistical analysis was performed by using One-way ANOVA followed by post hoc Tukey's multiple comparison tests. The statistical significance of data was defined as $p < 0.05$. HSCORE datas are presented as the mean $\pm$ standard error (SEM) of 3 independent experiments.

Results

Histomorphological changes

Histological sections of the ovaries from the control group (Fig. 1A) showed very good development of the ovarian follicles in different stages, normal blood vessels, and stromal cells in the interstitial area. The primary follicles with the oocyte surrounded by a single layer of cubical granulosa cells rested on the basement membrane (Fig. 1A). The secondary follicles showed normal histological features, typical morphology of oocytes with euchromatin nucleus, surrounded by zona pellucida (ZP), and a multilayered mass of granulosa cells. The innermost layer of the granulosa cells was adjacent to ZP, and the outermost layer of these cells rested on the basement membrane, which was adjacent to the stromal cells. The theca interna and the theca externa were seen.

The microscopic examination of the ovarian tissue sections of the stand-by group showed ovarian follicle degeneration (Fig. 1B). Thin and irregular ZP surrounded the primary follicles with degenerative oocytes, and congestion was observed in blood vessels. There were also histological alternations in the secondary follicles, including vacuolations in the ooplasm and granulosa cell layers.

The most evident histopathological change in the ovaries of the rats from the dialing group (Fig. 1C) was congestion. The microscopic examination of the ovarian tissue sections of this group showed the congestion of blood vessels and degeneration of follicles in different stages.

In some secondary follicles, vacuolation was observed in granulosa cells, and micronuclei formation was detected in oocytes. Congestion and degenerative theca and granulosa lutein cells (GLCs) and theca lutein cells (TLCs) were seen in the corpus luteum (Fig. 1C).

We categorized and counted healthy follicles using pre-established morphological criteria in order to investigate the effects of EMR on rat ovarian follicles (Fig. 2).

Primordial follicles determine an individual's ovarian reserve and reproductive longevity. Therefore, we looked at each experimental group's primordial and growing follicle count independently. Figure 2A shows an average of primordial follicle, while Fig. 2B shows an average of growing follicles per section. Radiation exposure dramatically decreased the number of average primordial and growing follicles in stand-by and dialing groups compare to control.

Both the stand-by and dialing groups demonstrated a substantial drop in total counts when all follicle classes were examined collectively in comparison to the control group.

Immunohistochemistry results

The rat ovaries from the control group did not exhibit any distinct immunostaining of Beclin-1 (Fig. 3A and D). Immunoeexpression of Beclin-1 was seen in the granulosa cells of the primary and secondary follicles and some GLCs and TLCs of the corpus luteum in the cortex of the ovaries from the stand-by group (Fig. 3B and E). In the dialing group, strong Beclin-1 immunostaining was detected in the granulosa cells of the ovarian follicles and the GLCs and TLCs of the corpus luteum in the ovarian cortex (Fig. 3C and F).

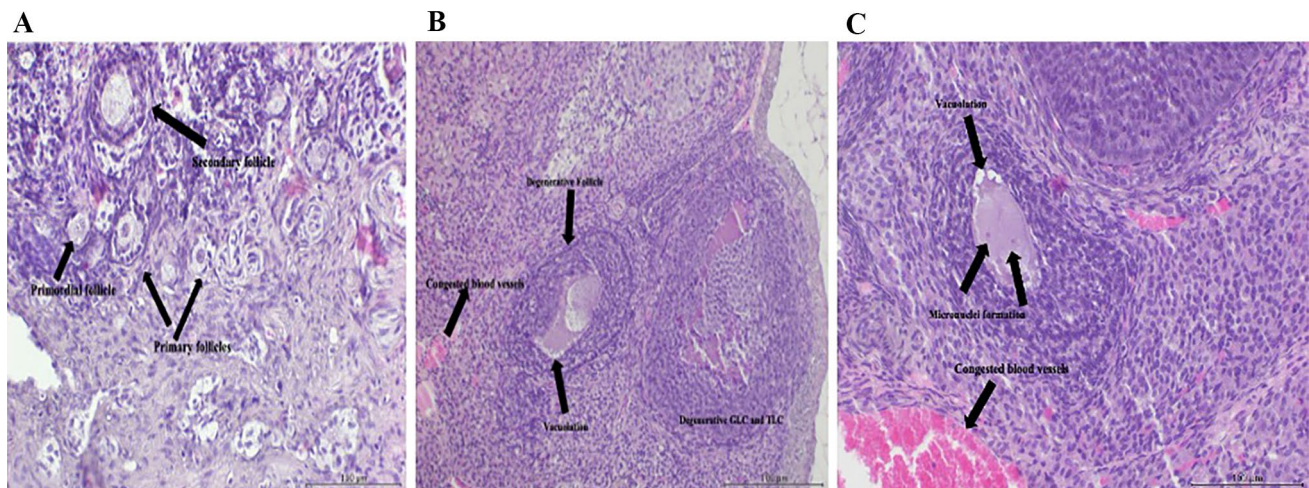


Fig. 1 Representative images of the rat ovarian sections stained with hematoxylin and eosin. The control group has a normal histological structure of the ovarian follicles (A). The stand-by group shows congested blood vessels, degenerative growing follicles with vacuolization in ooplasm and granulosa cells and degeneration in the corpus

luteum. (B). Congested blood vessels are seen in the dialing group (C This group presents with primary follicles with vacuolation in ooplasm and micronuclei formation. (Magnification: A and B = 10×; C = 40X)

Fig. 2 The number of the primordial and growing follicles in control, stand-by and dialing groups. There is a significant decrease in the number of primordial and growing follicles compare to the control group

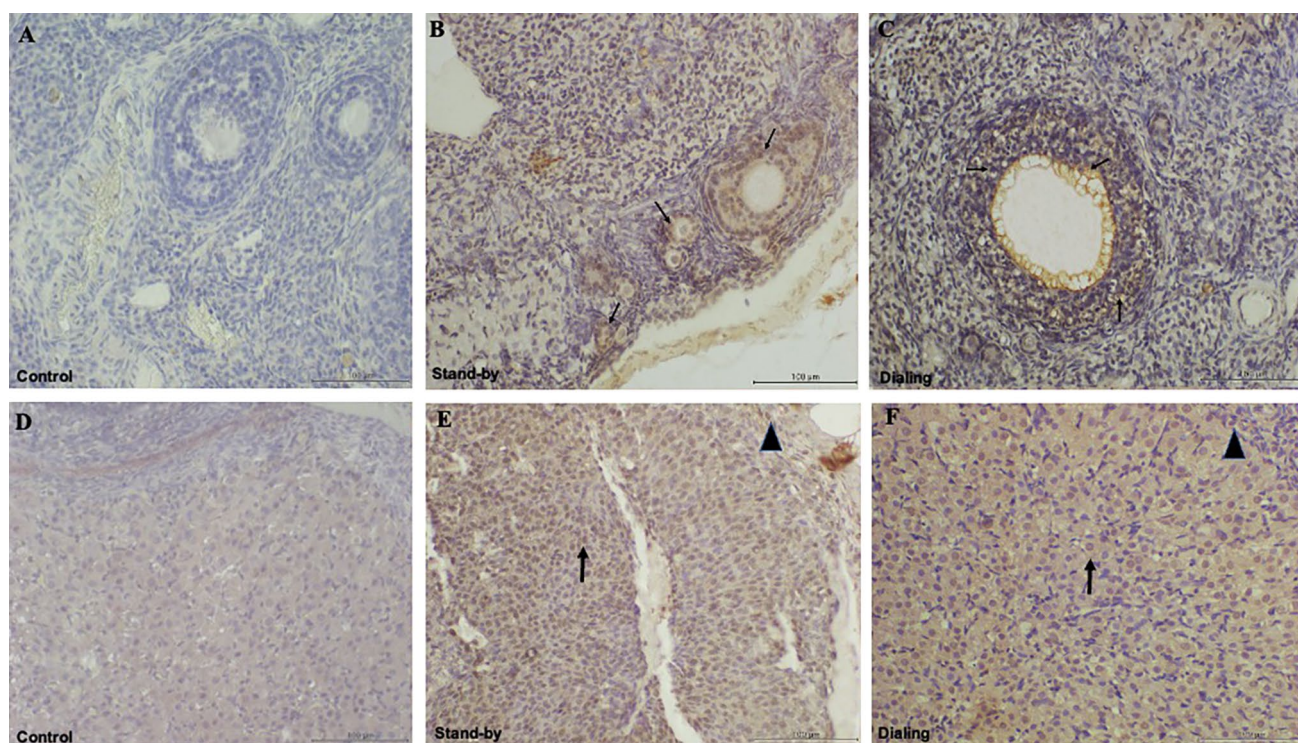
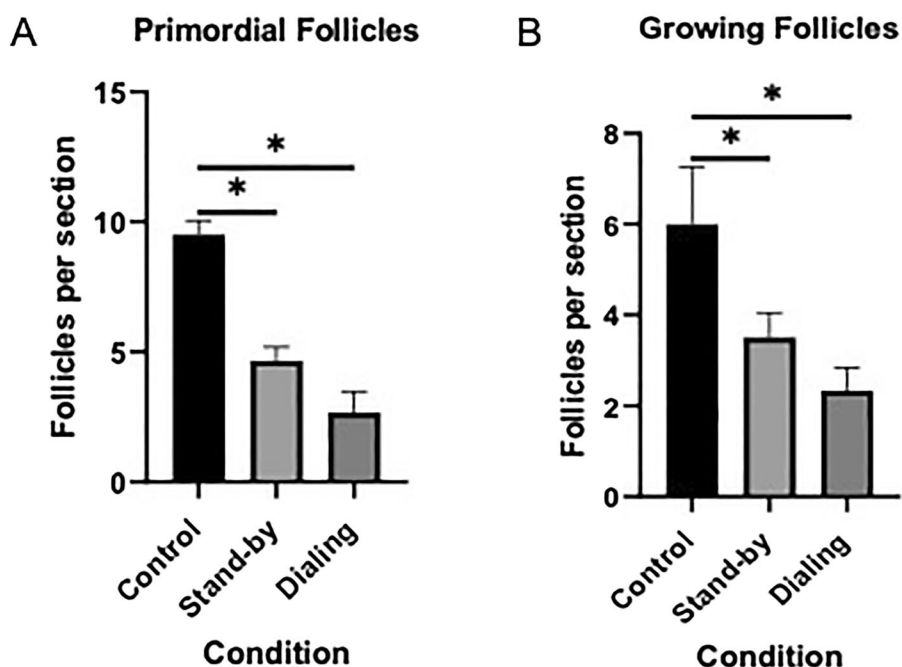


Fig. 3 Immunohistochemical distribution of Beclin-1 in rat ovarian tissue. There is no specific immunostaining of Beclin 1 in control groups (A and D). Immunostaining of Beclin-1 is seen in the granulosa cells (thin arrows) of the ovarian follicles, and granulosa lutein

cells (thick arrows) and theca lutein cells (arrowhead) of the corpus luteum in the ovarian tissues of the stand-by (B and E) and dialing groups (C and F). (A, B, C = 10X; D, E, F = 40X magnification)

When we analyzed the immunoexpression of Caspase-3, we observed no significant staining in the ovaries of the control group (Fig. 4A and D). Caspase 3 was moderately

expressed in the primary follicles' granulosa cells and TLCs and GLCs in the ovarian tissues obtained from the stand-by group (Fig. 4B and E). Strong Caspase-3 immunostaining

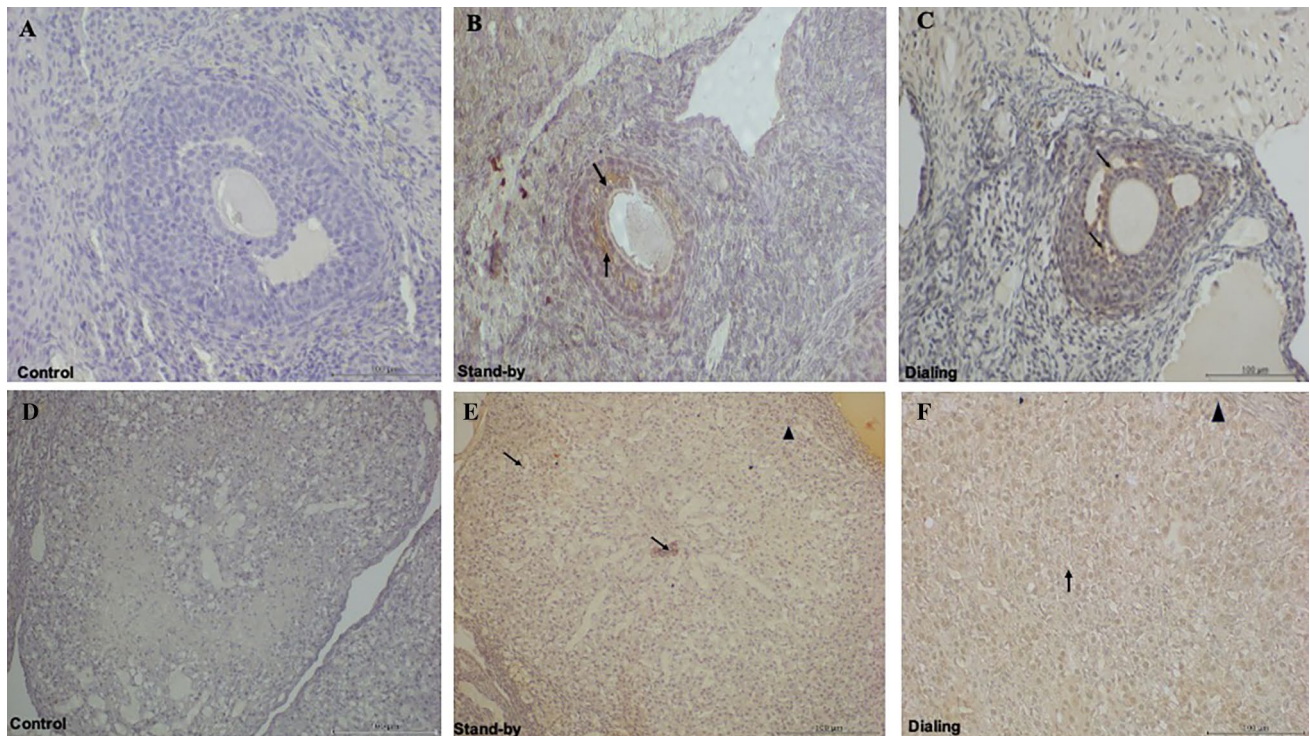


Fig. 4 Immunohistochemical distribution of Caspase-3 in rat ovarian tissue. There was not any specific immunopositivity in Caspase 3 expression in control (A and D). Caspase-3 immunostaining is observed in the ovarian follicles' granulosa cells (thin arrows), as

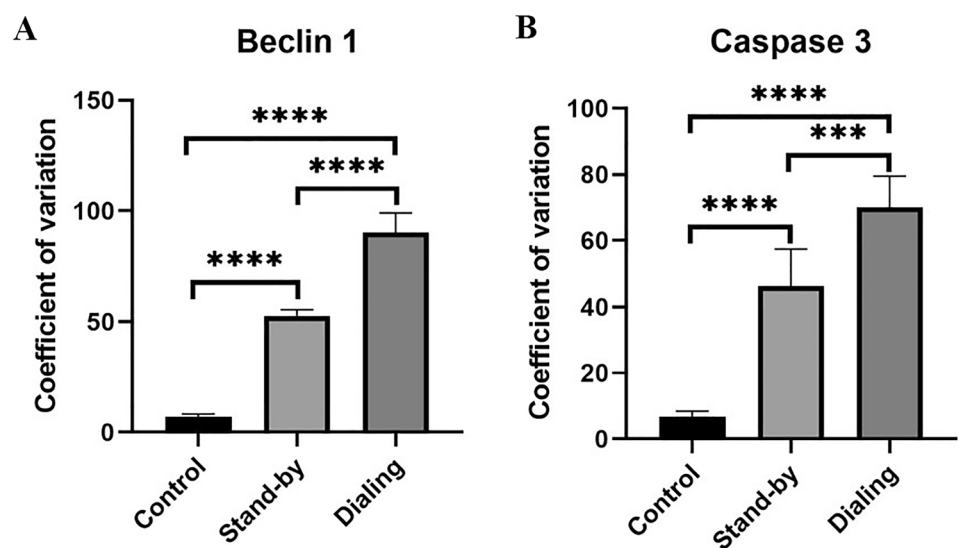
well as in the corpus luteum's granulosa lutein cells (thick arrows) and theca lutein cells (arrowhead) in the ovarian tissues of the stand by (B and E) dialing groups (C and F) (A, B, C = 10X; D, E, F = 40X magnification)

was present in the granulosa cells of the ovarian follicles and GLCs and TLCs in the rat's ovaries from the dialing group (Fig. 4C and F).

The HSCORE analyses showed that immunoexpression levels of Beclin-1 and Caspase-3 were significantly ($p < 0.05$) increased in the dialing group compared to the remaining two groups (Fig. 5).

There was not any specific staining of iNOS in the control group (Fig. 6A and D). The immunostaining of iNOS was detected in the different stages of ovarian follicles and corpus luteum in the ovaries of EMR-exposure groups (Fig. 6B and E), and it reached the highest expression level in the dialig group (Fig. 6C and F).

Fig. 5 The HSCORE analyzes of the expression levels of Beclin-1 and Caspase-3 immunostaining intensities in the ovarian samples. Data are presented as mean $\pm$ standard error. Beclin-1 (A) and Caspase 3 immunoreactivity (B) increased in the ovaries from the control to the dialing groups, reaching the maximum level in the dialing group. Asterisk: $p < 0.05$



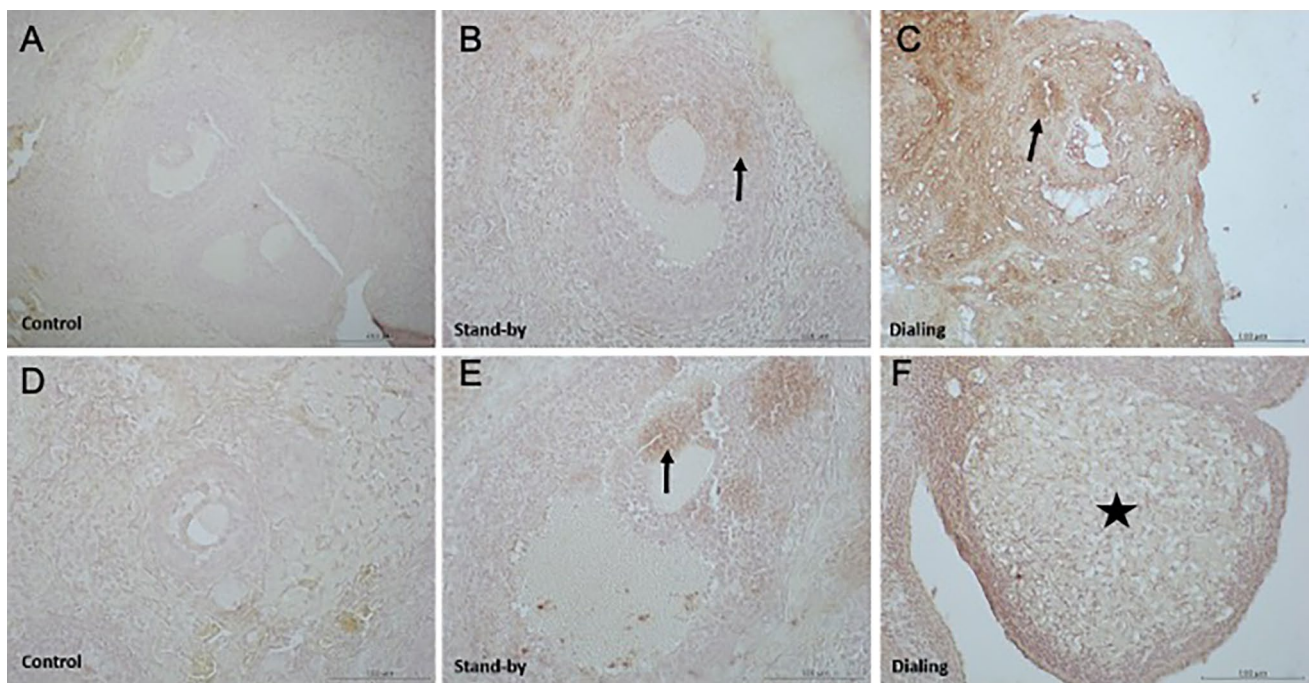


Fig. 6 Immunohistochemical distribution of iNOS in rat ovarian tissue. No staining in control (A and D). The immunostaining of iNOS is observed in the granulosa cells (arrows) in the stand- by (B and E)

of the ovarian follicles and corpus luteum (star) in the ovarian tissues of dialing groups (C and F) (Magnification: 40X)

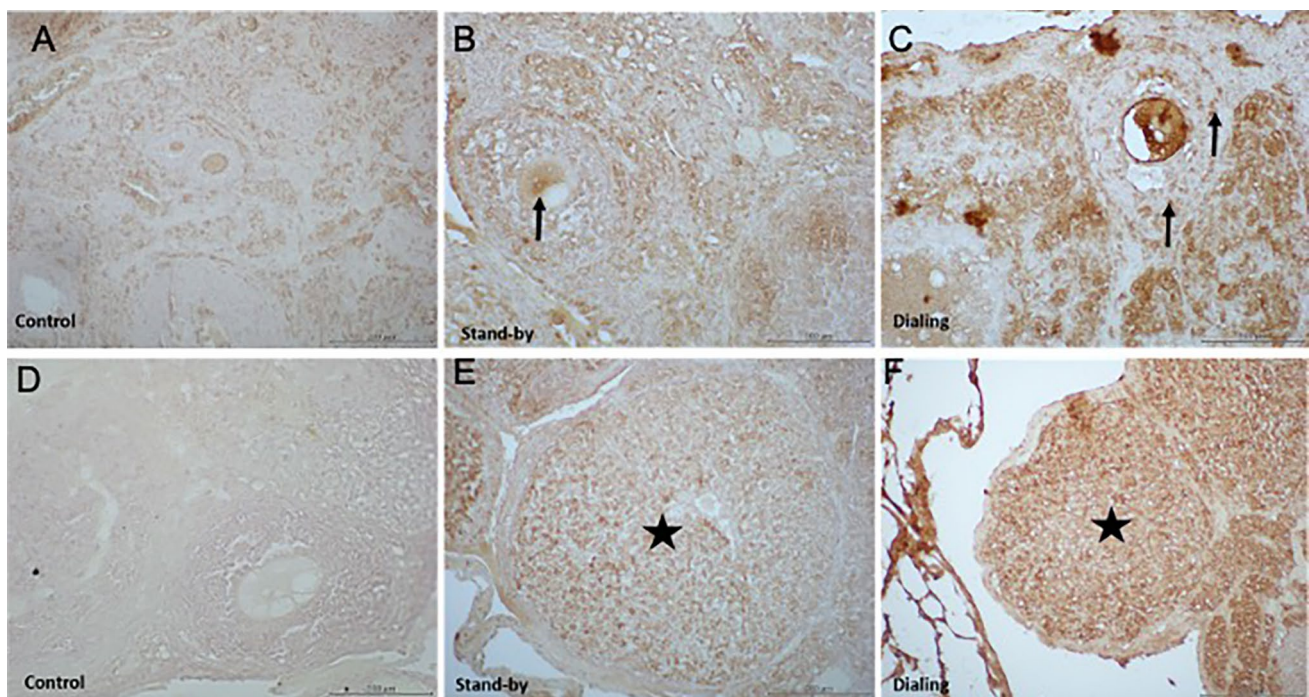


Fig. 7 Immunohistochemical distribution of FSH in rat ovarian tissue. FSH immunostaining is seen in the corpus luteum (star) and granulosa cells (arrows) of the ovarian follicles in the ovarian tissues of the stand-by (B and E) and dialing groups (C and F). (A, B, C=10X; D, E, F=40X magnification)

(A, B, C=10X; D, E, F=40X magnification)

The control group's ovarian sections showed low staining of FSH (Fig. 7A and D). Moderate immunostaining of FSH was detected in the ovarian follicles of the stand-by group (Fig. 7B and E). Furthermore, strong staining was observed in the granulosa cells of ovarian follicles and corpus luteum in the dialing group (Fig. 7C and F).

Furthermore, the dialing group exhibited significantly higher expression levels of FSH and iNOS in comparison to the stand-by and control groups demonstrated by HSCORE analyzes (Fig. 8).

Discussion

In our study, we investigated the effects of mobile phone EMR exposure for eight weeks in female rats on autophagic and apoptotic cells in the ovaries based on the expression of the Beclin-1 and Caspase-3 proteins. Firstly, we showed the harmful effects of EMR exposure on rat ovaries at the histological level. Secondly, the immunohistochemistry results showed that the autophagic and apoptotic cells, FSH, and iNOS expression in ovaries.

The electromagnetic field of mobile phones can be absorbed by various organs of the body depending on where they are carried. When carried in the waist region, the mobile phone's antenna is located close to the abdominal organs, which may cause biological interactions between EMR and the gonads (Miller et al. 2019).

One study has investigated the effect of low-frequency EMR on ovarian follicle development in rats. The researchers observed that oocytes with shrunken nuclei and narrower ZP in the EMF-exposed group. Apoptosis signs were seen in the granulosa cells of the EMF-exposed rats. They

concluded that EMF exposure might interfere with proper folliculogenesis (Roushangar and Rad 2007).

According to a study that examined the effects of low-frequency electromagnetic fields exposure during the developmental phase has been linked to ovarian reserve reduction, follicular development, oocyte differentiation, and folliculogenesis (Roushangar et al. 2014).

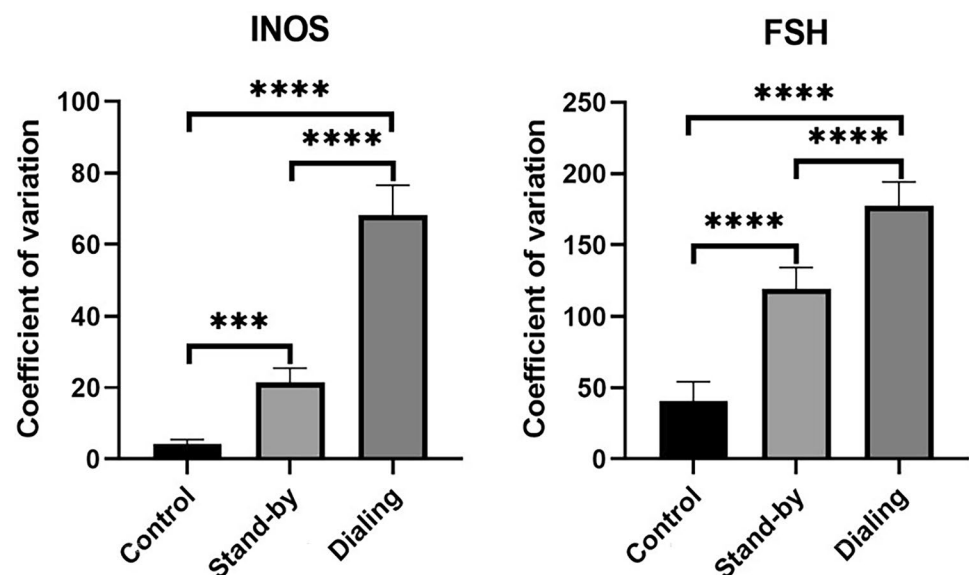
In a different study, the effects of prolonged exposure to the Wi-Fi internet frequency (2450 MHz EMF) on the development of puberty in female rats were also examined. It was found that this exposure during the prenatal stage led to postnatal growth limitation and delayed puberty (Sangun et al. 2015).

A recent study assessed the relationship between pregnant women's use of cell phones and the development of the fetus in the general population. After looking over 1378 medical documents for the investigation, it was found that 1368 moms (99.3%) had used their phones while pregnant. According to the study's findings, using a cell phone for calls for longer than 30 min per day while pregnant may be harmful to the developing fetus (Boileau et al. 2020).

It has been reported that RF-EMR exposure from mobile phone use could reduce the motility and viability of mature human sperm in vitro. On the other hand, the results of animal studies indicated that RF-EMR exposure from mobile phones could suppress sperm motility and viability (Yu et al. 2021).

In the current investigation, we used two distinct working modes—stand-by and dialing—to examine the effects of exposure to 2600 MHz EMF on rat ovaries. Degenerative changes, including congested blood vessels, primary follicles with fragmented oocytes, micronuclei formation in oocytes, vacuolation in ooplasm, and granulosa cells, were observed in the ovaries of the rats from the stand-by and

Fig. 8 The HSCORE analyzes of expression levels of FSH immunostaining intensities in the ovarian samples. Data are presented as mean $\pm$ standard error. The expressions of iNOS and FSH were significantly increased in the ovaries from dialing group compare to stand-by and control groups. Asterisk: $p < 0.05$



dialing groups. Additionally, degenerative TLCs and GLCs were seen in the corpus luteum in the stand-by and dialing group ovaries. The histological findings of our experimental groups clearly show that mobile phone radiation damaged the ovaries of female rats.

In their research, Grover and his colleagues used mice that were either exposed to total body irradiation (TBI), radiation directed at both ovaries (T2), or radiation directed at one ovary (left) with the contralateral ovary (right) left unaffected (T1). Sham mice were used as controls; they were treated in the same way as the other groups but were not exposed to radiation. Ovaries were removed two weeks after exposure, and each ovary's follicles were counted and identified through histological analysis. According to their findings, radiation considerably decreased the number of primordial follicles in the TBI and T2 groups as compared to the Sham cohort. These two irradiation groups did not differ much from one another. Targeted ovaries significantly reduced the number of primordial and developing follicles as compared to non-targeted contralateral ovaries in the T1 cohort (Grover et al. 2018).

We additionally investigated into how EMR exposure affected the number of follicles in the current study. The primordial follicle and developing follicles were separated. The number of primordial and developing follicles was significantly lower in the dialing and stand-by group than in the control group.

A woman's reproductive potential based on the quantity and the number of her remaining oocytes is known as an ovarian reserve (Zhang et al. 2016). Less inhibin B and anti-müllerian hormone (AMH) are secreted by granulosa cells when the oocyte and follicular pool decline. Follicle-stimulating hormone (FSH) rises earlier and more quickly during the follicular phase when inhibin B levels are lower. It has been stated that AMH, early follicular phase FSH, and inhibin B are indicators of ovarian reserve (Freeman et al. 2012). It's unclear if these indicators can indicate the possibility of reproduction. In several cohorts, the duration until menopause has been linked to AMH (Dolleman et al. 2013). Some studies in the literature investigated the ovarian reserve by using FSH, AMH, and inhibin B biomarkers (Jiao et al. 2021; Li et al. 2021; Steiner et al. 2017).

To ascertain how EMR affected ovarian reserve, we analyzed the immunoexpression of FSH in this study. The ovarian sections from two experimental groups showed increased FSH expression, which is evidence of poor ovarian reserve. Therefore, our results revealed that EMR exposure negatively affects the ovarian reserve.

Nitric oxide synthase enzymes (NOS) generate the signaling chemical nitric oxide (NO). NOS comes in three different isoforms: inducible NOS (iNOS), endothelial NOS (eNOS), and neuronal NOS. iNOS can promote the production of NO (Ortiz-Ortiz et al. 2009; Pi et al. 2003; Wang et al. 2013).

A family of NOSs, including neuronal NOS, iNOS, and eNOS, generate NO from its precursor L-arginine. Oxidative stress coincides with NOS activation, which causes NOS to uncouple and cause more oxidative stress (Otani 2009).

Shahin and colleagues examined the mechanism of action and long-term consequences of low-level microwave (MW) irradiation on male Swiss strain mice's ability to reproduce. The immunostaining of nitric oxide synthase (iNOS) in the testis was carried out in addition to sperm count and viability testing. They found that sperm viability and count were significantly reduced by MW irradiation. Additionally, the mice in the MW-irradiated group showed higher expression of testicular iNOS (Shahin et al. 2014).

In our study, we also investigated the iNOS expression to determine the oxidative stress level. The long-term EMR caused oxidative stress in the ovaries, demonstrated by the increased immunoexpression of iNOS in the stand-by and dialing groups.

Apoptosis also referred to as programmed cell death, and necrosis, or uncontrolled cell death, were once thought to be the two separate processes that caused cell death. However, in recent years, several additional forms of cell death have been identified, demonstrating that a cell can die through a variety of different pathways. Apoptosis is characterized by several distinctive morphological alterations in the cell's structure in addition to several enzyme-dependent (D'Arcy 2019).

Cellular constituents including macropoteins or even entire organelles are sequestered into lysosomes for destruction during the process of autophagy (Mizushima et al. 2008; Shintani and Klionsky 2004).

Trybus et al. shown that physcion causes an augmentation of the LC3 protein level, an increase in lysosomes, and autophagy vacuoles. Concurrently, pro-apoptotic action was discovered, and this mechanism relied on both a decrease in Bcl-2 expression and the activation of caspases 3/7.

One possible shared mechanism for physcion's anti-tumor activity is the stimulation of autophagy and apoptosis (Trybus et al. 2021).

Some studies in the literature have shown that granulosa cell death occurs due to apoptosis (Choi et al. 2011); however, under some conditions, autophagy may be induced by apoptosis in granulosa cells. These results suggest that both apoptosis and autophagy are involved in regulating granulosa cell death during ovarian follicular development and atresia (Lin and Rui 2010).

Granulosa cells have been found to contain some pro-apoptotic factors, such as TNF-alpha and proteins belonging to the Beclin-2 family (Matsuda et al. 2006). Research has indicated that granulosa cells produce vital chemicals for the upkeep and expansion of follicles. Therefore, follicular elimination is the outcome of granulosa cell death brought on by an apoptotic process. (Matsuda et al. 2012).

In isolated rat granulosa cells, examined the role autophagy plays in folliculogenesis and its connection to apoptosis. Autophagic vacuoles and LC3 were employed as indicators of autophagy, while cleaved Caspase-3 was utilized to indicate apoptosis. The researchers demonstrated that isolated granulosa cells expressed cleaved Caspase-3 and LC3 at all embryonic stages. These findings suggest that autophagy is stimulated in granulosa cells in association with apoptosis during folliculogenesis (Escobar et al. 2008).

The study used an exposure setup of 5 min on and 10 min off to examine the short-term (24-h) effects of 1800 MHz mobile radiofrequency radiation (RFR) (SAR = 2 and 4 W/kg). The researchers observed apoptosis, reactive oxygen species (ROS) generation, and DNA damage after exposure. The study's findings showed that short-term exposure to mobile phone RFR did not damage DNA in marginal cells and did not raise the rate at which cells undergo apoptosis (Yang et al. 2020).

Conclusion

In the current study we investigated the effects of EMR exposure on rat ovaries. We showed that the harmful effects of the EMR exposure by demonstrated the damaged histological architecture of ovaries- exposed to EMR. Additionally, the detrimental effects of EMR exposure is confirmed by the results of increased immunoexpression of FSH. Furthermore the oxidative stress was rised under the exposure of EMR that is concluded by the increased levels of iNOS.

Furthermore when we focused on the the mechanism under these effects we decided to investigate the apoptotic and autophagic cells. Therefore we also investigated the immunoexpression of Caspase 3 and Beclin 1. The higher expression of caspase 3 and Beclin 1 was seen in EMR-exposure ovaries.

Based on the findings of our study, we conclude that EMR exposure from mobile phones has harmful effects on the ovaries. Further studies are need to exactly determined the mechanism under the apoptosis and autophagy mechanisms are involved in this process.

Study limitations

However, some limitations should be considered when interpreting our data. The study's primary drawback is that it was conducted in an experimental rat model, which allowed us to draw conclusions that could not be applied directly in humans.

Author contributions B.K. and S.C. wrote the main manuscript M.A. and G.A. designed the experimental procedure E.A. prepared the figures.

Funding This work received no financial support.

Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interest The authors declare no competing interests.

Ethical approval Animals were obtained from Kobay Animal Breeding and Experimental Research Laboratory in Ankara, Turkey. The animal ethics committee approved the experimental protocol (Approved number: 338) of Kobay Company (Turkey).

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