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Hatching characters, behavioral and some blood changes of quail chicks exposed to mobile phone radiation during development.

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ABSTRACT: This study was designed to investigate the impact of radiation emitted by third-generation Huawei mobile phones operating at a 900 MHz radio frequency put inside the hatchery on hatching, behavioral and some blood changes of quail embryos, 632 fresh fertilized Japanese quail chick eggs were put into two hatcheries in a separate room. The control group (n=456) was not exposed to any phones, and the second group, consisting of 176 samples, was exposed to mobile phone radiation, was subjected to mobile phone radiation exposure. In this setup, the mobile phone was strategically positioned at the center, directly above the eggs, and called 15 minutes every 6 hours (60min/24hr), Eggs were left until hatching , the incubation period , hatchability %, weight of one day newly hatched quails and social and fear behavioral tests were performed at 3 days after hatching and the blood samples were collected from the quail. The obtained result, illustrated that,a significant increase in the dead embryos %, cortisol level, more time in belongingness, aggregation and fear behavior while, a significant decrease in hatchability%, incubation period, total protein, total albumin, globulin and serum phosphorous level period in exposed group than control group.

KEYWORDS: phone radiation, social behavior, fear behavior, belongingness, aggregation test.

1. introduction

In recent years, animal behavior has become an increasingly popular field of study, and its relevance to many other subjects is widely appreciated. in the fields of agriculture and veterinary medical sciences, this appreciation has been relatively slow. However, with the growth of intensive husbandry systems and the desire for higher standards of animal welfare, the subject has become important to all agricultural animals, including the poultry,The human population of Egypt continues to increase and animal proteins available are not sufficient to meet the requirements of human population, moreover, the price of meat is steadily increasing, so the raising of Japanese quail (CotunixJaponica) in Egypt is very important to help in solving the problem of a deficiency in animal protein [1]. The Japanese quail has become a species of considerable economic importance., owing to its compact size, low-cost rearing needs, and swift maturation relative to other domesticated poultry. Its remarkable adaptability to diverse husbandry environments further enhances its appeal in both commercial and research settings. Beyond its agricultural importance, the species has gained popularity as a laboratory animal, particularly in behavioral studies, due to its manageable size, ease of handling, and well-documented developmental patterns.[1] These characteristics make the Japanese quail an ideal model for a variety of scientific investigations. Mobile phones generate radiofrequency electromagnetic radiation (RF-EMR) that extends into the surrounding environment, raising concerns about potential health risks. Prolonged exposure to RF-EMR has been linked to various neurological and physiological changes, particularly in adolescents and young adults.[14]. Studies suggest that such exposure may contribute to behavioral disturbances, cognitive decline,

NVVJ., Vol. 5, Issue (2), 2025

and impaired brain function. The emitted radiation can interfere with neural activity, potentially affecting memory, attention span, and emotional regulation. These findings highlight the importance of understanding the long-term implications of frequent mobile phone usage, especially among younger populations whose developing nervous systems may be more vulnerable to radiation-induced effects. [6]. Mobile phones emit radiofrequency electromagnetic radiation (RF-EMR), which may have adverse health effects. Exposure to RF-EMR has been linked to neurological and physiological changes, as well as behavioral issues, particularly in adolescents and young adults. The radiation from mobile phones can potentially harm the brain, leading to cognitive decline and behavioral disturbances [6]. A substantial body of experimental research has focused on investigating the impact of electromagnetic fields (EMF) on the brain and nervous system. [4, 21, 7]. The current study hypothesized that Japanese quail chicks eggs exposed to mobile phone radiation during incubation process possess a negative impact on the one day-old chick biochemical parameters, percentage of hatchability, while on (three day old) chick social behavior.

2. Materials and Methods

2.1. Egg preparation

This experiment started from March to May 2023, a total of 632 fresh fertilized Japanese quail chicks eggs were obtained from a commercial farm in kafer-El-Sheikh, Egypt. Firstly, sterilization of hatching eggs should be performed before egg incubation by Fumigation with formaldehyde gas which has clearly demonstrated that this is an effective way of disinfection of hatching eggs to minimize bacterial contamination. The gas can be generated by several methods, but the most common way used in the poultry industry was the addition of formalin to potassium permanganate (KMnO4) in 2:1 ratio (v/w).The concentrations can be achieved by the reaction of 45 ml 40% formalin solution with 30 g potassium permanganate crystals per m3 or by the heating of 10 g of paraformaldehyde per m3 under controlled temperature [8] the fertilized eggs were transferred to the incubation phase, where they were maintained under controlled conditions at a temperature of 38.3 ± 0.2°C and a relative humidity of 60%, ensuring optimal embryonic growth and development in accordance with the standards outlined by [37].

2.2. Experimental design:

This experiment was designed to assess the impact of mobile phone radiation on the hatching process of fertilized eggs and newly hatched behavior which done as:- 632 Eggs were incubated into two separated room hatchery, The first control group without any phone exposure (n=456) and second group were exposed to mobile phone radiation (n= 176) by A third generation (3G) device (Huawei GR5) run on network frequency bands B1 mode (2100 MHz) [10]. In this experiment, the mobile phone was strategically positioned at the center, directly above the eggs which called 15 minutes every 6 hours (60min/24hr) and these ringing continue till the process of hatching occurs. (Figure. 1A&B) This mobile usually examined for network and charging. On the 15th day of incubation, the incubator was carefully opened, and the egg holder was removed. The eggs were then randomly distributed across the incubator floor to optimize conditions for the hatching process, as illustrated in Figure. 1A This arrangement aimed to provide the embryos with adequate space and airflow, facilitating successful hatching. The eggs remained in this position from day 16 to day 19, during which the hatching process was closely monitored. Upon completion, the newly hatched quails were thoroughly examined and photographed to identify any congenital abnormalities, with particular attention to deformities in the wings, beak, and legs.

Data collections.

1 Incubation period = The duration from when the egg is laid until the quail hatches.

2 Hatchability %= (number of hatched eggs/numbers of fertile eggs) × 100.[36]

3 Body weight of one day hatched quails.

4 Social behavior test done at 1-3days as at room temperature (36 °C). as

2.3. Social behavior test

2.3.1. Aggregation behavior test according to [41]

A cardboard box measuring 45 × 45 × 24 cm (length × width × height) was utilized as the experimental apparatus. The floor of the box was lined with a paper towel to provide traction and a clean surface for the chicks. To create distinct triangular spaces, four cardboard fences were placed at the corners of the box, forming compartments measuring 18 × 18 × 25 cm each. A video camera was mounted above the setup to capture the entire experimental process. For the test, four quails from either the experimental or control group were randomly selected and placed individually into the triangular compartments, separated by the cardboard fences. Once positioned, the fences were simultaneously removed, allowing the chicks to move freely. The camera recorded their movements, focusing on the aggregation behavior—the tendency of the chicks to group together. The time taken for two, three, and all four chicks to aggregate was carefully recorded, as depicted in Figure. 1. To ensure reliability, the procedure was repeated three times for both the experimental and control groups. The average aggregation time for each group was then calculated to evaluate potential differences in social behavior between the two conditions.

2.3.2. Belongingness test according to [41])

A cardboard box with the same dimensions as the one employed in the aggregation behavior test (45 × 45 × 24 cm; length × width × height) Figure. 1 was used, The floor was lined with a paper towel to ensure cleanliness and provide traction for the chicks. To create the testing environment, a cardboard fence was placed in one corner, forming a triangular space measuring 18 × 18 × 25 cm. On the opposite corner, a plastic mesh fence of identical dimensions was installed to allow visual and auditory contact between the chicks. A video camera was put above the apparatus to record the entire procedure. Prior to the test, the quails underwent a 1-minute acclimation period in a separate cardboard box of the same size but without the mesh fence, providing an open-field area for familiarization with the environment. Following acclimation, four quails from either the experimental or control group were selected. 3 of them were placed in the corner behind the mesh fence, representing the group to which the isolated chick belonged. The remaining chick was positioned in the opposite corner behind the cardboard fence. After a 30-second waiting period, the cardboard fence was removed, allowing the isolated chick to move freely toward the mesh fence and its companions. The time taken for the isolated chick to reach the mesh fence was recorded as a measure of its belongingness or social attachment. This test was repeated three times for both the experimental and control groups, and the average time for each group was calculated to assess potential differences in social behavior.

2.3.3. Tonic immobility (TI) test

This behavior performed twice at the age of (three days and at the age of 35days); The birds were transferred to a separate, quiet room for tonic immobility (TI) measurements. The TI was induced by inverting the bird on its back and applying a manual restraint until the bird stopped struggling. The tonic immobility (TI) test was conducted to assess the fear response in quails by measuring the duration of immobility after physical restraint. Each bird was gently placed on its back and held in this position for 10 seconds to induce the immobility reaction. Care was taken to minimize external disturbances—such as unnecessary noise or movement—and direct eye contact between the observer and the bird was strictly avoided, as previous research [17]. The TI duration was defined as the time from the moment the bird became immobile until it righted itself. If a bird remained immobile for

NVVJ., Vol. 5, Issue (2), 2025

300 seconds, the test was terminated, and the TI duration was recorded as 300. Conversely, if a bird failed to enter a state of immobility after five induction attempts, a TI score of 0 was assigned. The number of attempts required to induce TI was documented for each bird, providing additional insight into their susceptibility to fear.

2.3.4. Blood Parameters

At the conclusion of the experiment, a representative sample of quails (n = 15) from each group was randomly selected for further analysis. Each bird was carefully weighed to record body mass as an indicator of growth and overall health. Following the weighing process, the quails were humanely slaughtered in accordance with Islamic guidelines, a method recognized for its emphasis on minimizing pain and distress to the animals. [33]. Blood samples were collected from the slaughtered quails and transferred into test tubes, some containing an anticoagulant and others without, depending on the intended analysis. The samples were left at room temperature for 30 minutes to allow clot formation in the non-anticoagulant tubes. Following this, the tubes were refrigerated for 60 to 90 minutes to enhance serum separation. Subsequently, the blood samples were centrifuged at 3000 rpm for 10 minutes, resulting in the separation of the serum from the cellular components. Using a micropipette, the separated serum was carefully transferred into labeled Eppendorf tubes to avoid contamination. The collected sera were then stored at –20°C until further biochemical analyses could be performed. All measurements were conducted using a commercially available kit following the manufacturer's instructions to ensure accuracy and consistency in the results.

2.3.5. Chemical parameters

The concentrations of total serum proteins, albumin, inorganic phosphorus, and calcium were determined using a digital VIS/ultraviolet spectrophotometer, ensuring precise and reliable measurements. The analysis was conducted following standardized protocols to maintain consistency across all samples. Addtionallly, the albumin/globulin (A/G) ratio was calculated to evaluate the balance between these two major serum protein fraction, which can indicate the equation[38]

$$\textbf{A/G ratio} = \frac{\text{Albumin (g/dl)}}{\text{Globulin (g/dl)}}$$

Globulin values were calculated by subtracting albumin concentration from the total serum protein levels. This ratio provided valuable insights into potential physiological changes between the experimental and control groups

2.3.6. Serum hormones:

Serum triiodothyronine (T3) , thyroxin (T4) and cortisol hormones concentration were measured by radioimmunoassay with a kit which was produced by the Institute of Isotopes Co., Ltd. (Budapest, Hungary), and the samples were counted on Packard Gamma Counter (PerkinElmer Inc., Branford, CT, USA).

2.4. Statistical analysis

Data were tested for distribution normality and homogeneity of variance. Data were reported as means and standard error. In case of pre hatching using T test and the post hatched quail data were analyzed by one-way ANOVA with statistical package SPSS. The significance of difference among the different light groups was evaluated by Duncan test. The significance level was set at $P \leq 0.05$

2.5. Ethical approval

This research was conducted in strict accordance with the Animal Care and Use Committee (ACUC) guidelines of Medicine, Assiut University, Egypt, under the approved ethical number 04-2024-100240. All procedures involving hatching eggs and quails adhered to established ethical standards to ensure the humane treatment of the animals throughout the study. Proper care and management practices were implemented to minimize stress and discomfort, maintaining optimal conditions for incubation, handling, and post-hatching observations. The study prioritized animal welfare, ensuring that all experimental procedures

Table 1: Effect of mobile radiation inside incubator on some blood parameters of newly-hacted quails (one-day age)

Parameters	Control	Mobile	P-Value
Total Protein	5.2000	3.8533[b]	.005
Albumin	1.6933	1.4100[c]	.030
globulin	3.5067[a]	2.4433[b]	.015
A/G RATIO	0.4850.022	0.5860.074	.263
calcium	8.0667	8.1633	.601
phosphorous	4.2333[a,b]	4.0667[b]	.463
Ca/ph ratio	1.910.097	2.010.079	.438
Cortisol	.4800[b]	.7167.03480[a]	.005
T3	160.0	155.67	.337
T4	5.367	6.10	.463
T3/T4 Ratio	30.51	25.92	.305

Groups mean labeled different superscript letters (a, b) in the same column are significantly ($p \leq 0.05$) .

were carried out with the least possible disturbance while meeting scientific and ethical integrity requirements.

3. Results and Discussion:

3.1. Incubation period and hatchability%

The Fig. 2 and 3 illustrated that there were significant decrease in the percentage of hatchability and incubation period of embryos exposed to the mobile radiation (60% vs78%) and (17.9 % vs 19%) this data in agreement with Pawlak et al.,[24] who showed that embryos exposed to the EMF Showed accelerated pipping and hatching development, The observed differences between the control group and the electromagnetic field (EMF)-exposed group may be attributed to the stress response triggered by exposure to mobile phone radiation. EMFs, particularly at frequencies similar to those emitted by mobile phones, have been linked to physiological and developmental changes in various animal models. The potential stress-inducing effects of EMF exposure could explain the deviations in hatchability and embryonic development noted in this study. However, these findings contrast with the results reported by Sechman et al.,[29] , who found that electromagnetic field exposure did not significantly affect hatchability. A key difference between the studies lies in the experimental design. Sechman et al.'s study suggests that the absence of egg turning during incubation may have significantly influenced their findings.

3.2. Dead embryo %

Figure. 4 demonstrates a significant increase in the percentage of dead embryos in the group exposed to mobile phone radiation (24%) compared to the control group (12%), indicating a notable impact of electromagnetic exposure on embryonic viability. These findings align with previous research by (Batellier et al.,[5], (Roda et al., [28], and Vereshchako et al.,[35], they reported that mobile phone radiation can have detrimental effects on embryonic development, potentially leading to increased mortality rates. However, these results contrast with the findings of Pawlak et al.,[25], who observed no significant impact of 900 MHz electromagnetic field exposure on chicken embryo mortality. The discrepancy between studies may be attributed to differences in experimental conditions, such as radiation exposure duration, intensity, incubation environment, and species-specific sensitivity [30]. Furthermore, variations in embryonic resilience to electromagnetic fields across different bird species could explain the conflicting outcomes, emphasizing the need for further controlled studies to fully understand the biological effects of mobile phone radiation on avian development.

3.3. Body weight at hatching

Quails egg exposed to mobile phone radiation inside the incubator show significant decrease in the first day hatched body weight (Figure. 5) this data was in agreement with finding of Youbicier-Simo et al., [39] and Zareen et al., [40] who reported that, electromagnetic radiation emitted by mobile phones on chick embryos during incubation resulted in significantly lower body weights. This data may be due to the decrease (numerically but non-significant) in T3 and T4 and T3 / T4 ratio .

NVVJ., Vol. 5, Issue (2), 2025

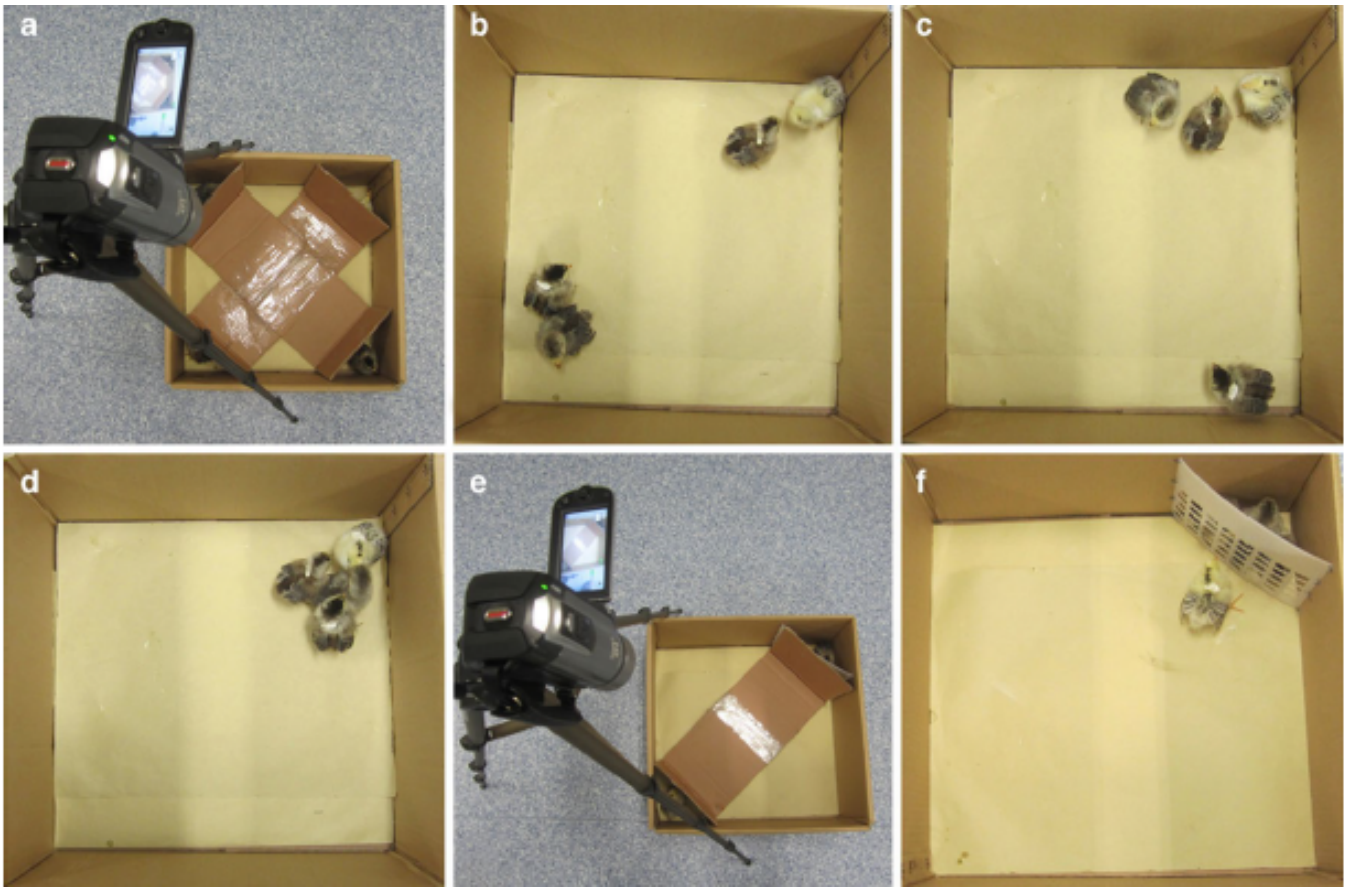

Figure 1: Aggregation test (a-d) and belongingness test (e and f)

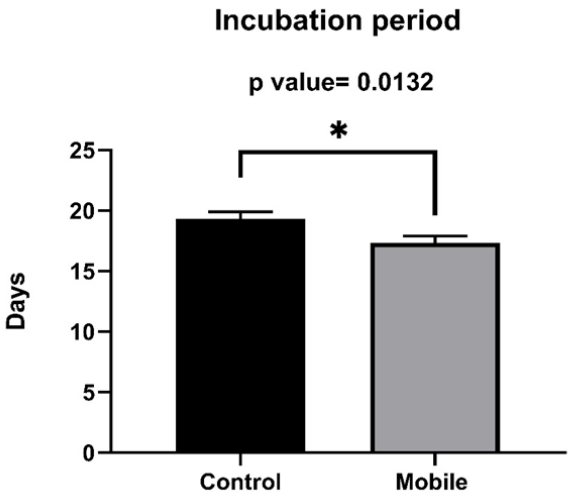

Figure 2: Effect of mobile radiation inside incubator on of incubation period

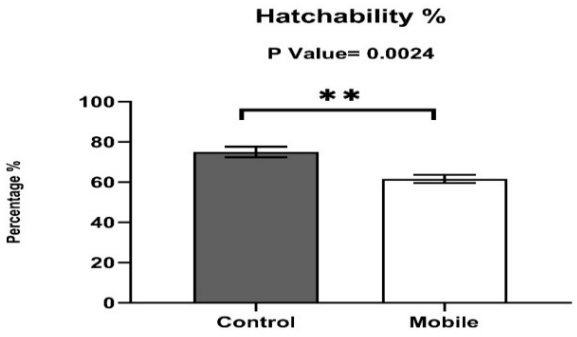

Figure 3: Effect of mobile radiation inside incubator on hatchability%

3.4. Social behavior:

The Aggregation and Belongingness tests were employed to assess social behaviors in three-day-old post-hatch quails, following the methodology described by Zhou et al.,[41]Our findings provide compelling evidence that exposure to mobile phone radiation during embryonic development negatively impacts social behavior in newly hatched quails. Specifically, quails subjected to electromagnetic radiation exhibited a notable increase in aggregation time, indicating a delayed response in seeking

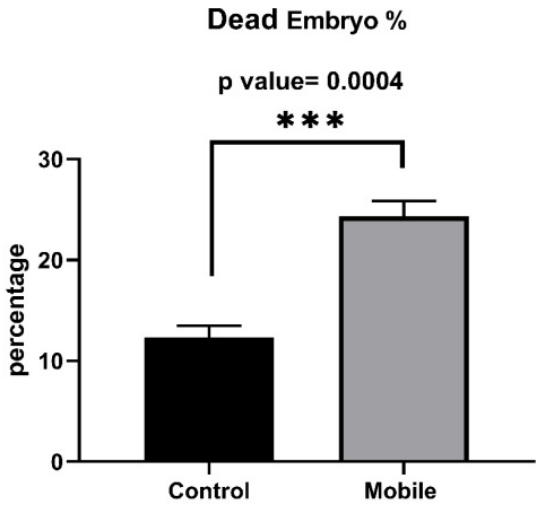

Figure 4: Effect of mobile radiation inside incubator on dead embryo%

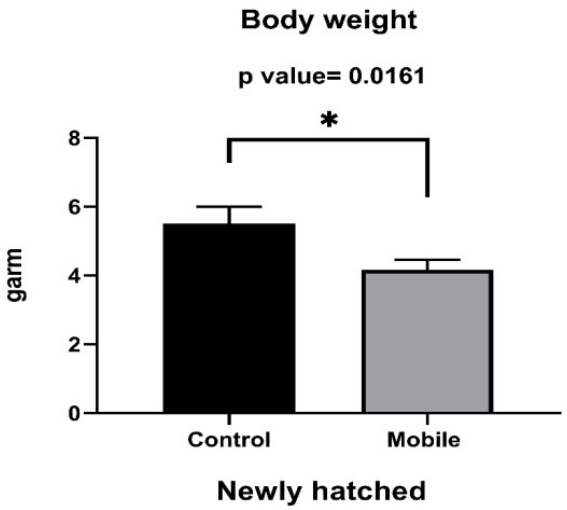

Figure 5: Effect of mobile radiation inside incubator on body weight Social behavior test:

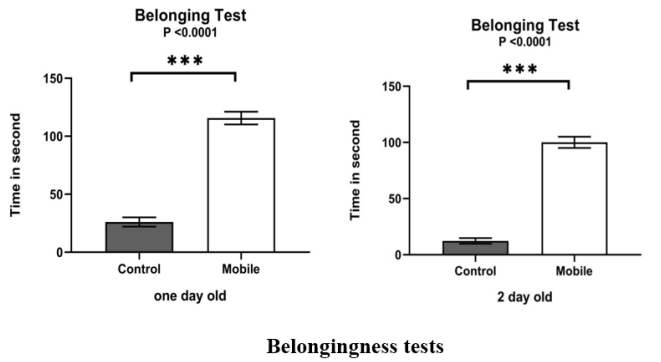

Figure 6: Effect of mobile radiation inside incubator on belongingness tests

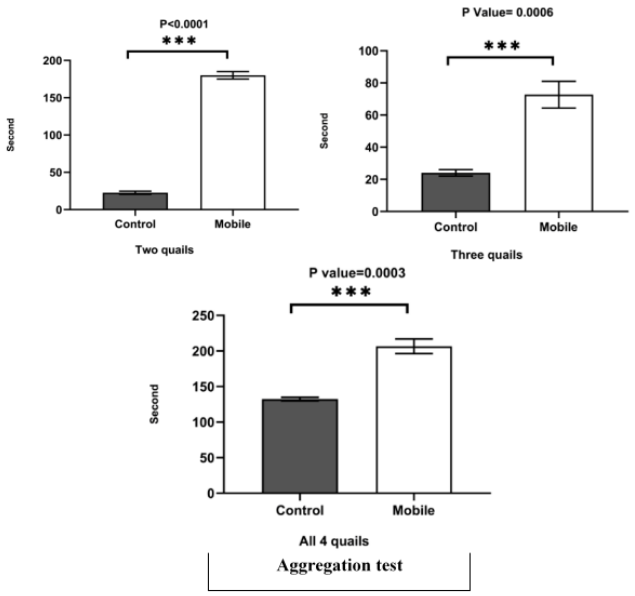

Figure 7: Effect of mobile radiation inside incubator on aggregation tests

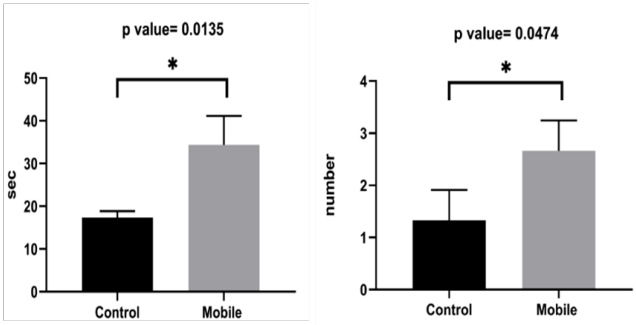

Figure 8: effect of mobile radiation inside incubator on tonic immobility test

social contact with their peers. Additionally, the belongingness test results revealed that radiation-exposed chicks took longer to approach their group members, suggesting impaired social recognition and affiliative behaviors.

Figure. 6 illustrates findings that align with the results reported by Haba et al.,[13] and Zhou et al.[42], particularly in the aggregation behavior of two, three, and four chicks as well as three and four chicks, respectively. However, our findings diverge from Zhou et al. [42]. regarding the aggregation time of two chicks, where they observed no significant difference between the radiation-exposed and control groups. Conversely, our results align with both Haba et al., [13] and Zhou et al. [42] in the Belongingness test. These findings may be attributed to the imprinting

NVVJ., Vol. 5, Issue (2), 2025

ability of chicks, which serves as a valuable indicator of recognition and memory functions in birds, as noted by Horn, [15] and Solomonia et al., [32]. The behavioral anomalies observed in newly hatched quails, such as delayed aggregation and belongingness, could be linked to abnormal neural development caused by the toxic effects of hypothyroidism during the late stages of embryogenesis [12]. Thyroid hormones (THs) play a pivotal role in brain maturation, and their deficiency during crucial developmental windows can lead to impaired intellectual growth and motor skill deficits. [9]. In the present study, the electromagnetic field (EMF) intensity was notably higher, and the duration of exposure was extended compared to previous research. These findings suggest that both field strength and exposure duration are critical factors in determining the extent of electromagnetic radiation effects on quail embryos.

3.5. Fear behavior: Tonic Immobility Test

There was significant increase in tonic immobility (Figure. 7) induction and duration in mobile phone in compare with control group. This data in agreement with Tomasova et al.,[34] who, showed that rats exposed to a sub lethal dose of gamma-rays exhibited heightened anxiety levels, as evidenced by increased avoidance behavior in elevated plus-maze tests. Radiation can influence animal behavior, with its effects depending on factors such as exposure level, timing, and genetic background.

3.6. Blood parameters

chemical parameters of serum:

3.6.1. Total protein and Total albumin and globulin

From the results tabulated at Table (1) it is clearly noticeable that the level of total protein and Total albumin and globulin in blood showed significant decrease in mobile phone group compared to control, these results are in accordance with those of Adebayo et al.,[3] and Fedotova and Turitsyna,[11] who showed noticeable decrease in total protein amount. And Fedotova and Turitsyna, [11] reported that Ionizing radiation reduces globulin levels. While, disagreed with the finding of (M et al., [18] and Adebayo et al., [2] revealed an increase in amount albumin for exposed group comparing with the control group.

3.6.2. Serum calcium (g/dl):

Results of our study in table (1) cleared that, there were no significant difference in serum calcium level in mobile phone group compared to control. Our findings are disagreement with Sieron-Stoltny et al., [31] observed that exposure to the electromagnetic field generated by a mobile phone decreased the calcium content in long bones and L4 vertebra of rats.

3.6.3. Serum phosphorus level:

The data demonstrated in Table (1) showed that, there was decrease in serum phosphorous level in exposed quail than control. The results of the serum phosphorous level in exposed quail were in contrast to those obtained by Nabighadim et al., [20], who found that a meaningful increment was seen in the level of phosphorus of exposed female rats compared to the control group.

3.7. Effect of radiation on serum hormones:

3.7.1. Thyroid hormones:

Tri-iodothyronine (T3) and thyroxine (T4), the primary hormones produced by the thyroid gland, play vital roles in regulating a broad spectrum of metabolic processes that significantly influence the growth and development of organisms. These hormones are central to maintaining energy homeostasis, primarily through their ability to increase the metabolic rate of cells. This metabolic stimulation enhances the body's capacity to produce energy, which is crucial for supporting various physiological functions, including tissue growth, neural development, and organ maturation.[19].

a-T3 and T4 levels and T3/ T4 ratio:

The data in Table (1) cleared that, the overall means values of T4 and T3levels and T3/ T4 ratio showed that, a non-significant difference between treatments. These

results were similar to those obtained by Rahimnia et al.,[27]reported that, T4, T3 showed no significant differences found among groups while, these results are in disagreement with the report of Pawlak et al., [23] who showed that T4 and T3 concentrations decreased markedly in embryos exposed to 1800 MHz EMF during embryogenesis

3.7.2. Cortisol level (ng/ml):

The activation of the Hypothalamic-Pituitary-Adrenal (HPA) axis is a central physiological mechanism that governs the stress response in vertebrates. When an organism encounters a stressor, the hypothalamus is stimulated to release Corticotropin Releasing Factor (CRF) and/or Arginine Vasotocin (AVT), which serve as key signaling molecules in initiating the stress cascade. These neuropeptides then act on the pituitary gland, prompting the secretion of adrenocorticotropic hormone (ACTH) into the bloodstream. Following ACTH release, the signal reaches the adrenal glands, stimulating them to produce and release glucocorticoids, with corticosterone being the primary glucocorticoid in birds and many other non-human vertebrates.[19]. The current study in Table (1&2) illustrated that, there were a direct relation between the mobile phone radiation used in experiment and the increase in serum corticosterone level in exposed quails. Significant ($p \leq 0.05$) increase in serum corticosterone level was recorded in quails exposed to radiation of mobile phone compared to control group. The current study are in agreement with Puvadolpirod and Thaxton, [26], Pawlak et al.,[24] and Pagadala et al., [22] who reported a significant increase in the serum corticosterone levels of RF-EMR exposed rats compared to the control. While, the results are in disagreement with Jezova et al., [16] who reported that corticosterone levels did not significantly differ across the groups.

Conclusion

Mobile phone radiation has potentially impaired adverse effects on the hatching process, performance, health status, and social and fear behavior, so special caution must be taken while using a mobile phone with special caution during pregnancy.

Conflict of Interest

The authors declare that they have no competing interests related to this study.

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