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Effects of Melatonin and Omega-3 Fatty Acids on Female Reproductive Hormones, Ovarian Follicles, and Blood Parameters in New Zealand White Female Rabbits

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Abstract | The present study aimed to investigate the impact of dietary supplementation with Melatonin (MT) and Omega-3 fatty acids on ovarian follicle development, blood hematology, female reproductive hormones, and reproductive histology in New Zealand White (NZW) female rabbits. Melatonin (MT) and Omega-3 fatty acids are essential for regulating follicular development, ovulation, and cell growth and proliferation. Many physiological processes in animals are regulated by Melatonin (N-acetyl-5-methoxytryptamine) (MT). According to recent research, MT influences the quantity and level of ovarian follicle maturation. This study was conducted at the Department of Surgery and Obstetrics, College of Veterinary Medicine, Basrah University, between January 15, 2025, and December 15, 2024. Fifteen female New Zealand White rabbits, each weighing around 3 kg and 12 weeks of age, were employed in this investigation. Three groups of five female rabbits each were randomly assigned to the adaptation experiment: The G1 (control group) without addition of supplement, G2 experimental group were treated by a single dose of Melatonin 2.5 mg/kg/day orally by capsule in evening and G3 experimental group of Omega 3 a single dose of it (1 mL/kg/day) orally by syringe in evening. Results showed that level of follicular stimulating hormone (FSH) in the female rabbits at (15) days increased significantly ($p < 0.05$) in G2 and G3 groups camper with G1, As well as the study appear the levels of FSH increased significantly ($p < 0.05$) at 30 days in G2 and G3 groups camper with G1 control group. The estrogen (E2) level was highest in the G2 and G3 groups ($p < 0.05$) at 15 days compared to the G1 group. On the other hand, the differences in E2 levels were significantly higher ($p < 0.05$) at 30 days in the G2 and G3 groups compared to the G1 group. The Luteinizing hormone (LH) level was the highest at 15 and 30 days in the G2 and G3 groups, significantly higher than in the G1 group. The results showed that the rate of WBC decreased significantly ($P < 0.05$) in the G2 group at 15 days compared with the G1 and G3 groups. On the other hand, the study showed that the value of white blood cells (WBCs) significantly decreased ($P < 0.05$) at 30 days in G2 and G3 compared with G1. As observed in the current study, the level of blood hemoglobin (Hb) was significantly increased ($P < 0.05$) in G2 at 15 days compared with G1 and G3. However, when compared to G1, the mean HB was substantially greater in G2 and G3 after 30 days ($P < 0.05$). Additionally, the study revealed that, at 15 days, platelet (PLT) levels in the G2 and G3 groups were considerably higher ($P < 0.05$) than those in the G1 group. The findings showed that, at 30 days, the rate of PLT was significantly higher ($P < 0.05$) in the G2 and G3 groups than in the G1 group. According to the current study, G2 had substantially more primary, secondary, and tertiary follicles at 15 days than G1, with a value of $P<0.05$. Additionally, the study showed that at 30 days, G2 had considerably more primary, secondary, and tertiary follicles than G1 ($P<0.05$). In the present study, at 15 days, the number of primary, secondary, and tertial follicles in G3 is significantly larger than that in G1 ($P<0.05$) while the study was showed the numbers of primary follicles and tertial follicles become increased significantly value ($P< 0.05$) at 30 days compared with G1. In conclusion, the present study demonstrates that Melatonin and Omega-3 are effective in enhancing ovarian follicle development, blood hematology (WBC, HB, and PLT), and reproductive hormones (FSH, LH, and E2) in New Zealand White (NZW) female rabbits.

Keywords | Ovary, Melatonin, Omega 3, Ovarian follicle

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INTRODUCTION

In developing countries, rabbits may have challenges in obtaining animal protein due to their use as farm animals (Oseni, 2012). A good foundation for economic selection is rapid puberty and high beginning fecundity. Age, breed, heterosis, and environmental conditions (such as temperature and photoperiod) all influence when an animal reaches puberty (Hafez and Hafez, 2013). Primordial follicle assembly is thought to be finished between weeks two and four of life, while ovarian follicle development in rabbits happens postnatally (Hutt *et al*., 2006). Around 10–14 weeks of age, females start puberty (Hulot *et al*., 1982; Rommers *et al*., 2006). At the same time, when the Rabbit tries to ride for the first time on days 60 to 70, the first signs of male sexual behavior emerge (Lebas *et al*., 1997). In all animal species, the development of reproductive and puberty features is strongly influenced by adequate nutrition (Elhammali and Elsheikh, 2014). Because management techniques and diet influence puberty, young animals vary significantly by the time they reach it (Daramola *et al*., 2007). Additionally, photoperiod is considered the most accurate indicator of when reproductive activity occurs (Rosa and Bryant, 2003; Muteka *et al*., 2006). The pineal gland, which secretes Melatonin, is the source of the knowledge on the duration of the day. The pineal gland converts Melatonin, a hormone that regulates photoperiod, from brain impulses. The primary cause of the decrease in the rate of ovarian follicles, resulting in fewer follicles in the ovary and impaired female reproductive performance in rabbits, may be aging and the associated dysfunction (Zakaria *et al*., 1983). In addition, there are longer average intervals between egg production (Grossman *et al*., 2000), follicular atresia, and apoptosis (Lillpers and Wilhelmson, 1993). Additionally, during the ovulation stage, the follicular pool is exhausted. Thus, the main objectives are to prevent ovarian dysfunction and to safeguard ovarian aging. The effects of Melatonin on the reproductive function of female animals have been documented in several studies. In the 1950s, Melatonin (N-acetyl-5-methoxytryptamine; MW 5 232) (MT) was initially identified in the pineal gland (Lerner *et al*., 1958). Numerous physiological processes in animals, such as female reproduction (Wang *et al*., 2014; Mohsen *et al*., 2024), innate immunity (Zhou *et al*., 2016), and the ability to scavenge reactive oxygen species (ROS) (Zhang *et al*., 2006), are regulated by melatonin (Reiter, 1991; Morgan *et al*., 1994). By binding to receptors (melatonin receptor 1, MT1, and melatonin receptor 2, MT2), one of them functions as an antioxidant and controls ovarian function. This is linked to an increase in phospholipase C (PLC) and a decrease in downstream molecules, such as cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP). Furthermore, MT has antioxidant (Galano *et al*., 2011), anti-cancer (Mehaisen *et al*., 2015), and antiradiation (Söderquist *et al*., 2016) qualities. MT may have an impact on ovarian function. MT-binding sites have been found in the ovaries, according to earlier research (Jia *et al*., 2016).

Recorder antioxidant treatment has been shown to improve hens' reproductive abilities and the amount of MT in their blood was inversely correlated with their age (Roland, 1979). Because Melatonin and its metabolites scavenge free radicals and have related antioxidant qualities, they encourage follicular maturation and ovulation (Williams, 1992; Dowling *et al*., 2010). Currently, scientists have discovered that mTOR signaling pathways regulate granulosa cell growth, ovulation, and ovarian function (Tamura *et al*., 2017). The impact of MT on ovarian follicle maturation or quantity (Patel *et al*., 2020). Omega-3 fatty acids are primarily located in the heart, brain, testes, retina, and immune system, and they also improve the reproductive function of females (Perdana *et al*., 2021; Skulas-Ray *et al*., 2019). There is strong evidence that Omega-3 fatty acids have anti-inflammatory and anti-cancer properties (Wang *et al*., 2018), enhance the immunological, cardiovascular, and cerebral systems, and offer additional health benefits for the bones, muscles, eyes, and nerves (Ohira *et al*., 2014).

The current study aims to investigate the effects of Omega-3 and exogenous melatonin administration on the reproductive hormones, ovaries, and uterine histology of females during various reproductive periods.

MATERIALS AND METHODS

The study's time frame was from December 15, 2024, to January 15, 2025, at Basrah University's College of Veterinary Medicine, Department of Surgery and Obstetrics. In the current investigation, 15 female NZW rabbits, weaned at 12 weeks of age and weighing approximately the same (3 kg), were used. Before the trial started, the female rabbits were housed in cages for ten days to allow for acclimatization. They were then split into three groups at random: (5 female rabbits /group): The G1 (control group n=5) without addition of supplement, G2 experimental group were treated by a single dose of Melatonin 2.5 mg/kg/day orally by capsule in evening (Sohnrey and Holtz, 2005) and G3 experimental group of Omega 3 a single dose of Omega 3 (1 mL/kg/day) orally by syringe in evening.

Hormonal evaluation

Blood samples was drawn 3 ml from the direct heart rabbits by syringe(3ml) from control and each treated groups supplement by anticoagulant tubes at day (0 day, 15 days and 30 days) and carried to a lab. to be separated by centrifuged of the samples (1500 r/min for 5 min.) and stocked under -20°C until assay to evaluated hormonal estimation (FSH, LH, P4, and E2) (Bancroft and Gamble, 2008).

Operation techniques ovariectomy

A 3 cm long surgical incision is made in the female's abdominal skin, around 1 inch below the umbilicus on the abdominal midline, while the area is aseptic. The female's fallopian tubes were palpated to locate both ovaries. You can snare the uterine horn by using a spay hook to sweep the abdomen caudal to a kidney. We were able to identify and visualize the ovary, suspensory ligament, and uterine tip. Hemostatic forceps are used to exteriorize the ovary through the appropriate ligament, maintaining traction and isolation. Check for fenestration in the mesovarium, a narrow, comparatively avascular region situated caudal to the ovarian vascular plexus. A hemostat carrying suture has passed through the fenestration in the mesovarium, as shown in Figures 1A, B, C.

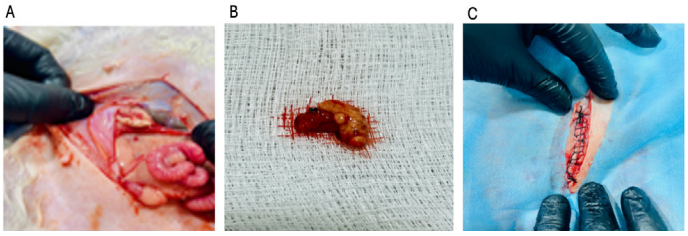

Figure 1: (A) An incision is made in the umbilical abdominal midline. (B) Operation techniques ovariectomy. (C) Suturing the umbilical abdominal midline.

Histopathological examination

At the end of the experiment period, the total body weight of the animals and the weight of their genital organs were recorded. Ovarian and uterine specimens (ovariectomy) were obtained, preserved in 10% neutral buffered formalin, cleaned, dehydrated, and embedded in paraffin. For histopathological analysis, the paraffin-embedded blocks were sectioned at a thickness of 5 microns and stained with hematoxylin and eosin (Anwar *et al.*, 1998). Using a light microscope (Olympus BX50, Japan), stained slices were inspected. The treatment was performed in the Department of Pathology's Histopathology Laboratory at the University of Basrah's College of Veterinary Medicine.

Statistical analysis

The data were statistically analyzed using the Student-Newman-Keuls multiple range test after a one-way ANOVA. When $P < 0.05$, the differences were deemed statistically significant.

RESULTS

Hormonal differences in female rabbits at different periods of the treatment

As shown in Table 1, the levels of FSH in the female rabbits at 15 days increased significantly ($p < 0.05$) in G2 and G3 groups compared with G1 (3.17±0.51, 5.42± 0.09 and 4.90±0.32), respectively. In addition to the study, the levels of FSH increased significantly ($p < 0.05$) at 30 days in the G2 and G3 groups compared with the G1 group (3.60 ± 0.39, 9.33 ± 0.32, and 7.31 ± 0.42, respectively). The E2 level was highest in G2 and G3 ($p < 0.05$) groups at 15 days of camp with G1 (511.04 ±9.22, 552.6± 52.16, 578.60± 42.11), respectively. The study also showed that the level of E2 increased significantly ($p < 0.05$) at 30 days in the G2 and G3 groups compared to the G1 group (516.03 ± 7.66, 662.90 ± 35.25, and 651.88 ± 29.23, respectively). The LH level was the highest at 15 days in the G2 and G3 groups, significantly higher than in G1 ($p < 0.05$). The present study revealed that the level of LH increased significantly ($p < 0.05$) at 30 days in the G2 group compared with the control and the G3 group, as shown in Table 1.

Blood parameter differences in female rabbits at different periods of the treatment

The results showed that the rate of WBC decreased significantly ($P < 0.05$) in the G2 group at 15 days compared with G1 and G3 (8.92 ± 0.22, 6.25 ± 0.11, and 7.25 ± 0.10, respectively). On the other hand, the study showed that the value of WBC significantly decreased ($P < 0.05$) at 30 days in G2 compared with G1 and G3 (9.03 ± 0.41, 3.25 ± 0.02, and 6.25 ± 0.19, respectively). In addition to the current study, the level of HB was significantly increased ($P < 0.05$) in G2 at 15 days compared with G1 and G3

Table 1: Reproductive hormone levels in female rabbits at different times of the treatment (mean ± SE).

Groups		Time after treatment/ days		
		0	15	30
FSH mIU/mL mIU/mL	G1	3.82± 0.11a	3.17±0.51a	3.60±0.39a
	G2	3.37±0.44a	5.42± 0.09 b	9.33± 0.32 c
	G3	3.81±0.51a	4.90± ±0.32b	7.31± 0.42b
E2 pg/mL	G1	512.08± 35.25 a	511.04 ±9.22 a	516.03±7.66 a
	G2	506.04 ±5.71a	552.6± 52.16 b	662.90± 35.25 b
	G3	502.08 ±4.47a	578.60± 42.11 b	651.88± 29.23 b
LH ng/mL ng/mL ng/mL	G1	49.97 ± 0.25 a	48.97 ± 2.27 a	48.17 ± 2.17 a
	G2	48.54 ± 0.27 a	52.37 ± 1.62 b	52.83 ± 0.72 b
	G3	49.23 ± 0.11 a	51.66 ± 0.45 b	48.97 ± 2.27 a

Different small letters vertically denote significant differences (P < 0.05) between groups.

Table 2: Effect of Melatonin and Omega-3 on some blood parameters of female Rabbits (mean ± SE).

Groups		Time after treatment/ days		
		0	15	30
WBC(103/mm3) mm3) mIU/mL mIU/mL	G1	9.11 ± 0.23A	8.92 ± 0.22a	9.03 ± 0.41a
	G2	8.25 ± 0.49A	6.25 ± 0.11c	3.25 ± 0.02c
	G3	7.92 ± 0.12A	7.25 ± 0.10 b	6.25 ± 0.19b
HB(g/dL)	G1	7.86 ± 0.06A	7. 56 ± 0.02a	7.04 ± 0.05a
	G2	7.33 ± 0.04A	8.12 ± 0.01b	9.83 ± 0.04c
	G3	7.86 ± 0.05A	7.96 ± 0.03a	8.06 ± 0.06b
PLT(10^3/µL)ng/mL ng/mL	G1	456.23 ± 38.65A	415.20 ± 1.46a	466.26 ± 3.89a
	G2	416.23 ± 11.89A	949.40 ± 29.39c	942.60 ± 43.05c
	G3	408.20 ± 61.20	697.00 ± 80.01b	890.40 ± 55.3b

Different small letters vertically denote significant differences (P < 0.05) between groups.

(7.56 ± 0.02, 8.12 ± 0.01, and 7.96 ± 0.03, respectively). On the other hand, the mean of HB was significantly increased (P < 0.05) in the G2 and G3 groups at 30 days compared with the G1 group. Moreover, the study showed that the level of PLT was significantly different (P < 0.05) in the G2 and G3 groups at 15 days compared with the G1 group (415.20 ± 1.46, 949.40 ± 29.39, 697.00 ± 80.01, respectively). The results illustrated that the rate of PLT was significantly higher (P<0.05) in G2 and G3 groups at 30 days compared with G1 (466.26 ± 3.89, 942.60 ± 43.05, 890.40 ± 55.3), respectively, as shown in Table 2.

Effect of the melatonin hormone on the number of ovarian follicles

According to the current study, G2 had significantly more primary, secondary, and tertiary follicles at 15 days than G1, with a value of P<0.05. Additionally, the study revealed that at 30 days, G2 had significantly more primary, secondary, and tertiary follicles than G1 (P < 0.05), as shown in Table 3.

Effect of Omega 3 on the number of ovarian follicles

In the current study, the G3 group's counts of primary, secondary, and tertiary follicles increased significantly (P < 0.05) at 15 days compared to the G1 group. While the study showed that the numbers of primary follicles and tertiary follicles increased significantly (P < 0.05) at 30 days in G3 compared with G1, as shown in Table 4.

Table 3: Effect of the addition of Melatonin on the number of ovarian follicles (mean ± SE).

Tertial follicles	Secondary follicles	Primary follicles	Group
2.50 ± 0.28A	3.50± 0.25a	9.75 ± 0.62a	G1
6.75± 0.47B	5.13± 0.47b	11.00 ± 0.40b	G2 at 15 days
7.00 ± 0.20B	6.50 ± 0.34c	12.75 ± 0.12c	G2 at 30 days

Different small letters vertically denote a significant (P<0.05) difference between groups.

Table 4: Effect of the addition of Omega 3 group on the number of ovarian follicles (mean ± SE).

Tertial follicles	Secondary follicles	Primary follicles	Group
2.23 ± 0.12a	2.22± 0.25a	7.23 ± 0.11a	G1
3.21± 0.10b	4.00± 0.40b	9.00 ± 0.12b	G3 at 15 days
5.13 ± 0.23c	4.50 ± 0.34b	11.75 ± 0.62c	G3 at 15 days

Different small letters vertically denote a significant (P<0.05) difference between groups.

Histology development differences in female rabbits at different stages of supplement

Hematoxylin and eosin staining of the ovary group treated with Melatonin revealed more abnormal primary and secondary follicles in the histological analysis, as well as hemorrhage and thickening in connective tissue, at 15 and 30 days compared with the Control group (Figure 2A). Ovarian slice displaying granulosa as a single layer of cuboidal cells and the main follicle (Figure 2B). In the current study, the group treated with Melatonin showed that the ovary was covered by epithelium, also containing simple cuboidal tissue (Figure 2C). The histopathological section of the Oviduct Melatonin group-treated shows hyperplasia in the mucosal layer and atrophy in some areas, as well as thickening and muscular thickening (Figure 2D).

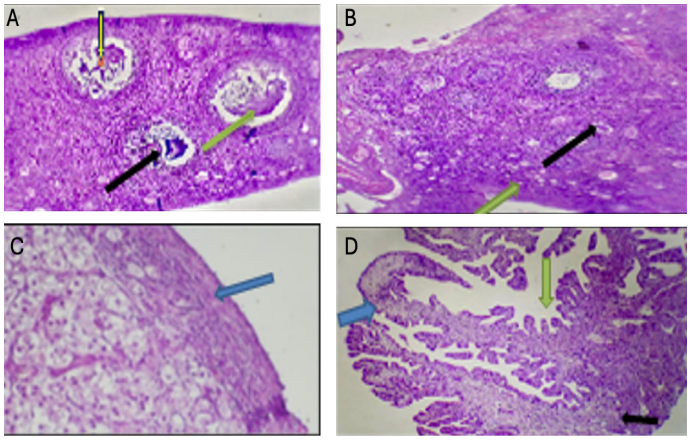

Figure 2: (A) Histopathological section of the ovary group treated with Melatonin, shows abnormal secondary follicle (yellow arrow), hemorrhage (black arrow), and thickening in connective tissue (green arrow), HandE stain x4. (B) Ovarian section treated with Melatonin, showing primordial follicle (black arrow), granulosa as a single layer of cuboidal cells (green arrow). (H and E stain, X4). (C) The ovary treated with Melatonin was covered with simple cuboidal epithelial tissue (blue arrow). (H and E stain, X4). (D) Histopathological section of Oviduct Melatonin group treated, shows hyperplasia in the mucosal layer (blue arrow), atrophy in some areas (green arrow), and thickening and muscularis in some animals (black arrow). (H and E stain x10).

Histopathological sections of the Uterus in the melatonin-treated group show mild vacuolation of endothelial cells, normal uterine glands with edema, and Congested blood vessels (Figure 3A). On the other hand, the study showed that ovarian sections treated with Omega-3 showed secondary follicles, with the zona pellucida appearing clearly (Figure 3B). A stratified epithelium is formed by the number of granulosa layers in the ovarian section displaying the preantral follicle (Figure 3C). The histopathological section of the oviduct group treated with Omega-3 shows mild hyperplasia of epithelial cells and mild thickening of the muscular layer with congested blood vessels (Figure 3D). Histopathological section of the uterus group treated with Omega, shows spaces between the muscular layer, atrophy of uterine glands, and mild hyperplasia of the endothelium layer.

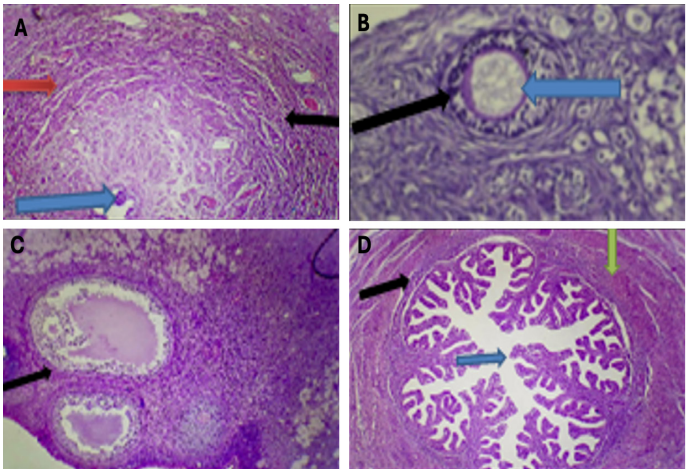

Figure 3: (A) Histopathological section of the Uterus in the Melatonin group-treated group shows Mild Vacuolation of endothelial cells (blue arrow), normal uterine glands with edema (red arrow), and Congested blood vessels (black arrow) (H and E stain X10). (B) Ovarian section showing secondary follicle (blue arrow), the zona pellucida (black arrow) appeared clearly (H and E stained with X40). (C) Ovarian section showing secondary follicle (black arrow) (H and E stain, X40). (D) Histopathological section of the oviduct group treated with Omega-3 showing mild hyperplasia of epithelial cells (black arrow) and mild thickening of the muscular layer (blue arrow) with congested blood vessels (green arrow) (H and E stain x4)

DISCUSSION

The rabbit is an excellent laboratory animal with numerous benefits for reproduction. Its size makes it easy to handle. The recent findings revealed a significant increase in PLT (103/µL) and HB (g/dL). These results agree with (Durotoye and Rodway, 1996), who found that melatonin treatment in rodents increased significantly in Hb and PLT. While our findings disagree with the report (Karimungi and Joshi, 2004), which reported that subcutaneous, Melatonin in ewes decreases the mean Hb, PLT, and PCV. Previous researchers recorded the changes in blood parameters over time, depending on the time of melatonin injection and blood collection from animals (Cheesbrough, 2005). In addition, MT possesses antiradiation (Söderquist *et al.*, 2016), anti-cancer (Mehaisen *et al.*, 2015), and antioxidant properties (Galano *et al.*, 2011). On the other hand, (Haldar *et al.*, 2001) mentions the toxic chemical of Melatonin responsible for the destruction of red blood cells, packed cell volume, and hemoglobin concentration. The significant increase observed in the platelet count

agrees with the report by reporters (Oseni, 2012) that human platelets are peripheral cells sensitive to Melatonin, which could be potentially employed in clinical studies. A significant increase was observed in the total WBC count of the melatonin-treated rabbits, further confirming the immune-enhancing role of Melatonin. Further supported by the finding (Pacchiarotti *et al*., 2016) that the injection of Melatonin increased all the immune parameters. Some reported that daily subcutaneous injection of Melatonin significantly increased the number of lymphocytes in peripheral blood and bone marrow (Tamura *et al*., 1998). The study also shows a considerable increase in HB (g/dL) and PLT (1×10^9/μL) in the Omega-3 group compared with the control group, because the heart, testes, retina, immune system, and central nervous system are the main organs that contain Omega-3 fatty acids (Perdana *et al*., 2021; Skulas-Ray *et al*., 2019). There is strong evidence that Omega-3 fatty acids have anti-inflammatory and anti-cancer properties (Wang *et al*., 2018), enhance the immunological, cardiovascular, and cerebral systems, and offer additional health benefits for the bones, muscles, eyes, and nerves (Ohira *et al*., 2014).

The present study showed beneficial impacts of dietary Melatonin and Omega-3 on the hormonal (FSH, LH, P4 and E2) which is related that melatonin treatment improves endocrine hormone levels by increasing FSH, decreasing LH and androgen levels, regulating lipid metabolism, reducing inflammation, and modulating the activity of certain enzymes like antioxidant enzymes and aromatase are all beneficial and lead to better assisted reproductive outcomes (Fraschini *et al*., 1968). The present study disagreed with (Lang *et al*., 1983) in rabbits, as it reported a significant reduction in the weight of reproductive organs and steroidogenesis following melatonin administration. The reduced steroidogenesis might be due to the action of Melatonin on multiple sites, i.e., the hypothalamus (reducing GnRH) and the pituitary (reducing FSH and LH), thereby affecting ovarian synthesis of E2. The hypothalamic-pituitary axis is the traditional target of Melatonin's activity. Researchers first reported that Melatonin's action on the hypothalamus is responsible for its antigonadal activity (Martin and Klein, 1976). Subsequent research revealed that the hypothalamus plays a role in the action of Melatonin (Theau-Clément *et al*., 1995). Discovered that melatonin action resulted in a lower GnRH level. The first direct action of Melatonin on the pituitary was reported by (Bernardi *et al*., 2012). Melatonin prevented neonatal rat anterior pituitary cells from releasing LH in response to GnRH. The ejaculate and semen volumes, sperm concentrations, percentage of living spermatozoa, and mass motility all increased after melatonin treatment. Light duration may have an impact on the hypothalamus-pituitary axis, which in turn affects the release of hormones and the generation of spermatozoa (both qualitatively and quantitatively) (Simopoulos, 2002). The effects of Melatonin on female animals' reproductive function have been documented in several studies. In the 1950s, Melatonin (N-acetyl-5-methoxytryptamine; MW 5 232) (MT) was initially identified in the pineal gland (Lerner *et al*., 1958). Numerous physiological processes in animals, such as female reproduction (Wang *et al*., 2014), innate immunity (Zhou *et al*., 2016), and the ability to scavenge reactive oxygen species (ROS) (Zhang *et al*., 2006), are regulated by melatonin (Reiter, 1991; Morgan *et al*., 1994). One binds to receptors (melatonin receptor 1, MT1, and melatonin receptor 2, MT2) to serve as an antioxidant and control ovarian function (Reiter *et al*., 2014). Researchers have demonstrated a propensity for Omega-3 supplementation to improve fertility and receptivity (Amiri-Jami *et al*., 2014). Additionally, it has been shown that using Omega-3 supplements during pregnancy supports the development of early neurons and regulates neurochemical features associated with growth, stress response, and cognitive processes. These factors are critical for the viability of newborns, particularly during the first few days of life. Myocardial infarction and chronic inflammatory or autoimmune illnesses are less common in those who consume large amounts of Omega-3 fatty acids from seafood. EPA and DHA are also thought to minimize the risk of cardiovascular disease (Amiri-Jami *et al*., 2014).

CONCLUSION

Administration of Melatonin and Omega-3 was evaluated on ovarian follicle development, blood hematology (WBC, HB, and PLT), and hormonal levels (FSH, LH, P4, and E2) in New Zealand White (NZW) female rabbits.

ACKNOWLEDGEMENT

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NOVELTY STATEMENT

The novelty of our study including understanding the effects of melatonin and omega-3 fatty acids on female reproductive hormones, ovarian follicles, and blood parameters in New Zealand white female rabbits.

AUTHOR'S CONTRIBUTION

These authors each contributed equally.

Generative AI or AI-assisted Technology Statement

The author(s) declare that no Genrative AI was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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