

Hormonal profile of Mediterranean green turtles (*Chelonia mydas*)

Şahver Ege HİŞMİOĞULLARI^{1*}, Tünay KONTAŞ AŞKAR², Muhammed Enes ALTUĞ³, Yaşar ERGÜN⁴

¹Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Balıkesir University, Balıkesir, Turkey

²Department of Nutrition and Dietetics, Faculty of Health Sciences, Çankırı Karatekin University, Çankırı, Turkey

³Department of Surgery, Faculty of Veterinary Medicine, Hatay Mustafa Kemal University, Hatay, Turkey

⁴Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, Hatay Mustafa Kemal University, Hatay, Turkey

Received: 18.12.2019 • Accepted/Published Online: 05.04.2020 • Final Version: 02.06.2020

Abstract: The beaches of Turkey are important nesting habitats of *Chelonia mydas* sea turtles and the determination of their health and disease status is critical in sustaining healthy populations. Limited data currently exist on the hormone values required to determine the status of reproductive capabilities of sea turtles. This study aimed to collect basic data regarding their hormonal profile and set reference limits for the Mediterranean population. Forty-nine free-ranging *C. mydas* sea turtles were used in the study and were classified into 3 groups according to their age (hatchlings, juvenile, adult). Adult turtles were also grouped according to sex. Adult females were further divided into 3 seasonal groups (summer, spring, and autumn), based on the dates of blood sampling. Plasma testosterone, oestradiol, progesterone, thyroxine (T4), triiodothyronine (T3), thyroid-stimulating hormone (TSH), cortisol, and corticosterone levels were determined by ELISA method. Significantly higher levels were observed in oestrogen ($P < 0.001$), progesterone ($P < 0.001$), cortisol ($P < 0.05$), corticosterone ($P < 0.05$), T3 ($P < 0.05$), and T4 ($P < 0.01$) in the adult turtles when compared with the other groups. The highest levels of oestrogen (280.2 ± 39.34), progesterone (274.2 ± 29.4), cortisol (2.26 ± 0.36), and corticosterone (2.94 ± 0.53) were determined in the adult female turtles during the spring season. This data could be used to protect the population of this endangered species by taking precautions against diseases via determining their blood hormone levels and taking precautions against reproductive diseases. Further work is required, but this research can expand the knowledge on the basic blood biochemistry of Mediterranean green turtles.

Key words: Steroid hormones, *Chelonia mydas*, green sea turtles, health status

1. Introduction

Sea turtles are large, air-breathing herbivores, inhabit tropical and subtropical seas of the world [1]. There are 7 known species of sea turtles [2] with the beaches of Turkey of primary importance for the species *Chelonia mydas*, followed by the beaches of Cyprus, Syria, and Israel [3–6]. It has been reported that approximately 70%–80% of *C. mydas* turtles are nested in the Mediterranean coasts of Turkey [3–7]. Numerous studies have been conducted on monitoring of sea turtles in the Mediterranean and determining the importance of beaches as their habitat [3–7].

Samandağ beach is an important breeding site for green sea turtles, whose Mediterranean breeding grounds are restricted to the beaches of Eastern Mediterranean. The beach is bordered by the Çevlik Town and a rocky area (this rocky area forms the border with Syria) in the north and the Meydan Village in the south [8,9]. Numerous studies have been conducted in the Eastern Mediterranean beaches, whereas very few studies have been conducted

on sea turtles nesting at Samandağ beaches and these studies are mostly on the number of nests, nest success, and partly spatial distributions of nests on the coast [10,11]. To ensure the continuity of the generation of these creatures, determining their health-disease status is of great importance besides protecting their breeding and feeding grounds [12–15]. Hormone levels are precise and reliable indicators of the health status of turtles and their populations, and the impact of environmental factors and stress [16–20].

The hormone levels (T4, testosterone, oestrogen, progesterone, and corticosterone) in various sea turtle species have been measured to reveal the organ and reproductive activities in living creatures [16,21,22]. There are a few studies on the reference hormone values required for determining the health-disease and reproductive function status of sea turtles in Turkey [15]. This study aimed to collect basic data on the hormonal profile in *C. mydas* turtles located on the Eastern Mediterranean coast of Turkey and set the reference limits.

* Correspondence: sahverege@balikesir.edu.tr

2. Materials and methods

2.1. Materials

Forty-nine free-ranging *C. mydas* sea turtles were used in the study and were classified into 3 groups according to their ages (hatchlings, juvenile, adult) with adult turtles being further grouped according to sex (male and female). Furthermore, adult females were separated into 3 seasonal groups such as summer (June–August), spring (March–May), and autumn (September–November) dependent on the dates of blood sampling. The blood samples taken from total 15 females that caught in sea and beach (4 spring, 7 summer, and 4 autumn), 5 adult males, 9 juveniles, and 20 hatchlings.

2.2. Animals and sampling procedures

The head of the caught turtle was restrained by pulling to the forward and downwards below the level of the plastron to facilitate filling of the sinus with blood by the gravitational force [23]. It has been reported that 4%–8% the weight of turtles consists of blood [23]. 10 mL of blood was taken from adults. However, 0.3–0.5 mL of blood was taken from dorso-cervical sinus via insulin needle in hatchlings.

To draw blood from the turtles, the dorsal neckline was cleaned with povidone-iodine and the vascular structure was determined using vascular ultrasound. After that the blood samples were taken directly from dorso-cervical sinus into blood tubes with sodium heparin under sterile conditions using vacutainer double-ended or normal 22-gauge needles in adults and juveniles and with the help of insulin needles in the hatchlings. These procedures carried out both at sea and on the beach. The blood samples were kept on ice and brought to the laboratory. Samples were then centrifuged at 3000 rpm for 10 min; plasma was removed and frozen at –86 °C until the analyses were performed.

2.3. Hormone analysis

While determining plasma hormone levels, the turtles had been determined as adult females (based on oestrogen, oestradiol, and testosterone levels) were divided into 3 groups (summer, spring, and autumn) based on the dates of sampling. Sex of turtles were determined via analysis of the blood testosterone levels. According to Bolton and Björndal [24], they assigned young *C. mydas* turtles as females with testosterone hormone values below 10 pg/mL whereas those above 20 pg/mL as males in south Bahamas study. In our study, sex determination conducted through young turtles due to their testosterone levels were found to be 15.8 and 17.8 pg/mL, respectively.

The plasma testosterone (catalogue no: 582601), oestradiol (catalogue no: 582251), progesterone (catalogue no: 582601), T4 (catalogue no: EIA- 4568), T3 (catalogue no: EIA- 4569), TSH (catalogue no: EIA- 4171), cortisol

(catalogue no: EIA- 1887), corticosterone (catalogue no: EIA- 4164) levels were determined by enzyme-linked immunosorbent assay (ELISA) using commercially available kits (Cayman Medical and DRG, USA) on a diagnostic instrument (BioTek Instruments, USA).

2.4. Statistical analyses

The results are expressed as mean \pm standard deviation (SD). The significance of differences among all steps of the groups was analysed by analysis of variance (ANOVA), if the F value was found to be significant, differences between means were then analysed with the post-hoc (Duncan) test. Differences were considered statistically significant when the P value was <0.05.

3. Results

In the present study, we determined the basic data for the hormone values of *C. mydas* that inhabit Turkey's coasts. The results regarding the hormonal profile in *C. mydas* are presented in Table 1.

Significant differences were observed in oestrogen ($P < 0.001$), progesterone ($P < 0.001$), cortisol ($P < 0.05$), corticosterone ($P < 0.05$), T3 ($P < 0.05$), and T4 ($P < 0.01$) levels in the adult turtles. Progesterone, cortisol, and corticosterone levels could not be determined in the hatchlings group (Table 1).

There were no significant differences between the hatchling, juvenile, and adult male turtles in oestrogen levels. The highest levels of oestrogen (280.2 ± 39.34), progesterone (274.2 ± 29.4), cortisol (2.26 ± 0.36), and corticosterone (2.94 ± 0.53) were determined in adult female turtles in the spring season. Also, the lowest oestrogen levels were found during the summer months at the time of nesting (45.5 pg/mL) and the corticosterone levels were found to be age and sex dependent ($P < 0.05$).

In addition, adult male turtles had significantly higher levels of plasma testosterone (146.3 ± 13.74) when compared with hatchlings, juvenile, and adult female groups. However, there was a significant difference ($P < 0.05$) among the plasma T4 levels of the hatchling, juvenile, and adult turtles, whilst T4 levels increased with age. T3 levels did not change significantly depending on age or sex.

4. Discussion

There are few studies which have addressed the issues related to basic hormonal profiles in different age classes of sea turtles in the Mediterranean [15]. Hence, this study was undertaken in order to determine physiological hormonal levels, which are important for their reproduction and establish reference ranges in blood.

Most of the studies conducted on the hormonal profiles in sea turtles are about reproductive endocrinology of nesting females. Plasma oestradiol, testosterone,

Table 1. Hormonal profile in the *Chelonia mydas* turtles.

	Hatchlings (n = 20)	Juvenile (n = 9)	Adult female			Adult Male (n = 5)
			Autumn (n = 4)	Spring (n = 4)	Summer (n = 7)	
Oestrogen (pg/mL)	29.2 ± 18.57 ^a	34.5 ± 16.04 ^{ad}	135,7 ± 21.01 ^b	280.2 ± 39.34 ^c	45.5 ± 10.23 ^d	26.9 ± 13.24 ^a
Progesterone (pg/mL)	ND	79.1 ± 18.2 ^a	185,7 ± 12.3 ^b	274.2 ± 29.4 ^c	97.5 ± 2 1.6 ^a	ND
Testosterone (pg/mL)	15.5 ± 2.24 ^a	17.8 ± 2.84 ^a	19.4 ± 3.48 ^a	65.6 ± 9.53 ^b	28.3 ± 4.17 ^c	146.3 ± 13.7 ^d
T3 (ng/mL)	0.55 ± 0.11 ^a	0.89 ± 0.11 ^b	1.08 ± 0.13 ^b	1.02 ± 0.09 ^b	1.07 ± 0.06 ^b	1.14 ± 0.23 ^b
T4 (ng/mL)	2.39 ± 0.36 ^a	4,84 ± 0,68 ^b	12.63 ± 2.11 ^c	8.51 ± 1.17 ^d	6.57 ± 2.09 ^d	6.16 ± 1.86 ^d
TSH (IU/mL)	0.005 ± 0.002	0.005 ± 0.003	0.007 ± 0.003	0.008 ± 0.001	0.005 ± 0.002	0.006 ± 0.01
Cortisol (ng/mL)	ND	1.09 ± 0.23 ^a	1.14 ± 0.29 ^a	2.26 ± 0.36 ^b	1.57 ± 0.23 ^c	2.14 ± 0.48 ^b
Corticosterone (ng/mL)	ND	0,63 ± 0,32 ^a	1.22 ± 0.46 ^b	2.94 ± 0.53 ^c	1.94 ± 0.56 ^d	2.04 ± 0.62 ^d

^{a,b,c,d} The difference is significant in the columns between the groups, which carries different superscripts (P < 0.05).
 ND: nondetected.

progesterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and corticosterone levels were measured in female *Caretta caretta*, *Lepidochelys kempi*, *Lepidochelys olivacea*, and *C. mydas* turtles [21]. However, there are no studies on plasma hormone levels of sea turtles in Turkey.

Oestrogen is a steroidal hormone secreted mainly by the ovaries in response to gonadotropin secretion in the mammals. It is released from *Teka interna* and granulosa cells of previtellogenic follicles in the mammals. It is essential for the development of sexual organs and secondary sexual characters and maintenance of development and its physiological effects in female mammals. It acts as the primary hormone in follicular development [25]. In the study, no difference could be found between the hatchling, juvenile, and adult male turtles regarding the oestrogen levels. The highest concentration of oestrogen was found to be 280.2 pg/mL in the adult females during spring (Table 1). In *Dermochelys coriacea*, Rostal et al. [22] observed a decrease in oestradiol and testosterone levels during the nesting cycle. In this study, the lowest oestrogen levels were found in the summer months (45.5 pg/mL), during the nesting season. The low summer values may be associated with taking the blood samples after the female turtle had finished nesting. Another study, Rostal et al. [26] determined a decrease in oestradiol levels in the female *C. caretta* turtles from beginning to the end of the nesting cycle, they also found a sudden increase in both testosterone and oestradiol in the spring. Guillette et al. [27] found the average value of oestradiol 17-β in sea turtles to be 255 pg/mL at beginning of summer. It has been reported that 4–6 weeks before migration from feeding grounds to mating and nesting areas, serum oestradiol-17 beta level increased significantly and remained high about 4 weeks, meaning a

period of increased vitellogenesis [28]. This accounts for the increase in the oestrogen level that was observed in the autumn in the study. Vitellogenesis is reported to be dependent on oestradiol 17-β in the reptiles. Increases in calcium and oestrogen levels within 4–6 months (spring season) before the reproduction period in *L. kempi* turtles are indicators of vitellogenesis [29]. Oestrogen injections administered to young *C. mydas* turtles that are raised in farms have been found to stimulate vitellogenesis [30]. It is also known that it is secreted from the placenta and adrenal glands in some mammalian species, its main source is luteal cells that form the corpus luteum. The normal physiological effect of progesterone hormone can be shaped after the target tissue receives oestrogen stimulation for some time [25]. While progesterone level could not be determined in the hatchlings and adult males in the study, the highest progesterone concentration was found in adult female turtles in the spring season (274.2 pg/mL). In the previously conducted studies, it was found that the progesterone hormone levels decreased significantly in the females from the beginning to the end of the nesting season. It had been reported that this was because the progesterone was the primary hormone that accompanied ovulation in sea turtles [28,29]. A sharp ascent was determined in 24–48 h following the nesting in various species of sea turtle (*L. olivacea*, *C. mydas*, and *C. caretta*). Regarding the ovulation after egg release and mating during the mating season, it was determined that the progesterone levels have risen [22]. Because the blood sampling was performed immediately after nesting, the progesterone levels were found to be low in females during the summer. The high progesterone levels found in the spring are likely to be due to mating, as reported by Rostal et al. [22].

Although testosterone is produced in small amounts in female mammals, the group of hormones that are intensely specific to males are called androgens. Testosterone is the most widely known from this group and has many derivatives. Testosterone is produced by the Leydig cells in the male gonads, although small amounts are also produced by the adrenal glands. It initiates the development of male characteristics. The hypothalamus and the pituitary gland are important in controlling the amount of testosterone [25]. In this study, the highest testosterone levels in the male adult turtles were found to be 146.3 pg/mL in the study. Licht et al. [31] found that the testosterone levels in male *C. mydas* turtles were highest during mating season (27–39 ng/mL). The highest testosterone levels in the adult females were 75.6 pg/mL in the spring. It has been found that there occurred a significant decrease in the testosterone level in female *L. kempfi* sea turtles from the beginning (314.7 pg/mL) to the end (22.3 pg/mL) of the nesting cycle [29]. Similar decreases were also found in *C. mydas* turtles [30,32]. This accounts for why the testosterone level in the females was found to be low in the summer in this study. This decrease during the nesting season was also observed in *C. caretta* and *D. coriacea* turtles [22]. Serum testosterone levels increased with the resumption of the activities of ovaries during the pre-mating period. Serum testosterone levels reached the maximum level with the start of the mating period (spring season) [29]. As the blood samples of the spring season were collected during the months of April and May which is the mating season of *C. mydas* turtles in the Mediterranean, accounts for the high levels of testosterone found in both the female and male adult turtles in this study.

Cortisol and corticosterone are released from the adrenal cortex by adrenocorticotropic hormone (ACTH) stimulation and synthesized from cholesterol. In sea turtles, corticosterone mediates the body condition, regulates the energy required for follicular development, nesting and migration, and regulates reproduction in female green sea turtles [32]. In the present study, the corticosterone levels were found to vary depending on the individuals age and/or sex ($P < 0.05$). In the green sea turtles during the nesting period, the high level of plasma corticosterone was found to decrease at the end of the period (1.85 ng/mL) [32]. The values found in the females during the summer (nesting period) in this study are similar to those reported by Hamann et al. [32]. The plasma corticosterone level increases during the emergence from the sea and a nest digging phase. It was stated that the increase during the nesting period was formed to facilitate lipid transfer to prepare the follicles in the ovary or prepare the reproductive organs for fertilization [33]. Potential roles for corticosterone and catecholamines in the regulation of metabolism and lipolysis in seasonally breeding organisms

are well documented. Specifically, increased corticosterone has been correlated with protein catabolism, hyperphagia, and lipolysis in migratory and/or fasting birds [34]. In *C. mydas* corticosterone increases glycerol release from adipose tissue suggesting that this hormone induces the mobilization of triglycerides accumulated in the adipocytes. Consequently, it causes the release of free fatty acid into the blood during nesting. Since female green turtles (*C. mydas*) are generally thought to be aphagic during the nesting season [35]. Corticosterone induces free fatty acids release from adipose tissue to compensate for the lack of food and the energy demands during courtship and nesting activities in sea turtles, indicating that corticosterone is an essential steroid hormone for energetic homeostasis of sea turtles during reproduction [36]. Gregory et al. [37] reported that the corticosterone levels in wild loggerhead sea turtles (*C. caretta*) were higher in the summer when compared with the winter, this is in line with the results of our study.

Adrenal glucocorticoids, like cortisol and corticosterone, are commonly used as an indicator of stress in vertebrates. Cortisol is a hormone that is released during periods of stress [16]. In this study, the cortisol levels were found to be highest in the adult females during spring (2.26 ng/mL) and lowest in the juvenile turtles (1.09 ng/mL). Recent studies report that there is a positive correlation between stress and corticosterone levels in *C. caretta* and *C. mydas* turtles [37,16], the same may be true for cortisol. Although no study on the cortisol levels of turtles has been found in the literature, cortisol may be responsible for regulating the body condition and providing energy source in long-term starvation like epinephrine and corticosterone [32].

The main hormones of the thyroid gland are T3 and T4 and when TSH stimulates the thyroid gland, T4 and T3 pass into the blood. The most common of these hormones is T4 (90%) with a small portion made up of T3 (10%). In the experimental studies conducted on various reptiles, thyroid hormones were found to be effective on growth and development, reproduction, metabolism, food intake, and behaviours [38]. The plasma T4 activity in vertebrates is reported to be associated with metabolic status. In many species of reptiles, seasonal changes have been observed in the plasma T4 levels [39]. In this study, it was found that there was a significant difference ($P < 0.05$) between the hatchling, juvenile, and adult turtle plasma T4 levels with levels increasing with age. The T4 level that Moon et al. [40] found in juvenile green sea turtles (9.4 ng/mL) are similar to T4 levels here. Licht et al. [31] found the T4 level in the male *C. mydas* turtles to be 9 ng/mL and it is higher than the level found in this study. Rostal et al. [29] found an increase in T4 concentration in the mating season in both male and female *L. kempfi* turtles. Also, they

found a significant increase in T4 levels in the females in parallel with the increased serum calcium concentrations. The increased T4 level was found to be associated with mating, ovulation, and vitellogenesis in the reptiles [30]. This shows that the high T4 levels found in females in the spring may be associated with vitellogenesis and mating period.

T4 is deiodinated to T3 in target tissue in many vertebrates. Although thyroid gland produces and secretes a very little amount of T3, 80% of T3 in circulation is made up of the deiodination of T4. T3, which is created in this way, is biologically active thyroid hormone in mammals, birds, and fish. T3 levels are very low or could not be determined in very few reptiles and the desert tortoise [38]. In our study, T3 levels did not change significantly and were neither age nor sex dependent. The T3 levels found in this study are similar to the ones found in the study on *C. mydas* [40].

In conclusion, this study determined the hormonal profile in *C. mydas* turtles found in the Eastern Mediterranean region. Hormone reference values are important in determining health-disease, nutrition, and

reproduction cycle in reptiles and sea turtles. For this reason, it will be possible to protect the population of this endangered species and take precautions against its diseases by determining the blood hormone levels of *C. mydas* turtle found in the Eastern Mediterranean coasts by age (hatchlings, juvenile, and adult) and sex (male, female), separately. The data obtained in this study may also be useful for monitoring the other endangered turtle species (*C. caretta*) found in the coasts of Turkey in terms of health-disease status.

Acknowledgements

This study was supported by a grant from the Scientific and Technological Research Council of Turkey (TÜBİTAK TOVAG 1050207). We would like to thank Assoc. Prof. Dr. Şinasi Aşkar for his field support. This study was conducted under the permission of both Turkish Ministry of Forestry and Environment (permission no. 414-5632) and Turkish Ministry of Food, Agriculture and Livestock (permission no. 01-2378) and also animal protocols were approved by the Local Animal Ethics Committee of Mustafa Kemal University (2005/023).

References

1. Lutz PL, Musick JA. The Biology of Sea Turtles. New York, NY, USA: CRC Press, 1997.
2. Mazaris AD, Schofield G, Gkazinou C, Almpanidou V, Hays GC. Global sea turtle conservation successes. *Science Advances* 2017; 3 (9): e1600730. doi: 10.1126/sciadv.1600730
3. Canbolat AF. A review of sea turtle nesting activity along the Mediterranean coast of Turkey. *Biological Conservation* 2004; 116: 81-91. doi: 10.1016/S0006-3207(03)00179-4
4. Türkozan O. Reproductive ecology of the loggerhead turtle, *Caretta caretta* on Fethiye and Kızılot Beaches, Turkey. *Chelonian Conservation and Biology* 2000; 3 (4): 686-692.
5. Casale P, Broderick AC, Caminas JA, Cardomna L, Carreras C et al. Mediterranean Sea turtles: Current knowledge and priorities for conservation and research. *Endangered Species Research* 2018; 36: 229-267. doi: 10.3354/esr00901
6. Casale P, Margaritoulis D. (editors). Sea turtles in the Mediterranean: Distribution, threats and conservation priorities. Gland, Switzerland: IUCN, 2010. pp. 294.
7. Hochscheid S, Kaska Y, Panagopoulou A (editors). Sea turtles in the Mediterranean region: MTSG Annual Regional Report 2018. Draft Report of the IUCN-SSC Marine Turtle Specialist Group, 2018.
8. Yalçın-Özdilek Ş. Status of sea turtles (*Chelonia mydas* and *Caretta caretta*) on Samandağ beach, Turkey: A five-year monitoring study. *Annales Zoologici Fennici* 2007; 44: 333-347.
9. Yalçın-Özdilek Ş, Özdilek HG, Ozaner FS. Possible influence of beach sand characteristics on green turtle nesting activity on Samandağ beach, Turkey. *Journal of Coastal Research* 2007; 23 (6): 1379-1390.
10. Yalçın-Özdilek Ş, Sönmez B. Some properties of new nesting areas of sea turtles in north-eastern Mediterranean situated on the extension of the Samandağ beach, Turkey. *Journal of Environmental Biology* 2006; 27 (3): 537-544.
11. Yalçın-Özdilek Ş, Yerli SV. Green turtle (*Chelonia mydas*) nesting and habitat threats at Samandağ beach, Turkey. *Chelonian Conservation and Biology*, 2006; 5 (2): 302-305.
12. Yalçın-Özdilek Ş, Sönmez B. Nesting characteristics at Samandağ and extended beaches, Turkey. *Marine Turtle Newsletter* 2011; 131: 7-11.
13. Türkozan O, Yalçın-Özdilek Ş, Ergene S, Uçar AH, Sönmez B et al. Strandings of loggerhead (*Caretta caretta*) and green (*Chelonia mydas*) sea turtles along the eastern Mediterranean coast of Turkey. *Herpetological Journal* 2013; 23: 13-17.
14. Yılmaz C, Oruç A, Türkozan O. Marine turtles (*Chelonia mydas* and *Caretta caretta*) nesting along the eastern Mediterranean coast of Turkey: Results from six years surveying. *Herpetological Journal* 2015; 25: 197-204.
15. Sözbilen D, Kaska Y. Biochemical blood parameters and hormone levels of foraging, nesting and injured loggerhead sea turtles (*Caretta caretta*) in Turkey. *Turkish Journal of Zoology* 2018; 42: 287-296.

16. Swimmer JY. Biochemical responses to fibropapilloma and captivity in the green turtle. *Journal of Wildlife Diseases* 2000; 36 (1): 102-110. <https://doi.org/10.7589/0090-3558-36.1.102>
17. Dickinson VM, Jarchow JL, Trueblood MH. Hematology and plasma biochemistry reference range values for free-ranging desert tortoises in Arizona. *Journal of Wildlife Diseases* 2002; 38 (1): 143-153. [doi: 10.7589/0090-3558-38.1.143](https://doi.org/10.7589/0090-3558-38.1.143)
18. Lopez-Olvera JR, Montane J, Marco I, Martinez-Silvestre A, Soler J et al. Effect of venipuncture site on hematologic and serum biochemical parameters in marginated tortoise (*Testudo marginata*). *Journal of Wildlife Diseases* 2003; 39 (4): 830-836. [doi: 10.7589/0090-3558-39.4.830](https://doi.org/10.7589/0090-3558-39.4.830)
19. Prieto-Torres DA, Hernandez JL, Bravo Henriquez AR, Alvarado MC, Davia MJ. Blood biochemistry of the breeding population of green turtles (*Chelonia mydas*) in the Aves Island Wildlife Refuge, Venezuela. *South American Journal of Herpetology* 2013; 8 (3): 147-154.
20. Montilla Fuenmayor AJ, Hernandez Rangel JL, Alvarado MC. Hematological values of the green turtle (*Chelonia mydas*) present in the high Guajira. *Revista Científica de Veterinaria* 2006; 16 (3): 219-226.
21. Hasbun CR, Lawrence AJ, Naldo J, Samour JH, Al-Ghais SM. Normal blood chemistry of free-living green sea turtles, *Chelonia mydas*, from the United Arab Emirates. *Comparative Haematology International* 1998; 8: 174-177.
22. Rostal DC, Grumbles JS, Palmer KS, Lance VA, Spotila JR et al. Changes in gonadal and adrenal steroid levels in the leatherback sea turtle (*Dermochelys coriacea*) during the nesting cycle. *General and Comparative Endocrinology* 2001; 122: 139-147. [doi: 10.1006/gcen.2001.7615](https://doi.org/10.1006/gcen.2001.7615)
23. Perpiñán D. Chelonian haematology 1. Collection and handling of samples. *In Practice* 2017; 39: 194-202.
24. Bolten AB, Bjorndal KA. Blood profiles for a wild population of green turtles (*Chelonia mydas*) in the Southern Bahamas: Size-specific and sex-specific relationships. *Journal of Wildlife Diseases* 1992; 28 (3): 407-413.
25. Alaçam E. Hormonların klinik kullanımı. In: Alaçam E (editor). *Evcil Hayvanlarda Doğum ve İnfertilite*. 4th ed. Ankara, Turkey: Medisan; 2002. pp. 41-54 (in Turkish).
26. Rostal DC, Paladino FV, Patterson RM, Spotila JR. Reproductive physiology of nesting leatherback turtle (*Dermochelys coriacea*) at Las Baulas National Park, Costa Rica. *Chelonian Conservative Biology* 1996; 2 (2): 230-236.
27. Guillette LJ Jr, Bjorndal KA, Bolten AB, Gross TS, Palmer BD et al. Plasma estradiol-17 beta, progesterone, prostaglandin F₂ and prostaglandin E₂ concentrations during natural oviposition in the loggerhead turtle (*Caretta caretta*). *General and Comparative Endocrinology* 1991; 82 (1): 121-130. [doi: 10.1016/0016-6480\(91\)90303-n](https://doi.org/10.1016/0016-6480(91)90303-n)
28. Wibbels T, Owens DW, Limpus CJ, Reed PC, Amoss MS Jr. Seasonal changes in serum gonadal steroids associated with migration, mating, and nesting in the loggerhead sea turtle (*Caretta caretta*). *General and Comparative Endocrinology* 1990; 79 (1): 154-164. [doi: 10.1016/0016-6480\(90\)90099-8](https://doi.org/10.1016/0016-6480(90)90099-8)
29. Rostal DC, Owens DW, Grumbles JS, MacKenzie DS, Amoss MS Jr. Seasonal reproductive cycle of the Kemp's ridley sea turtle (*Lepidochelys kempii*). *General and Comparative Endocrinology* 1998; 109: 232-243. [doi: 10.1006/gcen.1997.7026](https://doi.org/10.1006/gcen.1997.7026)
30. Owens DW. Hormones in the life history of sea turtles. In: Lutz PL, Musick JA (editors). *The Biology of Sea Turtles*. Boca Raton, FL, USA: CRC Press, 1997.
31. Licht P, Wood JF, Wood FE. Annual and diurnal cycles in plasma testosterone and thyroxine in the male green sea turtle *Chelonia mydas*. *General and Comparative Endocrinology* 1985; 57 (3): 335-344. [doi: 10.1016/0016-6480\(85\)90212-6](https://doi.org/10.1016/0016-6480(85)90212-6)
32. Hamann M, Schauble CS, Simon T, Evans S. Demographic and health parameters of green sea turtles *Chelonia mydas* foraging in the Gulf of Carpentaria, Australia. *Endangered Species Research* 2006; 2: 81-88.
33. Al-Habsi AA, Alkindi AYA, Mahmoud IY, Owens DW, Khan T et al. Plasma hormone levels in the green turtles *Chelonia mydas* during peak period of nesting at Ras Al-Hadd-Oman. *Journal of Endocrinology* 2006; 191: 9-14. [doi: 10.1667/joe.1.06790](https://doi.org/10.1667/joe.1.06790)
34. Hamann M, Limpus CJ, Whittier JM. Seasonal variation in plasma catecholamines and adipose tissue lipolysis in adult female green sea turtles (*Chelonia mydas*). *General and Comparative Endocrinology* 2003; 130: 308-316.
35. Jessop TS, Hamann M. Hormonal and metabolic responses to nesting activities in the green turtle, *Chelonia mydas*. *Journal of Experimental Marine Biology and Ecology* 2004; 308: 253-267.
36. Ikonomopoulou MP, Bradley AJ, Ibrahim K, Limpus CJ, Fernandez-Rojo MA et al. Hormone and metabolite profiles in nesting green and flatback turtles: Turtle species with different life histories. *Advances in Zoology* 2014; 50:3209. [doi: 10.1155/2014/503209](https://doi.org/10.1155/2014/503209)
37. Gregory LF, Gross TS, Bolten AB, Bjorndal KA, Guillette LJ Jr. Plasma corticosterone concentrations associated with acute captivity stress in wild loggerhead sea turtles (*Caretta caretta*). *General and Comparative Endocrinology* 1996; 104: 312-320. [doi: 10.1006/gcen.1996.0176](https://doi.org/10.1006/gcen.1996.0176)
38. Kohel AK, MacKenzie DS, Rostal DC, Grumbles JS, Lance VA. Seasonality in plasma thyroxine in the desert tortoise, *Gopherus agassizii*. *General and Comparative Endocrinology* 2001; 121 (2): 214-222. [doi: 10.1006/gcen.2000.7595](https://doi.org/10.1006/gcen.2000.7595)
39. Southwood AL, Darveau CA, Jones DR. Metabolic and cardiovascular adjustments of juvenile green turtles to seasonal changes in temperature and photoperiod. *Journal of Experimental Biology* 2003; 206: 4521-4531. [doi: 10.1242/jeb.00689](https://doi.org/10.1242/jeb.00689)
40. Moon D-Y, Owens DW, MacKenzie DS. The effects of fasting and increased feeding on plasma thyroid hormones, glucose, and total protein in sea turtles. *Zoological Science* 1999; 16: 579-586. [doi: 10.2108/zsj.16.579](https://doi.org/10.2108/zsj.16.579)