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Effect of seasonal variations on testes and serum protein expression of Desert Tortoise (*Testudo graeca cyrenaica* Linnaeus, 1758)

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Desert Tortoise; serum protein expression; testes.

Abstract: The present concentrating on the effect on seasonal variations of testes and serum protein expression of Desert Tortoise , and the results gave out that, The assayed the levels of testosterone hormone was pointedly raised within summer season otherwise the stages of testosterone hormone was decreased in hibernation season (winter season), and for histological section of testes of the adult tortoise (*T. g. Cyrenaica*) within winter season there is clear areas of edema, and necrotizing primary and secondary spermatocytes, and oligospermia, while during activity season (summer season A) demonstrating spermatogonia, primary and secondary spermatids, spermatozoa, and spermatocytes; conversely, the presence of protein bands and their levels were significantly reduced in the winter and heightened in the summer.

Introduction

Temperature is a significant environmental determinant that shapes numerous aspects of the biology of reptiles and other ectothermic organisms, influencing their life history strategies, ecological interactions, and physiological responses (Angilletta et al., 2002). Critical processes such as expansion rates (Avery, 1994; Arnold & Peterson, 1989; Litzgus & Brooks, 1998) and proliferation (Litzgus & Brooks, 1998; Rock & Cree, 2003) are particularly temperature-dependent. Reproduction in vertebrates is intrinsically multifaceted and evolutionarily diverse; variation in reproductive anatomy, as well as in the timing and regulation of reproductive cycles, is s widely recognized as a remarkable factor driving reproductive branching and speciation.

Reptilian species demonstrate a broad spectrum of reproductive strategies, which are often closely lined up with commanding environmental variables in taxa exhibiting seasonal reproductive designs, the gonadal cycle—consisting of the stages of recrudescence, summit reproductive activity (climax), and gonadal tranquility, appears to be temporally correlated in response to thermoregulatory pressures, active demands, and the anticipated duration of reproductive operation (Saint Girons, 1984). Experiential evidence from multiple lizard species has substantiated the occurrence of seasonal fluctuations in reproductive events, specifically in relation to circulating plasma steroid hormone concentrations (Arshalan et al., 1978; Amey & Whittier, 2000; Radder et al., 2001). this study aims to effect of seasonal variations on testes and serum protein expression of Desert Tortoise.

Experimental

Over winter and summer season between 2023 and 2024, gathered from Al-Jabal Al-Khadar (Khandola, southern of - Al-Bayda city eastern Libya) 5 males mature individuals, forty adults of Desert Tortoise (T. g. Cyrenaica), the animals anesthetized with diethyl ether and blood was gathered, centrifuged at 3000 g until 5 minutes and serum collected then preserved in refrigerator at -80ºC.

Testosterone **Hormone:** ELISA kit of the IBL-AMERICA company according to (Tietz, 1986).

Light microscopic examination:

At the end of the experiment, the animals were sacrificed by cervical dislocation, the tissues were immediately immersed in 10% formalin solution and aqueous Bouin's fixative for 24 hours, dehydrated in ascending grades of ethyl alcohol, cleared in xylene. The specimens were impregnated for three changes in paraffin and were finally embedded in paraffin wax (melting point between 56°C and 60°C). The paraffin blocks were cut by rotary microtome (Leica RM 2125) into sections at 5–7 µm- thick. The deparaffined sections were stained with Harri's hematoxylin and eosin(H&E) for general histological structure study according to Bancroft and Gamble (2008).

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE):

Electrophoresis was performed for 14 hours at a constant voltage of 200 V under denaturing conditions. Subsequently, the polyacrylamide gels were stained with a Coomassie Brilliant Blue R-250 solution (60 mg/L) according to the methods of Laemmli (1970) and Andrew (1986).

Results and discussion

Figure 2 illustrates the circulating testosterone concentrations in mature male *Testudo graeca cyrenaica* tortoises across seasonal periods: summer (the active season) and winter (the hibernation season). A significant increase in testosterone levels was observed during the summer, whereas the lowest concentrations were recorded during winter hibernation.

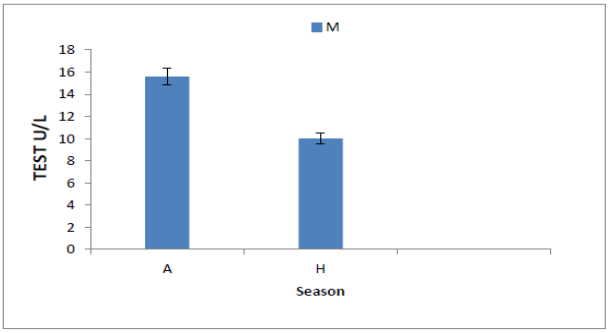

Figure (2): Effect of seasonal variation on testosterone hormone in (*T. g. Cyrenaica*) during A. summer season (Activity season) and (hibernation season H).

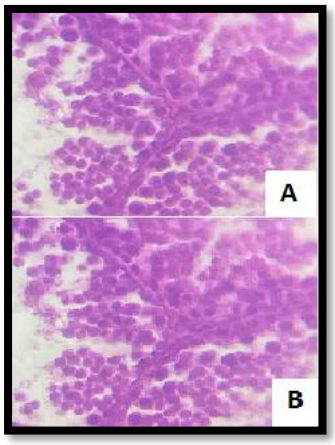

Figure (3A and B): Photomicrograph illustrates testis sections of (*T. g. Cyrenaica*), during summer seasons A (A activity season) showing spermatogonia, spermatozoa, and spermatids, and primary and secondary spermatocytes. (x40).

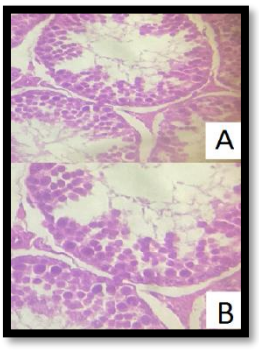

Figure (4A and B): Photomicrograph illustrates testis sections of (*T. g. Cyrenaica*) during winter season (hibernation season) (x40), there are clear areas of edema, necrotizing primary and secondary spermatocytes, and oligospermia.

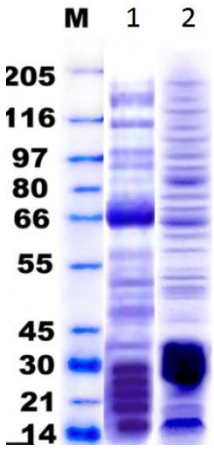

Figure (5) effect of seasonal variations on serum protein electrophoresis (*T. g. Cyrenaica*) during summer season A (A. activity season) and winter season (hibernation season).

Discussion

The physiology of T. g. Cyrenaica generally in north Africa and singularly in Libya Studies are relatively few, in spite of the fact that Information on this topic has been increasing over the rearmost ten years. This absence of understanding disserves of how physiological and ecological and divergences might emerge because of that the environment change in periods of seasonal difference, so , this research fixed on study the impact of seasonal differences on testes and serum protein expression of Desert Tortoise, The present study indicated that, The extent of seasonal effects and changes in testes and protein expression in the serum of the desert tortoise, and the results disclosed that, The assayed the levels of testosterone hormone was discernibly increased during summer season. Otherwise, the degrees of testosterone hormone were decreased in hibernation season (winter season).

Abdalhafid (2013) examined seasonal histological and molecular changes in the liver of male and female *Uromastyx acanthinura* (Bell, 1825) during summer and winter (hibernation). The study reported that hibernation induced significant hepatic damage, characterized by an increase in pigmentation cells and fatty liver degeneration compared to the active summer season. Following hibernation, the liver showed a reduction in fat, returning to a state near normal. At the molecular level, genomic DNA analysis revealed evident fragmentation during hibernation. Furthermore, the activity of the apoptosis-related enzymes caspase-3 and caspase-7 was significantly elevated in liver tissues during hibernation compared to the summer season.

Abdalhafid et al. (2018) investigated the impact of hibernation on the histological structure of the oviduct and liver in *Testudo graeca cyrenaica* across winter (hibernation) and summer (active) seasons. Their results demonstrated significant seasonal alterations. During hibernation, the liver exhibited substantial damage, appearing fatty and containing numerous degenerated cells. Similarly, the oviduct showed signs of atrophy and a marked reduction in the glandular component. In contrast, tissue samples collected after the hibernation period showed a return to a near-normal histological state, characterized by a significant reduction in fatty deposits in the liver and restored architecture in the oviduct.

In the presents study, Active spermatoid-and spermiogenesis were observed during summer. There were different kinds of metamorphosed spermatids and differentiation of sperm. However, during winter, there were a marked inhibition of both spermatogenesis and spermiogenesis. Some seminiferous tubules exhibited exfoliation of sex cells within the tubular lumina. The flourishing of testicular structure during summer and failure during hibernating season was confirmed by marked alterations in both testosterone hormonal level and genomic expression of protein during hibernation.

Conflict of Interest: The authors declare that there are no conflicts of interest.

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