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Examination of different blood parameters in *Testudo marginata* after the hibernation

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Table of Contents

Introduction

It might be safe to say that over the years, the class reptilia has not had great success in terms of publicity, however, the fascination humans have shown relating to these animals verges on the immeasurable. People's perception to the class reptilia is influenced by the countless venomous species that have posed a huge threat to the human race. Consequently many people, do not realize that turtles and tortoises are in fact reptiles, since the latter two are perceived to be relatively harmless, primarily because this order does not exhibit a predominant venomous characteristic. 'There are 12 families and approximately 255 species of Chelonia, most of them recognized by CITES as threatened or endangered'.[1] The order testudines (sometimes referred to as chelonia by some authors)[2], includes turtles and tortoises, were their charcteristic outer shell has bestowed recognition by many authors. Members of the order Testudines, are sometimes the focus of many cultural beliefs associated with longevity and good luck. Owing to their relatively long life-span, these animals have become renowned in certian cultures. In association with their longevitity, their use as a food source such as turtle soup, together with their medicinal properties used to treat kidney and liver aligments, has lead to a decline in wild populations rendering their scarcity more desirable in the reptilian trade.[3]

Turtles and tortoises are frequently popular in animal enthusiast households as low maintanence pets. This does not imply that tortoises and turtles in the pet trade, rests upon the mainstream single testudines households. Specimens of Testudines, are valuable investments and are of great focus and importance to dedicated collectors. Because of their dwindeling numbers breeders of the Testudines order are faced with difficulties in maintaining blood lines and keeping populations alive because of the diminishing gene pool. The longevity of this species implies a longer maturation time of these animals, coupled with the hinderance of the most diserable trait of Testudines, that is, their shell, breeders are compelled to select parents for future generations using external features. Conservation programs should be made available informing breeders and enthusiasts of the scientific chracteristics for identifying the sex of hatchlings. Selection solely based on phenotypical characteristics might be considered to be too

long a period for a viable breeding of the species, since a minimum of three years would be required for the latter features to be fully developed.

The chief goal of this thesis is to attempt to determine wether a trend in haematological parameters might distinguish between male and female adult tortoises. As a result, the author hopes to provide a low cost, reliable and scientific means of sexing tortoises using simple blood tests. Another goal is to examine wether there is a difference in blood parameters between the sexes, after hibernation to breeding season. Ultimately the author hopes this study can be extended to determine if the same approach can be used to distinguish the sexes of hatchling tortoises.The most common species encountered in the pet trade, which is the Testudo genus, will be tackled here, with the hope that the same technique can be extended to other species and relatives of this group.

A land dwelling member of the order Testudines is commonly referred to as a tortoise, as opposed to the other two groups, turtles and terrapins which are respectively classified as salt and fresh water dwelling Testudines. Testudo is a genus of tortoises comprising five main species and several sub species. These include Testudo; greca, horsfieldi, hermanni, marginata and kleinmanni and can be found primarily distributed in Northern Africa, Western Asia and Europe as an indigenous species. Noteworthy is the Greek tortoise which is known to be the most widespread encompasing several subspecies and morphs which have been identified. Of the order testudo the oldest lineage is that of the Russian tortoise scientificlly known as Testudo horsfieldi and owing to the distinct shape of its carapace and plastron, it occupies a new genus[4]. Within the order Testudo the genus Chersus has been created in an effort to link the Egyptian (klainmanni) and Marginated tortoises primarily because these species share similar DNA sequences.[5] Similarly Testudo hermanni has its own genus Eurotestudo which together with the Russian tortoise, has fossil records to support the age of this lineage.[6] For the purpose of this research subject the species Testudo marginata will be used as the research subject.

Literature Review

Testudo Ecological background

Testudo species share similar preferences in habitat and requirements. Primarily, in nature this species can be found in Mediterranean oak forests, arid land and rocky hillsides. These habitats offer an abundance of plants to satisfy the herbivorous diets of these tortoises as well as, of course, the quintessential sunlight.[7] Typically in Mediterranean regions, the staple food source of a wild tortoises are, grasses, flowers and a quantity of fruit and vegetables.[8] It is widely understood that the dietry requirements for Testudo species include a high calcium and fiber content, low protein and fat.[9] Most of the bodily water requirements are obtained from the ingested plants, implying that these tortoises can survive long periods of time in arid conditions.[10] Not withstanding this characteristic, pools of water facilitate the intestinal movements of most reptiles, including tortoises, thereby prompting tortoises to seek a water source.[11]

Typical of many reptiles, tortoises have an immeasurable requirement for sunlight including a vast spectrum of UV (ultraviolet) radiation. Drs. Forest and Smith maintain that UVA and UVB wavelengths (280-400nm), are amongst the most essential factors tortoises need in order to sustain healthy development and maintanence.[12] Also, breeding behaviour and hibernation are believed to depend primarily on daylight hours implying that sunlight is not only required for metabolic processes however it is also understood to inflict a major impact on a tortoise's behaviour[13]. Biologically it is an accepted prinicipal that cold-blooded animals, such as tortoises, rely on sunlight as a means of warming their body in order to maintain a healthy temperature for homeostasis together with digestion[14]. In the preceeding chapter, reference was made to a Testudines primary characteristic which is its outermost shell. This attribute suppliments a major role in the body temperature regulation (thermoregultion) of the animal. The outer most shell contributes to two major factors for the well being of the tortoise; the first being a mechanical means of protection, the second and perhaps most important is maintaining body

temperture by trapping heat in order to maintain essential metabolic processes. Exposure to UV radiation is also essential to what is commonly referred to as the basking period for tortoises. This period implies a chemical process by which tortoises utilize calcium and Vitamin D, together with UV radiation as a catalyst, to promote physiological bone development particularly in hatchlings and growing tortoises.[15]

When conditions are ideal, most egg laying behaviour takes place when ground temperature is preferential to the species, usually in the region of 38-41°. This ensures suitable egg incubation, however, these periods are highly variable depending on geographical location.[16]Thus the importance of sunlight to a breeding tortoise does not only lie in its role for metabolism, but it also determines the behavioural changes that go with breeding season.

Basic anatomy

The tortoise skeleton is quadrupedal and generally has pentadactyl limbs which hold the body off the ground despite being more laterally positioned than in mammals. The legs also play a role in protecting more sensitive structures like the neck, by sealing all openings of the shell, leaving only the thick, keratinized and horny surface of the skin to the exterior. Scutes are keratinized plates that make up the shell, which is covered in epidermal tissue. The plastron, which is the bottom half of the shell, is flat since this is the surface that must be parallel to the ground for locomotion to take place. The upper half of the shell, the carapace, has a convex shape and is responsable for housing and protecting all the internal organs. The skull and shell are formed by dermal ossification, while the beak is similar to the avian beak. The upper beak is made of keratinized horny substance and wraps around the osseus jaws. No teeth can be found in the oral cavity of tortoises, but the beak is still essential in the prehension of food. As with most reptiles, tortoises have thick, scaly and impermeable skin which protects them from most of the harsh conditions of a mediterranean tortoises' habitat.

Tortoises have a single coelomic cavity which is divided horizontally by a pleuroperitoneal membrane. This allows for the separation of the lungs dorsally, from the viscera ventrally, while a vertical membrane separates the left and right lungs with clear distinction. The pleurperitoneal membrane however has no role in ventilation movements, so intrapulmonary pressures are regulated by visceral, limb and limb girdle movements. Testudinas do not posess a muscular diaphragm, leaving no true abdominal and visceral cavities. This means that the heart can be found immediatly cranial to the liver, on the midline right above the plastron. The heart also bears its unique features in that only three chambers are present, which are; the paired atria and one functionally divided ventricle.

The gastrointestinal system is more or less similar to that of mammals. Starting with the buccal cavity, food is passed through the pharynx and moved along a muscular oesophagus eventually leading to the simple fusiform stomach. Chyme then makes its way to the small intestine where the pancreatic and liver secretions are deposited before entering the large intestine and cloaca. The cloaca is a unique feature to reptiles and birds. This is the point at which the urogenital and digestive tracts meet and end. The end of the colon is separated from the urodeum by a distinct fold, the latter of which is emptied into the proctodeum. The proctodeum is the most caudal part and receives its contents from the bladder, colon, genital organs and ureter.

The reproductive system is considered mature at about 15 years of age, although this is dependant on rate of growth and size. Being oviparous, marginated tortoises produce hard shelled eggs, the origin of which, are from the two ovaries that are held up by the dorsal coelomic membrane. In the oviducts, eggs produced by the ovaries, receive their albumin from the upper part of the oviduct, while the lower oviduct calcifies the shell before entering the cloaca. The single hemipenis of males is not involved in urination, but protrudes from the base of the cloaca. The paired testes change in size, seasonally and in the presence of females, and can be found cranioventrally to the kidneys within the coelomic cavity. Sperm is transferred to the female along a groove in the erected penis.

Sex determination

Identifying the gender of marginated tortoises can be a simple task due to their sexual dimorphism. This however, is only expressed after at least five years of age, sometimes even longer depending on when puberty is reached. As hatchlings or juveniles, all testudinas share features that resemble closely the female version of the species, making it impossible for differentiation at this age.[17]

During competition for female insemination, males typically engage in combat, which is suggestive as to why male testudines are larger than females.[18] Male choelonians have a large cloacal penis, which can measure up to three or four inches when erect. This may take place either during copulation, or in cases of stress when it is often accompanied by urination. The penis may be described as being a darkly coloured and spade shaped organ, bearing a median raphe.[19] The plastron in males, generally has a more concave shape than that of females, which serves to facilitate the copultion process. Females display a more flat plastron.[20]

The tail can also be suggestive of gender. In females, the tail is shorter than in males, and the distance from the caudal edge of the plastron, to the cloacal opening is generlly shorter. Males have a longer and more broad tale which may end in a sharper point, and is thicker at the base than in females.[21]

Using radiography, one can use the presence of eggs as confirmation of female gender, although this tool is not very effective in determining sex.[22] Ultrasonography can also be used in sex determination by inserting a probe in the inguinal fossa, and identyfying the female reproductive tract[23], or by locating the testes in adult males.[24] An indirect method of sex determination is by determining incubation conditions such as temperature and oxygen tension. It has been suggested that the sex ratio can be manipulated by setting different temperatures when incubating eggs, and that environmental conditions can indicate sex ratios in naturally incubating eggs.[25]

Reproductive physiology

Most female testudinas exhibit annual reproductive cycles, however those in tropical regions have been known to be fertile all throughout the year, due to the minimal climate changes.[26]This suggests that climatic factors play a major role in the reproductive physiology of tortoises, were temperature, humidity, food supply and rainfall all contribute to the reproductive physiology. Testudo species are mainly found in temperate zones, which means the environmental conditions during winter are an especially important factor when studying the reproductive cycle.

According to Dr.G.Kuchling, 'the female reproductive cycle can be divided into four phases of (1) follicular enlargement, (2) ovulation and intrauterine period, (3) nesting period and (4) latent period'. Usually vitellogenesis begins in late summer or autumn and continues until completed in the following spring, interrupted only by the winter hibernation period.[27] Liver lipids and proteins are mobilized for vitellogenesis and allow yolk accumulation to be completed before or right after hibernation. These lipid and protein levels in the female can reduce up to ten times lower than in males.[28]

In males, spermatogenesis takes place during the warmer months of summer, while during winter time there is a state of germinal inactivity. This cycle is rather uniform and is typical of testudines living in temperate regions. 'Spermatozoa are stored in the epipdidymis where they may be found from the onset of spermiation, onward through Autumn, Winter and Spring, and sometimes early Summer.

In summary, females ovulate in spring while spermatogenesis begins at this time and is completed by early autumn. Oviposition takes place during late spring and early summer, after which folliculogenesis commences in preparation for the next breeding season. Mating season follows the hibernation period in the winter months, making the male and female cycles asynchronous.[29]

Haematologic parameters to be investigated

Glucose

Tortoises living in temperate zones have marked variations in glucose levels, which is due to the hibernation season. After hibernation, what is known as a 'post-hibernation surge' in glucose takes place, were energy reserves are mobilized for feeding to begin again.[30]

Creatinin

Creatinin is a parameter used to evaluate renal function in most animals, however, with respect to tortoises, it is not considered to be very telling, as it is neither actively secreted, nor absorbed by the renal tubules.[31]

Uric acid

Nitrogenous waste comes mainly in the form of uric acid and ureates in the reptilian animal. Evaluating uric acid levels seems to be the most reliable method for detecting renal disease, however, only during extensive kidney damage can elevated levels be detected.[32]

Sodium (Na)

In Mediterranean species, sodium levels peak after hibernation in April and reduce in June after spring water intake. Thus, sodium levels are subject to considerable seasonal variation and depend on the hydration status of the animal.[33]

Potassium (K)

Similarly to sodium, potassium is at its highest during the post-hibernation period and remains at peak during the warmer months. During hibernation lower levels of potassium can be observed, while higher levels can be witnessed during dehydration due to reduced renal excretion.

Triglycerides

Triglycerides may be high during female reproductive activity and low right before hibernation. Trigylcerides are evaluated together with cholesterol, and high concentrations can be observed in females suffering from follicular stasis, but in general females have higher mean cholesterol.

Chlorine (Cl)

Sodium chloride and bicarbonate ions account for more than 85% of the osmotically active components in plasma of all reptiles. Chloride plus bicarbonate contribute 80-80% of the anions. Chloride levels above 115 mEq/liter typify lizards and snakes; other reptiles have lower levels. Osmotic pressure and plasma sodium and chloride attain maximum values during the winter.[34]

Calcium (Ca)

Serum calcium may be raised considerably prior to egg laying by up to 400%.[35]However, protein levels also increase during this time, which could signify the use of calcium in un-ionized form. In male and immature testudines, calcium levels do not seem to exceed 3.6 mmol/l which suggests that pathological hypercalcaemia is uncommon. Despite this, plasma calcium levels are extremely variable and may be suggestive of nutritional and health status.

Phosphate (P)

'Phosphate will be elevated if there is an excess of dietry excess of phosphate relative to calcium, for exmple in metabolic bone disease.' Interpretation of blood phosphate however, is not a simple task since finding a physiological range is subjective.[36]

Magnesium (Mg)

'During estrus in females the yolk precursor, plasma vitellin, appears in the blood causing increased protein, calcium, and magnesium levels. Other seasonal shifts in metabolic capabilities result in dramatic cyclic changes in blood levels of metabolites such as glucose'.[37]

Total protein

According to previous studies, the level of total protein in the blood of females is significantly higher then that of males during the same summer period. This is presumably due to yolk

formation. On the other hand, changes in total protein concentrations may be due to other underlying disorders such as anorexia, parasitism and malnutrition.

Aspartaminotransferase (AST)

Elevation of AST may indicate liver disease, however some studies have shown that this enzyme is not organ specific, and that high levels have been detected in heart muscle and kidneys, besides the liver.

Alanine aminotransferase (ALT)

ALT levels can be detected in the kidney as well as the liver, but changes in concentrations have rarely been documented even in the case of disease.[38]

Alkaline Phosphatase (AlkPhos)

Previous studies have suggested that although AlkPhos is present in the kidney and intestine, serum levels suggest that primary sources may be tissues that have not been investigated yet, possibly being bone or reproductive organs.[39]

Lactate dehydrogenase (LDH)

LDH can be found in a variety of tissues including the liver, kidneys, skeletal and heart muscles as well as lower levels in the gut. Elevated levels are often discovered in sick animals with tissue damage including septic arthritis, renal failure and stomatitis.[40] Artificial elevation of LDH may be associated with haemolysis.[41]

Creatine kinase (CK)

Previous studies have shown high concentrations to be present in skeletal and cardiac muscle, together with smaller amounts in the kidney. Elevated levels indiciate muscle damage which may be associated with injection sites from either the jugulal vein or tail.[42]

Own investigations

Materials and methods

Animals

Adult Testudo marginata where used for the investigation, using seven males and seven females for comparison. The gender of each tortoise was determined using the tail features as mentioned in earlier. The body weight and plastron length of each specimen were recorded together with their corresponding chip number using a chip reader. The general status of each tortoise was determined by examining the shell, skin, natural orifices including oral cavity, nares, eyes and cloaca, together with the limbs and reflexes.

Figure 1 Microchip reading

Figure 2 Body weight measurement

Figure 3 Plastron length

Housing

These tortoises were kept in similar housing conditions, in outdoor terrariums between the first week of April and late Autumn when suitable weather conditions permitted.The animals were housed in three separate groups. Two males kept with three females, another two males kept with the remaining four females, leaving the remaining three males housed alone. The enclosures measured 2x3.2m and had walls measuring 65cm high. The tortoises all had sufficient shelter in which to spend the night, suitable water facilities, together with an adequate egg laying area.

Figure 4 Tortoise housing

Blood sampling method

After disinfecting the skin with alcohol, blood was collected using a syringe and needle from the subcarapacial (subvertebral) site and stored in a heparinised tube at 8°C until transport to the department of oncology and pathophysiology at Szent Istvan Univeristy in Budapest.

Figure 5 Blood collection site

Figure 6 Blood sampling method

Measuring blood glucose

The blood sugar was measured at the place of sampling, with less than 30 seconds between collection and measurement. A simple hand glucometer was used to measure the blood glucose level of each tortoise by preparing the machine and administering one drop of fresh blood. The result was available within seconds.

Figure 7 Glucometer measurement

Blood test

Blood was collected twice, twelve days apart, from each tortoise and recorded together with their microchip number. The first sample was taken after the end of the hibernation period. The parameters to be examined in the laboratory are as follows; AST, ALT, AP, TP, LDH, CK, TG, glucose, uric acid, creatinine, Na, K, CL, Ca, P, Mg.

Goals of this experiment

The author wishes to examine wether a difference in haematologic parameters can be noted between the sexes of Testudo marginata, as well as, wether the parameters studied, show any differences from the end of hibernation period to the breeding season. Ultimately the author would like to examine the difference between genders using the mentioned blood parameters.

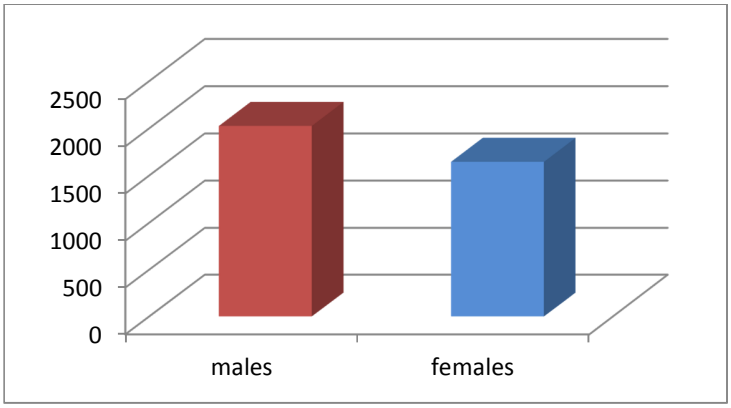
Results and discussion

Our study focused on the different blood parameters changing in Testudo marginata tortoises, after hibernation. We examined 7 adult males (age between 12 and 18 years) and 7 adult females (age between 10 and 17 years). The tortoises were in hibernation until the 12th of April and blood was collected on the 15th and 27th of April. During this period the animals were kept in an outdoor terrarium and fed ad libitum with greens and grasses. Fresh drinking water was supplied every day.

The temperature was 17-26 ^{o}C at daytime and 8-13 ^{o}C at midnight, with clear sunny skies during the examination period.

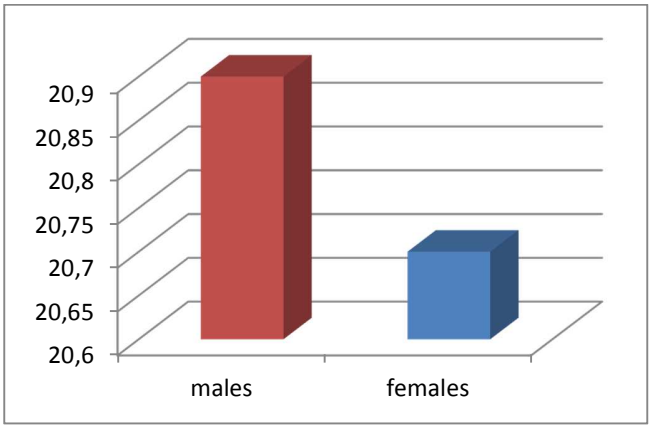
At the time of the first blood collection, animal weight and carapace length was measured. The males weight average was 2024,8 g (1382-3104 g), and female was 1644,8 g (974-3034 g) (diagram 1.).

Diagram 1. Average weight of males and females



The plastron length average in males was 20,9 cm (19-23,5 cm) and in females 20,7 cm (18-24,5 cm) (diagram 2). These results were harmonized with that found in other literature.

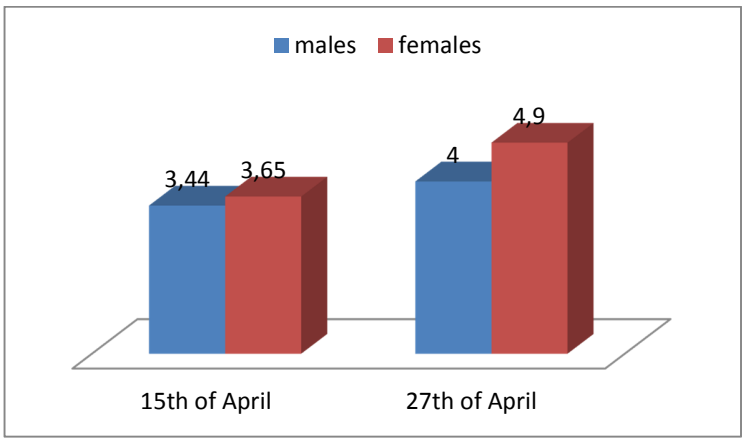
Diagram 2. Average of plastron length



In all cases, blood was collected during the late morning hours (10.00 and 12.30 o' clock). The blood sugar levels were tested 1-4 minutes after the blood sampling. The other parameters were tested in the biochemistry laboratory.

At the end of hibernation the average of blood sugar level in males was 3,44 mmol/l (the range is 2,4-4,6 mmol/l) and in females 3,65 mmol/l (the range is 2,3-4,8 mmol/l). After the second sampling, 12 days later, we measured higher blood sugar levels. In males it was 4 mmol/l (the range is 3,3-4,6 mmol/l) and in females 4,9 mmol/l (the range is 2,9-6,0 mmol/l) (diagram 3). Blood sugar level was lower at first examination compared to the higher values after the second sample collection 12 days later.

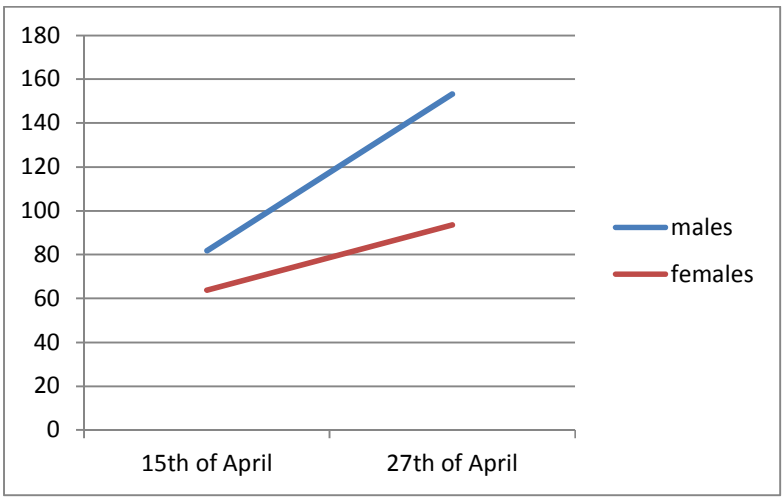
Diagram 3. Average blood sugar levels after hibernation in two separate samplings



It is interesting how the blood sugar level differed between the two blood collection times. The average in males was 0,55 mmol/l (the range is 0-1,2 mmol/l) and in females was 1,25 mmol/l (the average is 0,1-2,2 mmol/). It seems that the blood sugar levels fall within the normal range. At the end of hibernation, the blood glucose levels are at the lower end of the optimal level and increase gradually 12 days later, reaching the physiological level.

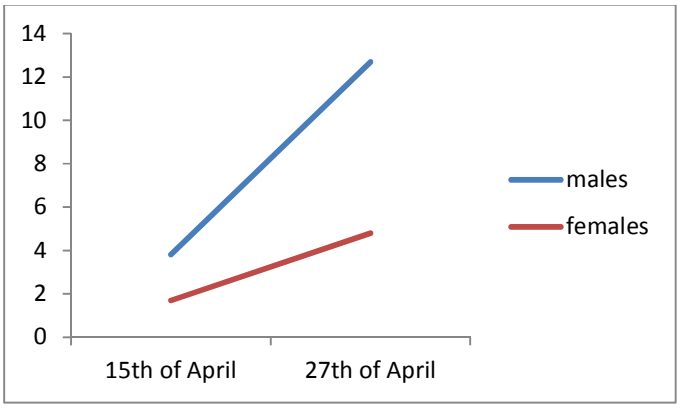
The average AST level was 81,71 IU/l (the range is 50,0-144,0 IU /l) in males and 63,85IU/l (the range is 88,0-398,0 IU /l) in females at the end of hibernation. The levels were higher in most cases for both genders except one female who showed a lower level at the second sampling. The AST levels were higher in males (153,28 IU /l) than in females (93,57 IU /l).

Diagram 4. Average AST levels in males and females



The ALT was higher in males than females. The average ALT level was 3,8 IU/l in males (the range is 1,0 and 9,0 IU/l) and lower (1,7 IU/l) in females. The levels were higher 12 days later, giving 12,7 mmol/l in males and 4,8 mmol/l in females (diagram 5). The increase in levels was 3 IU/l in females and four times higher in males (12,7 IU/l).

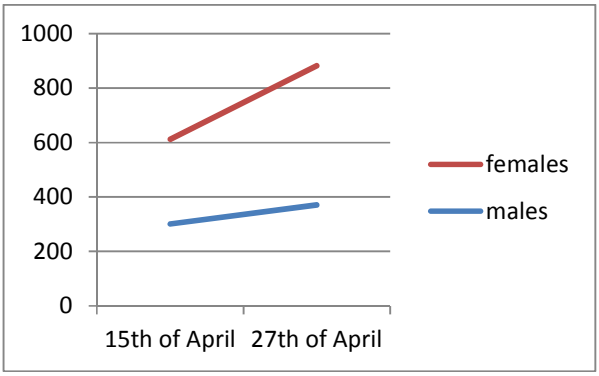
Diagram 5. Average ALT levels in Testudo marginata



The AP level was similar in males and females at the end of hibernation giving values of 300,8 IU/l for males and 311,7 IU/l for females. However, at the time of second sampling, it is clear that the rate of increase of AP was different between males and females. The AP levels

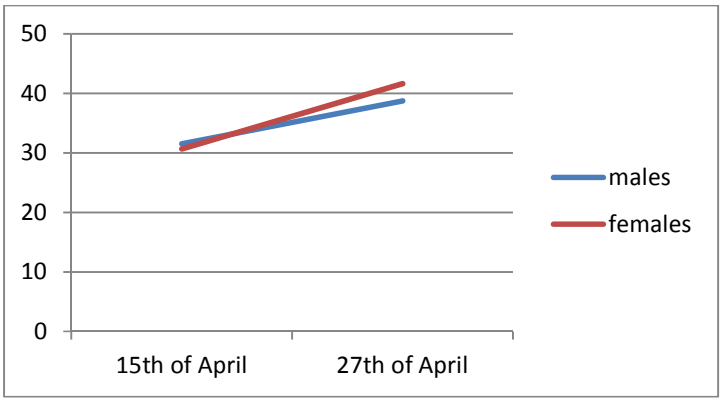
were higher in females (511,2 IU/l) than males (370,5 IU/l) during the second sampling (diagram 6).

Diagram 6. AP levels at the end of hibernation



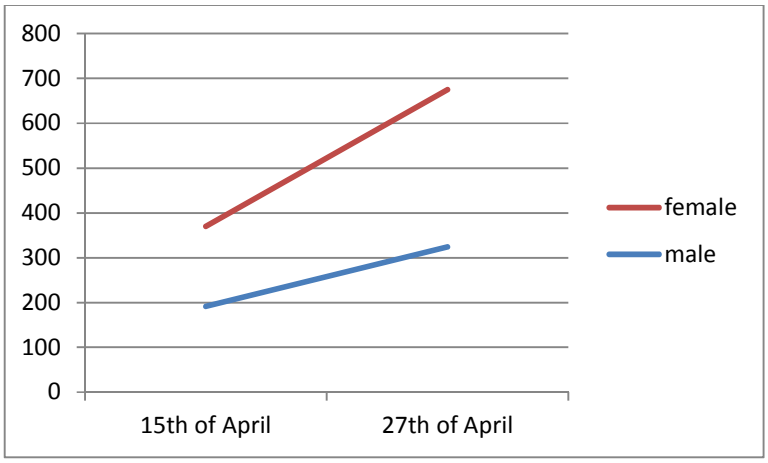
The total protein level also increased after the end of hibernation in both genders. In females the average was 30,71 g/l (the range is 20,9-42,4 g/l) while in males was 31,52 g/l (the range is 23-42,9 g/l). After 12 days we can found higher levels in both genders. In the males it was 38,77 g/l and in the females it was 41,62 g/l (diagram 7). A viable explanation for this is that female folliculogenesis commences after burmation.

Diagram 7. The level of total protein in Testudo marginata after hibernation



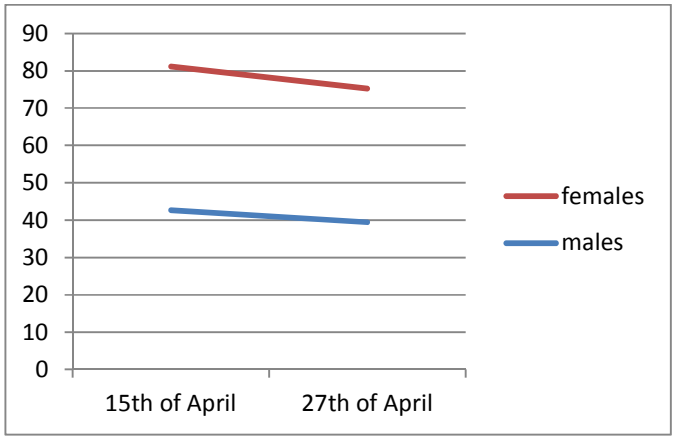
The uric acid is an important parameter in the blood when evaluating the kidney function. In our case the level was 191,4 mmol/l in males and in females it was 178,7 mmol/l after the end of hibernation. Twelve days later the uric acid level was higher in both sexes (males average 324,3 mmol/l, females average 350,9 mmol/l), however one can note the more drastic increase in females (diagram 8).

Diagram 8. The average of uric acid level in Testudo marginata



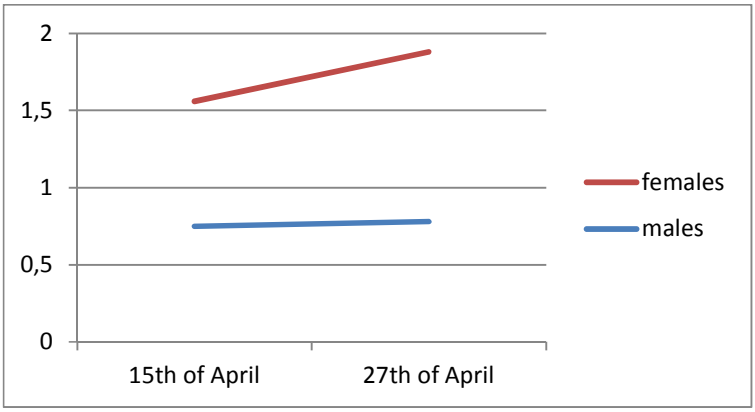
The creatinin level was 42,7 mmol/l (the range is 40-56 mmol/l) in the male and 38,4 mmol/l (the range is 35-41 mmol/l) in the females. When we checked the creatinin level 12 days later we can see the lower levels in both genders (39,4 mmol/l in males and 35,8 mmol/l in females) (diagram 9).

Diagram 9. The average creatinin levels in Testudo marginata



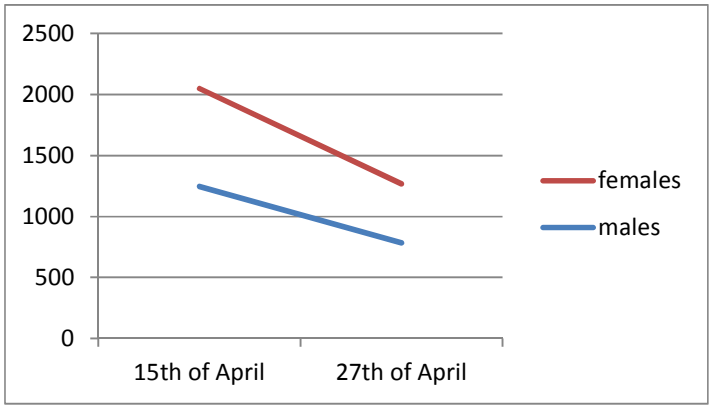
Phosphorus is an important element in the blood were average levels of 0,75 mmol/l in males, and 0,81 mmol/l in females were recorded at the end of hibernation. At the time of the second sampling the value was found to be higher in both genders (0,76 mmol/l in males and 1,1 mmol/l in females), but in females the increase was more prominent (diagram 10. According to literature, this is a physiological change in females which occurs before and during breeding season.

Diagram 10. The average level of phosphorus in Testudo marginata



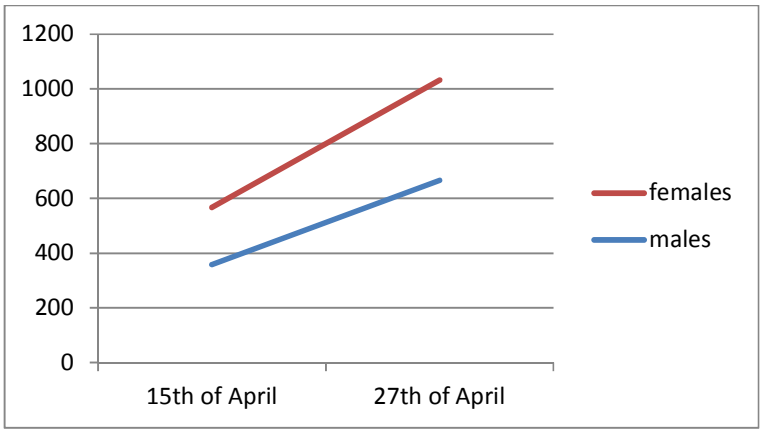
The creatinin kinase was high in both genders, but in males (1247.85 IU/l (extremes 800-3741 IU/l) lower values were recorded than in females (801,57 IU/l (extremes 94-3056 IU/l)). This parameter shows a decrease (785,14 IU/l in males and 481 IU/l in females) after 12 days (diagram 11).

Diagram 11. The average levels of creatinin kinase in Testudo marginata after the hibernation



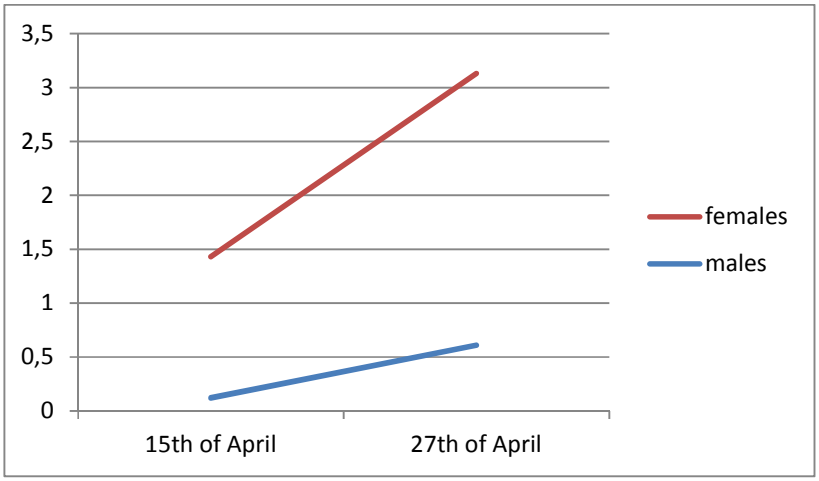
The LDH level in males (358,2 IU/l) and females (208,5 IU/l) was lower at the end of hibernation, but this parameter increases in the blood after 12 days. The females average was 365,4 IU/l while in males it was considerably higher (666,4 IU/l) (diagram 12).

Diagram 12. The average of LDH level in Testudo marginata at end of hibernation



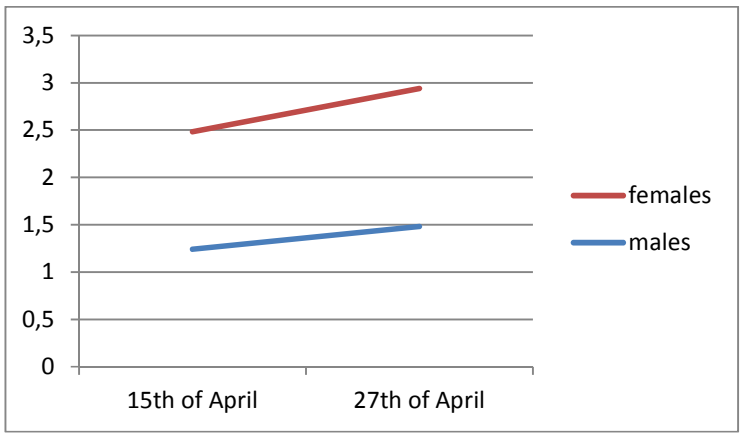
There was a marked difference in triglyceride levels between males (0,12 mmol/l) and females (1,31 mmol/l) after awakening from the hibernation period. At the second sampling 12 days later, the parameter increased but the contrast between the genders was extremely prominent. In males the average of triglyceride levels was 0,61 mmol/l and in females was 2,52 mmol/l (diagram 13).

Diagram 13. The triglicerid levels in the Testudo marginata



Calcium levels were similar in males and females at the time of awakening from burmation. The value increased slightly in both genders, 12 days later (males 1,48 mmol/l, females 1,46 mmol/l) (diagram 14).

Diagram 14. The calcium levels in the Testudo marginata



The remaining elements; Na+, K+, Cl- and Mg++ are demonstrated in the tables (see below) and in the diagram 15 and 16.

Tables. The different elements in the blood

Males

	Cl-	Na+	K+	Mg++
15/04/15	105,2	135,5	5,6	1,9
27/04/15	106,1	135,5	5,7	2,2

Females

	Cl-	Na+	K+	Mg++
15/04/15	102,8	133,8	5	2,2
27/04/15	100,2	130,5	6,2	2,7

Diagram 15. The average levels of different elements from the blood in males

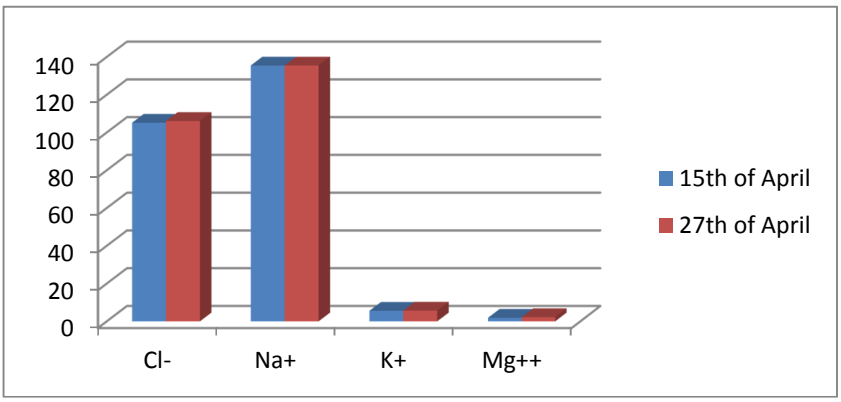
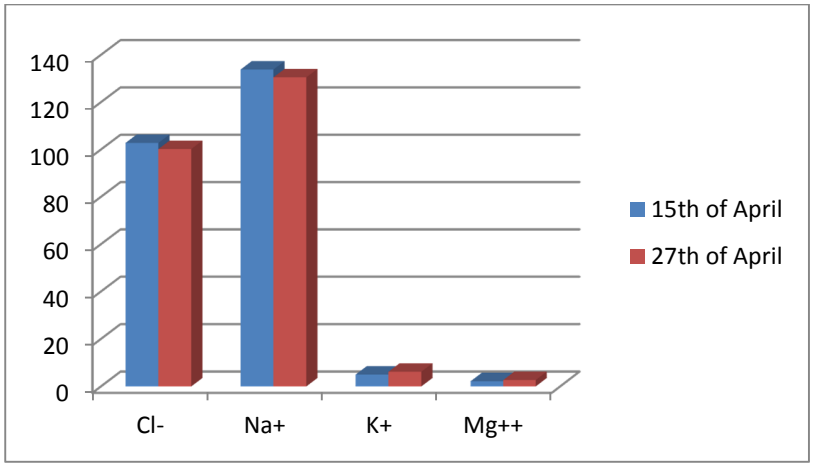


Diagram 16. The average levels of different elements from the blood in females Testudo marginata



Conclusion

After discussing the results we can conclude by evaluating the trends which are apparent in this study. The glucose values obtained show that there is an increase in blood sugar levels in both genders between the two sampling dates. This is understandable considering the minimal feeding taking place during hibernation, which is then compensated by food intake and energy mobilisation after awakening. Males showed higher values for AST than females throughout the study however an increase was noted in both genders between the samplings. ALT and AP also increased in both genders however ALT was higher in males while AP was higher in females. TP showed higher values in both genders between the two samplings, however, females gave higher values of TP which has previously been related to the onset of folliculogenesis. Uric acid gave higher values between samplings but especially in females, while creatinin gave lower values. Phosphorus showed only a slight increase in concentration however this increase has been noted in other studies to be related to breeding season and hibernation. Creatinin kinase showed a major decline in both sexes, more so in females. LDH was found to be considerably higher in males despite having increased in females also. Magnesium and triglyceride both showed an increase in value in both genders between the two samplings. Calcium showed only a slight increase after hibernation in both sexes.

Appendices

Table 1. Male tortoise values, 15/04/15

Chip/ parameter	98610	0	7505000	80104	13238	11115	53683
Weight (kg)	2.506	3.104	2.222	1.382	1.652	1.570	1.1738
Length (cm)	22	23.5	21	19	20	20	21
Glucose (mmol/l)	2.7	4.6	3.1	2.4	4.1	3.4	3.8
AST (U/l)	82	50	90	59	144	82	65
ALT (U/l)	3	3	6	2	1	9	3
AP (U/l)	43	58	228	248	459	338	312
TP (g/l)	36.8	28.4	23	42.9	33.8	24.7	31.1
Uric acid (µmol/l)	200.7	138.1	185.8	174.4	314.4	140.1	186.7
Creatinine (µmol/l)	42	40	56	42	40	40	39
P (mmol/l)	0.83	0.60	0.60	0.82	0.90	0.88	0.68
CK (U/l)	1313	800	1028	528	3471	967	628
LDH (U/l)	275	141	476	26.5	661	302	388
Mg (mmol/l)	1.9	1.9	2.6	1.8	1.6	1.7	1.8
Triglyceride (mmol/l)	0.1	0.1	0.2	0.1	0.1	0.2	0.1
Na (mmol/l)	133	138	132	142	133	133	138
K (mmol/l)	6.19	3.5	7.27	6.4	5.68	5.31	5.34
Ca (mmol/l)	1.18	1.38	1.37	1.26	1.14	1.27	1.09
Cl (mmol/l)	101	102	101	111	104	105	113

Table 2. Female tortoise values 15/04/15

Chip/Parameter	00	53958	14554	82584	84453	78936	57358
Weight (kg)	3.034	1.776	1.582	1.162	1.538	1.448	9.74
Length (cm)	24.5	22	21	19	2.1	20	18
Glucose (mmol/l)	2.3	3.5	3.8	3.5	4.4	3.3	4.8
AST (U/l)	70	52	58	78	105	39	45
ALT (U/l)	1	2	3	2	1	1	2
AP (U/l)	15	246	199	539	415	387	240
TP (g/l)	31.4	23.4	25.1	42.4	37.7	34.1	20.9
Uric acid (µmol/l)	117.0	96.3	268.4	195.4	183.1	254.5	136.3
Creatinin (µmol/l)	36	35	39	41	45	37	36
P (mmol/l)	0.68	0.81	0.88	1.19	0.86	0.80	0.49
CK (U/l)	457	768	765	152	3056	94	319
LDH (U/l)	179	167	207	231	369	168	139
Mg (mmol/l)	1.9	2	2	2.6	2.7	2.6	1.9
Triglyceride (mmol/l)	1.3	1.7	1.3	0.8	2.4	1.6	0.1
Na (mmol/l)	140	135	136	135	132	127	134
K (mmol/l)	3.88	4.45	5.13	5.93	5.59	5.71	4.81
Ca (mmol/l)	1.25	1.11	1.19	1.23	1.28	1.25	1.41
Cl (mmol/l)	107	103	105	103	98	98	106

Table 3. Male tortoise values 27/04/15

Chip/ parameter	98610	0	7505000	80104	13238	11115	53683
Weight (kg)	2.506	3.104	2.222	1.382	1.652	1.570	1.1738
Length (cm)	22	23.5	21	19	20	20	21
Glucose (mmol/l)	3.3	4.3	4.3	3.3	4.1	3.8	4.6
AST (U/l)	94	103	398	139	137	106	88
ALT (U/l)	15	7	42	8	5	6	6
AP (U/l)	334	178	301	286	571	579	345
TP (g/l)	23.5	56.9	39.4	43.2	38.0	37.3	33.1
Uric acid (µmol/l)	199.4	395.2	224	379.6	444.9	393.8	233.4
Creatinine (µmol/l)	33	42	52	40	38	35	36
P (mmol/l)	0.88	0.67	1.02	0.77	0.76	0.65	0.74
CK (U/l)	772	34	3666	535	105	148	236
LDH (U/l)	387	165	2274	424	627	372	416
Mg (mmol/l)	1.9	3.1	2.6	2.1	2.0	1.9	1.9
Triglyceride (mmol/l)	0.4	1.0	1.4	0.6	0.2	0.3	0.4
Na (mmol/l)	133	132	141	139	134	136	134
K (mmol/l)	4.51	5.10	6.50	6.10	5.60	6.60	5.60
Ca (mmol/l)	1.57	1.55	1.42	1.49	1.47	1.53	1.36
Cl (mmol/l)	104	103	110	114	105	106	101

Table 4. Female tortoise values 27/04/15

Chip/Parameter	00	53958	14554	82584	84453	78936	57358
Weight (kg)	3.034	1.776	1.582	1.162	1.538	1.448	9.74
Length (cm)	24.5	22	21	19	2.1	20	18
Glucose (mmol/l)	2.9	5.5	6.0	4.9	5.6	4.6	4.5
AST (U/l)	102	80	128	90	66	102	101
ALT (U/l)	1	2	6	6	5	5	9
AP (U/l)	527	625	393	679	439	549	367
TP (g/l)	54.0	43.9	39.2	40.8	30.0	41.1	41.5
Uric acid (µmol/l)	115.6	322.9	707	361.4	211.1	360.3	378.1
Creatinin (µmol/l)	40	32	36	34	33	39	37
P (mmol/l)	0.72	1.17	2.42	0.82	0.97	0.94	0.66
CK (U/l)	68	238	2552	119	254	40	96
LDH (U/l)	245	278	613	375	227	329	491
Mg (mmol/l)	2.9	3.2	2.4	2.8	2.5	2.9	12.8
Triglyceride (mmol/l)	4.0	3.7	3.1	1.4	1.9	3.2	0.4
Na (mmol/l)	136	127	130	129	130	132	130
K (mmol/l)	5.46	6.50	6.80	6.60	6.01	6.10	6.50
Ca (mmol/l)	1.40	1.44	1.20	1.61	1.52	1.66	1.45
Cl (mmol/l)	105	96	100	100	99	100	102

Abstract

During this experiment the author hoped to find a trend in some haematological parameters before and after hibernation in Testudo marginata species which would give some indication on the sex of the animal. Using external characteristics, 14 animals were studied and determined to be male or female. Blood samples were collected two successive times after the end of hibernation and the parameters were analyzed and compared between the sexes. In this way the author hoped to find an emerging trend that would help in the recognition of the sexes of tortoises using scientific methods, and thus avoid the subjective method of relying on external features.

During the study, several parameters were found to differ between the sexes, but another significant observation was the extent at which the increase or decrease in values varied between the sexes. It was found that females exhibited more rapid changes in values than otherwise observed in males. A notable parameter was that of total protein, were females initially exhibit lower levels at the end of hibernation than males, however, at the time of second sampling, this value increased dramatically in females. As is suggested in other literature, this change is due to the onset of folliculogenesis and it would be interesting to investigate if these changes can be observed in hatchlings.

As a result of this study, the author hopes to have shed some light on the changes in the various blood parameters after the hibernation period, and followed these values progress after 12 days. The author wishes to draw ones attention to the differences between male and female Testudo marginata and their blood values, in order to furthur investigations that could ultimately lead to the distinction between male and female tortoises, especially in hatchlings.

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I would firstly like to thank my tutor Dr. Gal Janos for his help and guidance throughout this entire study. Without his contribution this would not have been made possible. My parents for their constant support and patience with me throughout my studies. Carina Vassallo for her companionship and encouragment through the whole process, Nicole Fenech Adami for her support and help throughout my studies in Budapest and my brother for his help in formatting and final touches. Many thanks also to my partner Thomas Farrugia for his encouragment and help throughout my studies and thesis together with his constant patience during my darkest hours. I would also like to thank all my friends and family who have shown their support and enthusiasm in making this possible.

Declaration of the above thesis

Underisgned Angelika Fenech, student of Szent Istvan University Faculty of Veterinary Medicine, declare that this thesis under the title:

Examination of different blood parameters in *Testudo marginata* after the hibernation

Is the result of my own research work. I agree that with the respect of my copyright, interested people can use both the printed and electronic version, placed into the central library of the veterinary faculty Budapest, Szent Istvan Univeristy.

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