

## Interrelations of Fat Bodies and Liver to Reproduction in Female *Acanthodactylus boskianus*

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**ABSTRACT.** Studies were undertaken to examine the interrelationships of fat bodies to reproductive activity in the lizard *Acanthodactylus boskianus*. The data obtained indicate, that non-reproductive females had the highest percent of females with dissectable fat bodies. Reproductive females (classes II and III) showed various degrees of fat consumption. Winter animals exhibited a low percent of dissectable fat bodies while Summer animals showed a higher percent. This was attributed to the capacity for lipid replenishment, being greater in Summer. Excessive utilization of fat bodies becomes apparent when animals become ovigerous where the percent of animals with dissectable fat bodies becomes zero both in winter and Summer. The utilization of lipid stores during vitellogenesis is also indicated by the declines recorded in **Lipidosomatic index** as well as in hepatic lipid content. The total free amino acids in liver displayed a decline during vitellogenesis attributed to the synthesis of vitellogenin. Hepatic protein content declined during vitellogenesis which might be due to active export of proteins to the growing follicles. The importance of such changes in relevance to reproductive activity were discussed.

### Introduction

Extensive investigations have been carried out on reproductive activity in reptiles. All such works stressed on the importance of fat bodies in vitellogenesis. This was reported in temperate lizards e.g. *Anolis carolinensis*<sup>[1]</sup>, *Dipsosaurus dorsalis*<sup>[2,3]</sup>, *Sceloporous jarrovi*<sup>[4,5]</sup> and *Amphibolurus fordii*<sup>[6]</sup>. Fat bodies were indicated<sup>[7]</sup> to decline in female *Uta stansburiana*, in late winter and early spring when deutoplasm was deposited in the ovarian follicles. Extirpation of the fat bodies in pre-breeding

females inhibited follicular development, while extirpation in females just becoming active, caused increased follicular atresia and retardation in yolk deposition.

Tropical species, displayed an inverse relationship between fat bodies and gonadal activity. This was demonstrated in *Agama agama*<sup>[8]</sup>, in *Ameiva festiva*, *Ameiva quadrilineata*<sup>[9]</sup>, *Sceloporus malachiticus*<sup>[10]</sup> and *Anolis*<sup>[11]</sup>.

Utilization of lipids for vitellogenesis appears to be mediated by liver which was found to play an important role in this respect. This can be judged from the hypertrophy demonstrated in livers of female *Uta stansburiana* in which vitellogenesis had been induced by injecting estradiol-17 B. The same function ascribed to liver was also reported in *Takydromus tachydromoides*<sup>[12]</sup> and *Sceloporus jarrovi*<sup>[4]</sup>. It was also, demonstrated<sup>[13]</sup> that tropical lizard *Chamaeleo jacksoni*, exhibited a maximal liver weight at the onset of vitellogenesis, and reached a maximum during gestation.

Liver performs as well the important function of synthesizing a calcium binding phospho-protein, vitellogenin. Once synthesized, it is secreted to the circulation to be conveyed to the ovaries. It is generally found in blood of female lizards to an extent which depends on the nutritional state<sup>[14-16]</sup> which promotes increased levels of vitellogenins in sera of females.

In Saudi Arabia, very limited works have been carried out to examine the reproductive activity in lizards. Moreover, works concerning functional relationship between fat stores and liver during vitellogenesis are lacking. Therefore, the present work was undertaken to examine the relationship of reproductive activity to liver in a semitropical lizard, *Acanthodactylus boskianus*.

## Material and Methods

### Animal

The lizard used in this work is the semitropical lizard *Acanthodactylus boskianus* (Family: Lacertidae). Monthly trips were made to Wadi Fatima to the close surrounding of Makkah region, at the west of Saudi Arabia, where animals were caught by hand. Only adult females (SVL  $\geq$  6.8 cm) were used in this work.

### Treatment

Animals were sacrificed the next day after collection. The Snout Vent Length (SVL) was measured to the nearest 1 mm. The body weights were recorded to the nearest 0.01 gm. Livers, fat bodies and ovaries were excised from sacrificed animals and were weighed to the nearest mg.

### Reproductive Status

Animals were assigned to one of four reproductive classes based on measurements and examinations of the ovarian follicles<sup>[17]</sup>, according to the following:

#### Class I

Non-reproductive (No eggs in oviducts, ovaries contain small follicles  $\leq$  2.4 mm)

**Class II**

Reproductive (No eggs in oviducts, ovaries contain at least one yolky follicle, 2.5-7.0 mm).

**Class III**

Reproductive (No eggs in oviducts, ovaries contain at least one preovulating follicle in late vitellogenesis, 7.1-8.5 mm).

**Class IV**

Reproductive (animals contain corpora lutea in the ovaries and/or eggs in the oviducts).

Females in classes II, III and IV are considered reproductive, while females in class IV only were considered ovigerous.

**Lipido, Gonado and Hepato Somatic Indices (LSI, GSI and HSI).**

The weights of fat bodies, ovaries and livers (mg) were referred to the body weight (gm)  $\times 100$ , to give LSI, GSI and HSI.

**Methods**

Liver samples were weighed then homogenized in distilled water (1:10) using Elvehjem homogenizer, fitted with teflon pestle. Homogenation was undertaken at 4°C. Samples of liver homogenates were used for subsequent determinations.

**1. Total lipids**

Determinations were performed using the phosphovanillin method of Knight *et al.*<sup>[18]</sup>.

**2. Total proteins**

Determinations were undertaken using the method of Lowry *et al.*<sup>[19]</sup>.

**3. Total free amino acids**

Determinations were carried out using the method of Moore and Stein<sup>[20]</sup>.

**Statistical analysis**

Values are given as averages  $\pm$  standard error. Significance of the changes was tested using the student "t" test<sup>[21]</sup>.

**Results and Discussion**

Studies undertaken on reproduction in reptiles, have pointed out the importance of fat stores for reproductive activity. Female reptiles were shown to put fat into their eggs at the expense of their fat bodies<sup>[2,7,9,12]</sup>.

The study of the monthly variations in autopsied females with dissectable fat bodies revealed that a high percent occurred in the period March through August

(100%) (Table 1). This occurred when a high percent of non-reproductive females was observed. This observation suggests that fat bodies are being developed and loaded with lipids when ample food was available.

TABLE 1. Monthly variations in the percents of non-reproductive, reproductive, and ovigerous female *A. boskianus* and the percent of animals with dissectable fat bodies per each reproductive phase.

Month	Autopsied Females	Non-reproductive class I			Reproductive class II + III + IV			Ovigerous class IV		
		No.	%	% of animals with dissectable fat bodies	No.	%	% of females with dissectable fat bodies	No.	%	% of females with dissectable fat bodies
Jan.	5	1	14.29	100	4	57.14	0	3	42.86	0
Feb.	6	0	0	0	6	100	16.67	0	0	0
Mar.	4	1	25	100	3	75	67.	1	25.	0
Apr.	4	2	50	0	2	50	0	2	50	0
May	5	2	40	100	3	60	100	0	0	0
June	5	4	80	100	1	20	100	0	0	0
Jul.	5	3	60	100	2	40	50	2	40	50
Aug.	5	2	40	100	3	60	100	0	0	0
Sept.	6	0	0	0	6	100	33.33	3	50	33.33
Oct.	5	1	20	0	4	80	50	1	20	0
Nov.	6	3	50	33.33	3	50	33.33	1	16.67	0
Dec.	4	2	50	0	2	50	0	1	25	0

As animals became reproductive with follicle sizes ranging from 2.5 to 8.0 mm in classes II & III, consumption of fat bodies occurred. However the data recorded indicate a non-exhaustive consumption. The greatest decline in percent of females with dissectable fat bodies was recorded in winter animals (October-February) where replenishment of exhausted fat seems to be limited. Conversely, reproductive female *A. boskianus*, caught in the period March through September possessed a variable percent of animals with dissectable fat bodies, which tended to exhibit a value, higher than that recorded in winter animals. This strengthens our suggestion that, although reproducing females invariably consume lipid stores, however replenishment seems to take place during that period characterized by availability of food.

The heavy consumption of lipid stores becomes apparent when one considers the percent of ovigerous females (class IV) with dissectable fat bodies (Table 1). In these animals the percent was zero, except some irregular observations recorded in ovigerous females caught in July and August, which might be due to an early start of lipid replenishment after the reproductive cycle is over.

Observations made on autopsied females revealed that no yolky ova would be recorded in the presence of corpora lutea. When only corpora lutea degenerate, yolky follicles make their appearance, implying that a time lapse should separate each two consecutive clutches, while in the same time reproductive activity remains invariably continuous throughout the year.

It appears that the clutching profile noted in *A. boskianus* allows sufficient time for fat bodies to develop and suitable replenishment of lipids consumed with reproduction. Other reptiles where clutches are produced without time lapse between each two consecutive clutches, fat bodies do not seem to be important for reproductive activity<sup>[7]</sup>. These animals were indicated to exhibit little if any replenishment of fat between clutches. It appears that in such animals, lipids are transferred to ovaries, which will furnish the requirements necessary for each clutch. The several clutches produced in this case, apparently take place with yolky ova in the presence of corpora lutea.

Measurements of the indices of lipidosomatic, gonadosomatic and hepatosomatic in *A. boskianus* carried out in the present work have revealed that LSI declines as non-reproductive females pass to the reproductive phase (classes II, III and IV), assuming its minimal value in the ovigerous females. Apparently, lipids are mobilized both to liver and ovaries indicated by the increases recorded in HSI and GSI. However as animals become ovigerous, a sharp decline would occur in GSI while a moderate decline takes place in HSI (Fig. 1). These observations support the above-mentioned view that fat bodies are important for reproduction in *A. boskianus*.

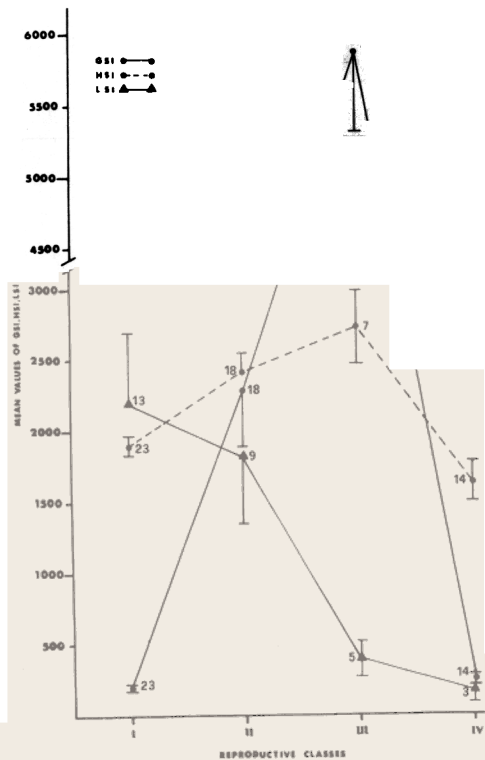


FIG. 1. Variations in the indices GSI (●—●), HSI (○- - -○) and LSI (▲—▲) in *A. Boskianus* during the four reproductive classes. Numbers are the sizes of observations.

The biochemical analyses of vitellogenic macromolecules and ultrastructural information as a function of season or estrogen stimulation are however meagre<sup>[23]</sup>. Since liver has a primary role in the synthesis and the supply of important vitellogenic substance<sup>[12,14,16]</sup>, examinations of the changes in lipids, proteins and total free amino acids in liver of *A. boskianus* were determined both seasonally as well as a function of the reproductive status.

The monthly records of hepatic lipid content (Fig. 2) revealed that liver exhibited three waves of high lipid content; January through April, July through October and November through January. When these changes are considered in the light of monthly variations in the percent of reproductive animals with dissectable fat bodies, one would observe that the high hepatic lipid content coincides with the relatively high percent of females with dissectable fat bodies. This relationship might indicate that as stated above, lipid stores are mobilized to liver beside ovaries. This strengthens the view of the role of liver in vitellogenesis and gonadal activity. Support of this view can be withdrawn when one considers the changes in hepatic lipid

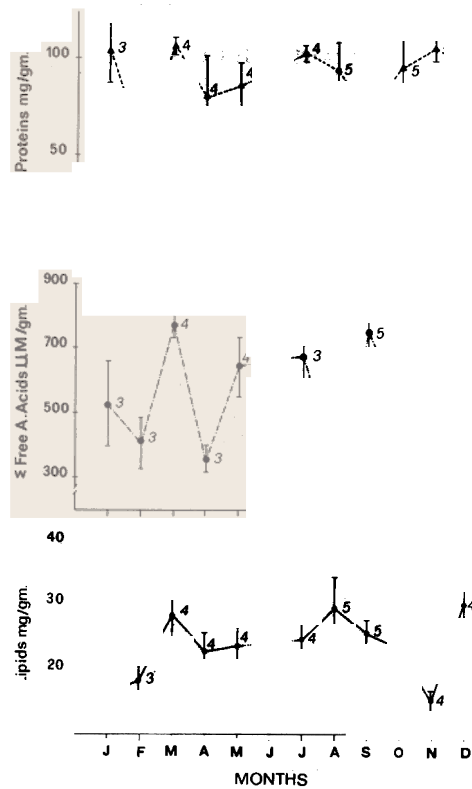


FIG. 2. Monthly variations in the levels of hepatic proteins (mg/gm) ▲- - -▲, lipids (mg/gm) ●- - -● and total free amino acids (μM/gm) ●- - -●. Vertical bars are S.E.M. Numbers are the sizes of observations.

content versus reproductive classes of *A. boskianus*. The data obtained (Fig. 3) demonstrate that a relatively high lipid content occurs in non-reproductive females (follicle size  $\leq 2.4$  mm). However as vitellogenesis proceeds a moderate decline occurs in hepatic lipid content, which exhibited a very low level as females become ovigerous.

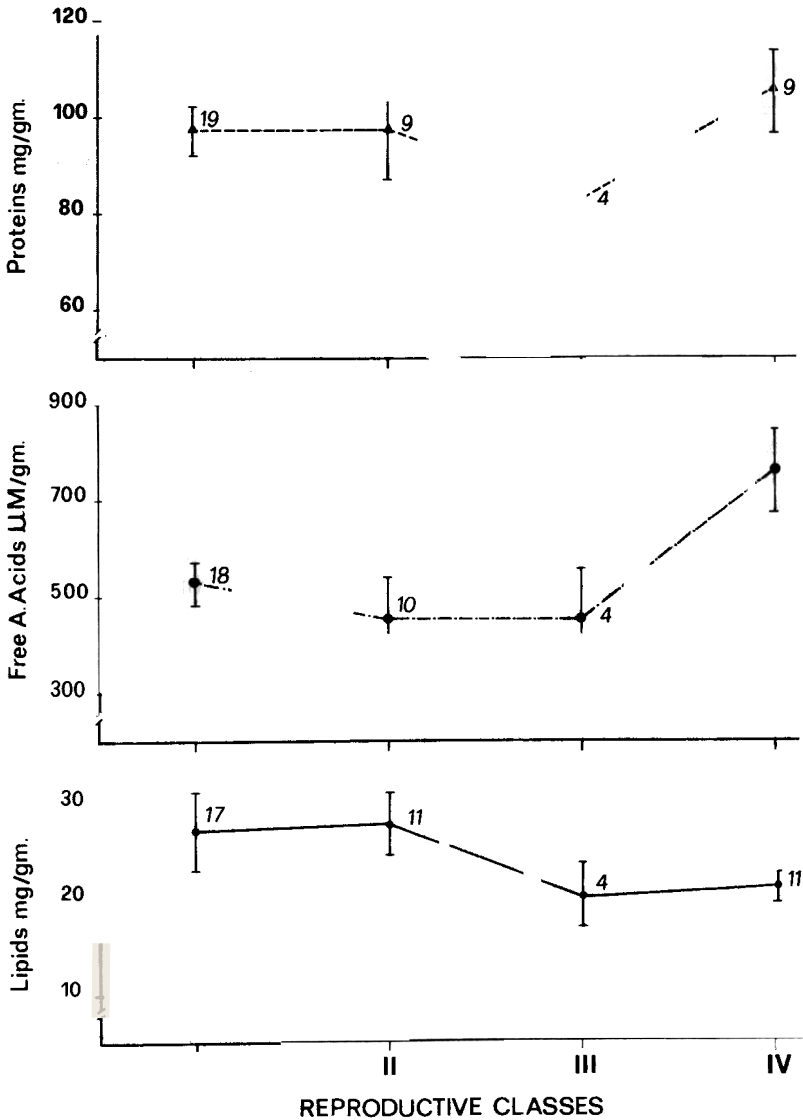


FIG. 3. Levels of hepatic proteins (▲-----▲), total free amino acids (●-----●) and total lipids (●-----●) versus different reproductive classes in *A. boskianus*. Vertical bars are S.E.M. Numbers are the sizes of observations.

It appears that fat mobilization to ovaries occurs either directly from fat stores or indirectly *via* liver, since good supply of lipids to vitellogenic ovaries is essential to meet the demands of the ovaries. It was demonstrated that lipid bodies, consisting of phospholipids develop a very complex structure and increase in amount with the growth of follicles. These phospholipids apparently are utilized for the construction of cellular lipoprotein membranes of cell organelles and various other metabolic activities concerned with maturation of follicular epithelium and oocyte<sup>[24-26]</sup>.

During vitellogenesis, the liver was found to exhibit a decline in its protein content as vitellogenesis proceeds (Fig. 3). Then, it increased when animals became ovigerous. On the other hand monthly fluctuations were recorded which gave peak values at March, July and November. On the other hand, while the total free amino acid content of liver exhibited monthly fluctuations (Fig. 2), it showed a significant decline during vitellogenesis (classes II, III). It then increased when animals were in the ovigerous stage (class IV). These results clearly indicate that liver is actively utilizing free amino acids for the synthesis of proteins important for vitellogenesis. However, it appears that liver once synthesizes such proteins would deliver it to the circulation, which explains why the hepatic protein content exhibited a decline during vitellogenesis. These changes are clearly indicative of adaptation of liver for providing various substances such as lipids, proteins and even amino acids to the growing follicle *via* the circulation. The follicular epithelium by specific uptake mechanisms (as well as through biosynthetic capacity) can maintain the nutritional requirements of the growing previtellogenic oocyte in the reptilian ovary.

In this connection we might refer to the pronounced increase which would occur in  $\alpha$ -globulin electrophoretic component after estrogen administration to *Sceloporus cyanogenys*<sup>[27]</sup> which might be interpreted as demonstrating vitellogenin induction. A plasma component was also noted in the serum of *Dipsosaurus dorsalis*<sup>[28]</sup>. It might be assumed in this respect that the liver in *A. boskianus* under the action of estrogen would synthesize vitellogenin and immediately convey it to the circulation. Vitellogenin was also demonstrated in different species of reptiles, e.g. *Thamnophis sauritus* and *T. elogaus*<sup>[29]</sup>, *Pseudemys scripta* and *Uta stansburiana*<sup>[30,31]</sup>.

It appears that vitellogenin reaches the growing follicles by means of the capillary network embedded within the thecal layer of the follicle<sup>[32]</sup>. It would then leave the capillaries and translocates to the oocyte surface<sup>[33]</sup>. It was reported that in amphibia, vitellogenin appears to be incorporated by an extensive micropinocytotic process<sup>[16,34,35]</sup>. The information in this connection in reptiles is however lacking and need an extensive work to clarify the process.

#### References

- [1] Dessauer, H.C., Seasonal changes in the gross organ composition of the lizard *Anolis carolinensis*, *J. Exp. Zool.* **128**: 1-12 (1955).
- [2] Minnich, J.E., Seasonal variation in weight/length relationship and fat body size in the desert iguana *Dipsosaurus dorsalis*, *Copeia*. 359-362 (1971).



- [3] Mayhew, W.W., Photoperiodic responses of female fringetoe lizards, *Science* **134**: 2104-2105 (1961).
- [4] Goldberg, S.R., Reproductive cycle of the ovoviparous iguanid lizard *Sceloporus jarrovi*, *Herpetologica* **27**: 123-131 (1971).
- [5] Goldberg, S.R., Seasonal weight and cytological changes in fat bodies and liver of the iguanized lizard *Sceloporus jarrovi*, *Copeia*. 227-232 (1972).
- [6] Cogger, H.G., Reproductive cycles, fat body cycles and sociosexual behaviour in the mallee dragon *Amphibolurus fordi* (Lacertilia: Agamidae), *Aust. J. Zool.* **26**: 653-672 (1978).
- [7] Hahn, W.E. and Tinkle, D.W., Fat body cycling and experimental evidence for its adaptive significance to ovarian follicle development in the lizard *Uta stansburiana*, *J. exp. Zool.* **158**: 79-85 (1965).
- [8] Chapman, B.M. and Chapman, R.F., Observations on the biology of the lizard *Agama agama* in Ghana, *Zool. Soc. Lon. Proc.* **143**: 121-132 (1963).
- [9] Smith, R.E., Experimental evidence for a gonadal-fat body relationship in two teiid lizards (*Ameiva festiva*, *Ameiva quadrilineata*), *Biol. Bull.* (Woods Hole) **134**: 325-331 (1968).
- [10] Marion, K.R. and Sexton, C.J., Reproductive cycle of the lizard *Sceloporus malachiticus* in Costa Rica, *Copeia*. 517-526 (1971).
- [11] Licht, D. and Gorman, G.C., Reproduction and fat cycles in Caribbean Anolis lizards, *Univ. Calif. Publ. Zool.* **95**: 1-52 (1970).
- [12] Telford, S.R., Seasonal fluctuations in liver and fat body weights of the Japanese lacertid *Takydromus tachydromoides* Schlegel, *Copeia*. 681-688 (1970).
- [13] Lin, E.J., Fat body and liver cycles in two tropical lizards *Chamaeleo hohneli* and *Chamaeleo jacksoni* (Reptilia, Lacertilia, Chamaeleontidae), *J. Herpet.* **13**(1): 113-117 (1979).
- [14] Wallac, R.A. and Jared, D.W., Studies on amphibian yolk. VII. The estrogen-induced hepatic synthesis of a serum lipophosphoprotein and its selective uptake by the ovary and transformation into yolk platelet proteins in *Xenopus laevis*, *Dev. Boil.* **19**: 488-526 (1969).
- [15] Wallac, R.A. and Bergink, E.W., Amphibian vitellogenin properties, hormonal regulation of hepatic synthesis and ovarian uptake and conversion to yolk proteins, *Am. Zool.* **14**: 1159-1175 (1974).
- [16] Holland, C.A. and Dumont, J.N., Oogenesis in *Xenopus laevis* (Daudin). IV. Effects of gonadotropin estrogen and starvation on endocytosis in developing oocytes, *Cell Tissue Res.* **162**: 177-184 (1975).
- [17] Dehlawi, G.Y., Female reproductive cycle in the lizard *Acanthodactylus boskianus*, (*In press*) (1986).
- [18] Knight, J.A., Anderson, S. and James, M.R., Chemical basis of the sulphophosphovanillin reaction for estimating total lipids, *J. Clin. Chem.* **18**(3): 199 (1972).
- [19] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J., Protein measurement with the Folin phenol reagent, *J. Biol. Chem.* **193**: 265-275 (1951).
- [20] Moore, S. and Stein, H.W., A new method for the determination of total free amino acids, *J. Biol. Chem.* **179**: 367 (1948).
- [21] Snedecor, G.W., *Statistical Methods*, 4th ed., The Iowa State College Press Amer., Iowa (1966).
- [22] Darevsky, I.S., Seasonal change of fat bodies and gonads in some lizards of the Arax River Valley in Armenia, *Zool. Zh.* **39**: 1209-1217 (1960).
- [23] Dessauer, H.C., Blood chemistry of reptiles: Physiological and evolutionary aspects, in: Gans, C. and Parsons, T.S. (eds.), *Biology of the Reptilia*, Vol. 3, Academic Press, New York, pp. 1-72 (1970).
- [24] Guraya, S.S., Further morphological and histochemical studies on the yolk nucleus and associated cell components in the developing oocyte of Indian wall lizard, *J. Morphol.* **124**: 283-293 (1968).
- [25] Guraya, S.S., Histochemical study of associated lipid changes in the follicular epithelium and oocyte of growing previtellogenic follicle in the ovaries of snakes, *Zool. Jb. Anat.* **96**: 183-192 (1976).
- [26] Guraya, S.S., Distribution, composition and functional significance of lipids in the follicular epithelium of growing oocyte in the snake *Lycodon aulicus* ovary. A histochemical study, *Acta Morphol. Neerl. Scand.* **15**: 287 (1977).
- [27] Suzuki, H.K. and Prosser, R.K. III, The effects of estradiol valerate upon the serum and bone of the lizard *Sceloporus cyanogenys*, *Proc. Soc. Exp. Biol. Med.* **127**: 4-7 (1968).
- [28] Gerstle, J.F. and Callard, I.P., Reproduction and estrogen-induced vitellogenesis in *Dipsosaurus dorsalis* (Comp.), *Biochem. Physiol.* **42A**: 791-801 (1972).

- [29] **Dessauer, H.C. and Fox, W.**, Changes in ovarian Follicle composition with plasma levels of snakes during estrus, *Am. J. Physiol.* **197**: 360-366 (1959).
- [30] **Urist, M.R. and Schjeide, O.A.**, The partition of calcium and protein in the blood of oviparous vertebrates during oestrus, *J. Gen. Physiol.* **44**: 743-756 (1961).
- [31] **Hahn, W.L.**, Estradiol-induced vitellogenesis and concomittant fat mobilization in the lizard *Uta stansburiana*, (Comp.), *Biochem. Physiol.* **23**: 83-93 (1967).
- [32] **Dumont, J.N.**, Oogenesis in *Xenopus laevis* (Daudin). I – Stage of oocyte development in laboratory maintained animals, *J. Morphol.* **136**: 153-179 (1972).
- [33] **Dumont, J.N.**, Oogenesis in *Xenopus laevis* (Daudin). VI. The route of injected tracer transport in the follicle and developing oocyte, *J. Exp. Zool.* **204**: 193-217 (1978).
- [34] **Wallac, R.A. and Dumont, J.N.**, The induced synthesis and transport of yolk protein and their accumulation by the oocyte in *Xenopus laevis*, *J. Cell. Physiol. Suppl.* **72**: 73-89 (1968).
- [35] **Brumett, A.R. and Dumont, J.N.**, Intracellular transport of vitellogenin in *Xenopus oocytes*: An autoradiographic study, *Dev. Biol.* **60**: 482-486 (1977).

## العلاقات التبادلية للأجسام الدهنية والكبد بالتكاثر في أنثى السحلية « أكانثوداكتيلاس بوسكياناس »

خميس عبد الرحيم و غازي يحيى دهلوي

قسم الأحياء ، كلية العلوم التطبيقية ، جامعة أم القرى ، مكة المكرمة ، المملكة العربية السعودية

أجريت الدراسات لقياس العلاقات بين النشاط التكاثري في السحلية أكانثوداكتيلاس بوسكياناس . ولقد دلت النتائج على أن أعلى نسبة إناث تحتوى على أجسام دهنية كبيرة وجدت في الإناث غير المتكاثرة . أما الإناث المتكاثرة (المراحل التكاثرية ٢، ٣) فقد اتضح بها درجات استهلاك الأجسام الدهنية بشكل متفاوت حيث كانت نسبة الإناث ذوات الأجسام الدهنية الكبيرة صغيرة في الشتاء وكبيرة في الصيف ، وقد أعزى ذلك إلى القدرة على تعويض الأجسام الدهنية ومحتواها وبصفة خاصة أثناء الصيف . ويتضح استهلاك الأجسام الدهنية عند دراسة الحيوانات التي تمر بمرحلة الإباضة حيث كانت نسبة الحيوانات ذوات الأجسام الدهنية متدنية (صفر/).

وبدراسة التغيرات في محتوى الكبد لكل من الدهون الكلية والأحماض الأمينية الكلية وكذلك البروتينات الكلية سواء شهرياً على مدار العام أو بالنسبة للمراحل التكاثرية المختلفة التي تمر بها الأنثى ، فقد وجد أن الدهون تستهلك أثناء عمليات تكوين المح (المراحل التكاثرية ٢، ٣) حيث ينخفض محتوى الكبد من الدهون . كما انخفضت محتويات الكبد لكل من الأحماض الأمينية والبروتينات أثناء تكوّن المح مما يعزز الاعتقاد باستهلاك الأحماض الأمينية سواء لإمداد المبيض بحاجاته أو تكوّن البروتين « فيتلوجين » وهو لازم لعمليات تكوين المح . ويدل انخفاض بروتينات الكبد على زيادة تصدير البروتينات أثناء تكوين المح والتي ينخفض معدلها في الحيوانات التي تمر بمرحلة الإباضة حيث يرتفع محتوى الكبد من البروتين والأحماض الأمينية ، توطئة لدورة جديدة .