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## OVARIAN CHANGES DURING THE REPRODUCTIVE CYCLE OF THE *TUPINAMBIS MERIANAE* LIZARD RAISED IN A TEMPERATE ENVIRONMENT

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**R E S U M E N.** — El ciclo reproductor de hembras de *Tupinambis merianae* criadas en ambiente templado consiste de tres fases gonadales: previtelogénica, vitelogénica y postovulatoria o lútea. Durante la fase previtelogénica, que ocupa gran parte del verano, el otoño e invierno, los ovarios son pequeños, pesan menos de 4 g y contienen solamente folículos no vitelogénicos, los cuales se desarrollan lentamente a partir de los nidos germinales, alcanzando unos 5 mm de diámetro. Debido a una maduración gonadal asincrónica entre ambos sexos, las cópulas acontecen cuando los ovarios son aún previtelogénicos. Luego sobreviene una corta e intensa vitelogénesis, durante la cual los folículos crecen uniformemente incrementando unas 6 veces su diámetro (cerca de 30 mm), mientras que la masa ovárica total alcanza unos 400 g. El proceso culmina entre los 15 y 20 días, con una ovulación generalizada y la subsiguiente oviposición. Como consecuencia, los ovarios postovulatorios aparecen reducidos a unos 5 g, con una superficie casi enteramente cubierta de cicatrices foliculares, las cuales pronto desarrollan cuerpos lúteos. La detención del ciclo ovárico, con atresia folicular previtelogénica masiva, como resultado del aislamiento de las hembras, permite concluir sobre la existencia de estímulos sexuales promotores de la vitelogénesis.

Palabras claves: *Tupinambis*, ciclo reproductivo, fases ováricas, vitelogénesis, estímulos sexuales.

**A B S T R A C T.** — The reproductive cycle of *Tupinambis merianae* females raised in a temperate environment consists of three gonadal phases: previtellogenetic, vitellogenic and postovulatory or luteal phases. During the previtellogenetic phase, which takes great part of the summer, autumn and winter, the ovaries are small, weigh less than 4 g, and contain only non-vitellogenetic follicles, which develop slowly from germinal beds, until they are almost 5 mm diameter. Due to an asynchronous gonadal maturation in both sexes, matings occur when ovaries are still previtellogenetic. Then comes a short and intense vitellogenesis, during which follicles grow steadily increasing around 6 times their diameter (close to 30 mm), while the total ovarian mass reaches almost 400 g. The process culminates between the 15<sup>th</sup> and 20<sup>th</sup> day, with a widespread ovulation and a subsequent oviposition. As a consequence, postovulatory ovaries appear reduced to 5 g approximately, with a surface almost entirely covered with follicular scars, which soon developed luteal bodies. Blockade of the ovarian cycle, with a massive previtellogenetic follicular atresia, as a result of females isolation leads to conclude on the existence of sexual stimuli promoting vitellogenesis.

Keywords: *Tupinambis*, reproductive cycle, ovarian stages, vitellogenesis, sexual stimuli.

### INTRODUCTION

The genus *Tupinambis* (Squamata: Teiidae) includes a group of large carnivorous lizards which are found in the South American plain (Presch, 1973).

Historically, *Tupinambis merianae* and *Tupinambis rufescens*, representing the most southern populations of the group (Ceï and Scolaro, 1982), constituted a

valuable resource for the indigenous communities, who took advantage of their leather, meat and fat (Donadío and Gallardo, 1984; Norman, 1987). However, in the last decades they began to be threatened by an intense and sustained traffic of their leather, causing international concern and leading to the inclusion of both species in the Appendix II of CITES.

More recently, implementation of captive breeding programs (Mercolli and Yanosky, 1990; Noriega *et al.*, 1996), and a better understanding of their biology, have encouraged a rational use of these saurians, with good prospects for their preservation and cultural reevaluation.

In correspondence with a strong seasonal activity, the reproductive cycle of *Tupinambis* in subtropical and temperate climates involves spring mating, a single annual egg laying and a relatively large egg clutch (Donadío and Gallardo, 1984; Mercolli and Yanosky, 1990; Noriega *et al.*, 1996).

It is clear that the group reproductive strategies and their environmental determinants can be more deeply understood from a detailed study of their gonadal cycle. Concerning this, basic aspects of the gonadal cycle and gametogenesis are adequately described in males (Paz *et al.*, 1993; Noriega *et al.*, 2002), although the opposite seems true for the female sex. The available information only consists of a superficial description of the female gonad in *Tupinambis merianae* (formerly *T. teguixin*) (Yanosky and Mercolli, 1991) and the ovarian cycle of *Tupinambis rufescens* (Fitzgerald *et al.*, 1993).

The present work describes the main ovarian changes during the reproductive cycle of *Tupinambis merianae* raised in a temperate environment. In addition, it includes experimental evidence on the existence of sexual stimuli that promote vitellogenesis.

## MATERIALS AND METHODS

This study was carried out in a *Tupinambis merianae* population adapted to captivity ("El Gringo" hatchery) in Sa Pereira, province of Santa Fe (Argentina). Thermal and hydric regimes of the region have been previously described (Noriega *et al.*, 2002).

The reproductive stock, with a male-to-female ratio of 1:5, and a surface per individual of 2 m<sup>2</sup>, was put in outdoors enclosures fenced with masonry walls 1.2 m high, supplied with sunshade and shelters. The *ad libitum* feeding mostly consisted of bovine meat supplemented with eggs and in-season fruits.

The present study was carried out with 19 females with 32 cm snout-vent length (SVL) —or larger— because females of this size are known to be fertile (Yanosky and Mercolli, 1992; and unpublished observations). Eleven of them were killed at definite stages of the reproductive cycle (Noriega *et al.*, 1996) to study main anatomical and histological ovarian changes (Table 1): *Hibernation* (June, n = 2); *Hibernation emergency* (September, n = 4); *Post-mating* (October, n = 2; females showing external anatomical changes, Noriega *et al.*, 1996); *Post-ovulation* (November, n = 2; females showing oviducal eggs); *Post-eclosion* (February, n = 2). The seven remaining were reproductively isolated, to see whether external anatomical changes concomitant to vitellogenesis (Noriega *et al.*, 1996), take place under sexual isolation. From these, an individual selected at random, was killed for a detailed anatomical and histological gonad examination.

Immediately after the animals were killed, both ovaries were removed, fixed in Duboscq-Brazil solution for 48 hours, and then kept in 80° alcohol. For each sample, gonadal weight was determined to the nearest 1 mg. Besides, diameter of the 10 biggest follicles was measured in each ovary. Previtellogenic follicles (less than 5 mm diameter) were mea-

sured with an ocular micrometer to the nearest 0.01 mm, and vitellogenic follicles (around 27 mm diameter) were measured with a vernier caliper to the nearest 0.1 mm accuracy. Alterations caused by fixation were disesteemed on the presumption of a similar effect on all fixed material.

For histological studies, ovarian portions of each sample were prepared using the common paraffin embedding techniques. Yolk-free samples were dehydrated with 96% alcohol, butanol and benzene. In yolked samples, a bath in 1% celloidin dissolved in methyl salicylate was inserted between the butanol and benzene steps. Material sectioned at 7  $\mu$ m was stained with haematoxylin-eosin, Heidenhain's azocarmine anilin (Azan) and van Gieson.

## RESULTS

**I. Gonadal Changes During Reproductive Cycle.**— Left and right ovaries showed weight differences lower than 20%, and similar size follicles, evidencing a synchronous behavior throughout the reproductive cycle. Three gonadal phases could be distinguished based on the follicular morphology: previtellogenic, vitellogenic and postovulatory or luteal phases (Table 1).

*Previtellogenic Phase.*— It was the longest stage, and lasted at least 8 months.

It took most of the summer, autumn and winter. Ovaries were small, with a total weight lower than 4 g. Follicles grew slowly from the germinal bed until they were almost 5 mm of diameter (Fig. 1A).

The germinal bed, a persistent structure at the dorsal ovarian surface, was composed of a thick cortical epithelium containing oogonias and an underlying medullar area in which young follicles were released (Fig. 2A).

The smallest ones exhibited a single layer of cuboidal follicular cells (granulosa) which became stratified and polymorphic as they developed (Fig. 2B). Pyriform cells, the largest granulosa cells, exhibited a voluminous nucleus and a long thin apex contacting the oocyte vitelline envelope (Fig. 2B). These cells appeared earlier at the vegetal pole of the oocyte. A thin and vascular theca completed the follicular wall.

During this phase, the oocyte cytoplasm developed a dense fibrillar mesh lacking yolk inclusions (Fig. 2B).

*Vitellogenic Phase.*— At the end of hibernation and after a short period of adaptation, lizards began sexual activity, which culminated with matings (mainly in October) (see also, Noriega *et al.*, 1996).

After 15 to 20 days following mating, females showed a remarkable abdominal expansion and behavior changes, which preceded imminent ovulation and subsequent oviposition. These modifica-

Reproductive cycle period	Follicular phase	Average follicular diameter (mm) (a)	Average gonadal weight (both ovaries) (g)
Post-eclosion (February) (n=2)	Previtellogenesis	2.69 $\pm$ 0.27	2.12
Hibernation (June) (n=2)		3.02 $\pm$ 0.71	2.92
Hibernation emergency (September) (n=4)		4.52 $\pm$ 0.49	3.86
Post-mating (October) (n=2)	Vitellogenesis	26.9 $\pm$ 0.88	390.8 (b)
Post-ovulation (November) (n=2)	Luteal	1.28 $\pm$ 0.31	5.39

**Table 1.** *Tupinambis merianae* ovarian phases during reproductive cycle in a temperate environment. (a) Corresponding to the ten biggest follicles of each ovary. (b) Value calculated from the average follicular weight and an estimated population of 40 mature follicles.

tions observed systematically in hatchery, were related to a rapid follicular growth (Noriega *et al.*, 1996). Near ovulation, steadily developed follicles had increased approximately 6 times their diameter, and the total ovarian mass had reached nearly 400 g (Fig. 1B; Table 1). There were also groups of small white follicles, possibly previtellogenic ones (Fig. 1B). This remarkable growth resulted from an intense vitellogenic process, which heavily filled the oocyte cytoplasm with yolk granules. These were large and polyhedral, except for the peripheral region where they were more rounded and considerably smaller (Fig. 2C). Meanwhile, the granulosa became a single cubic cell layer and the thickened theca showed two layers: an inner vascularized and cellular layer and an outer fibrous layer.

*Postovulatory or Luteal Phase.*— Due to the oviparous condition of this lizard, ovulation would have occurred shortly before oviposition. Since it involved all mature follicles, the ovaries were drastically reduced to a total weight of some 5 g and appeared almost entirely covered by depressions corresponding to the follicular dehiscence sites (Fig. 1C). Groups of previtellogenic follicles remained at this stage (Fig. 1C).

Soon, the follicular scars became compact luteal bodies constituted by a thick cellular stratum (Fig. 2D). The granulosa cells filling the follicular cavity, became hypertrophied forming luteinic cells. The theca expanded into the luteal mass forming connective septae. The two thecal layers were separated by ample vascular sinuses.

**II. Vitellogenesis Suppression By Sexual Isolation.**— Close temporary relationship between mating and vitellogenesis suggested that certain sexual stimuli might induce the vitellogenic process. To evaluate this possibility, a group of fertile females ( $n = 7$ ) was deprived of sexual interactions by isolat-

ing them from the reproductive stock before their hibernation emergency.

Even a month after egg laying in the reproductive stock had concluded no isolated female exhibited abdominal expansion indicative of a vitellogenic process nor had oviposited.

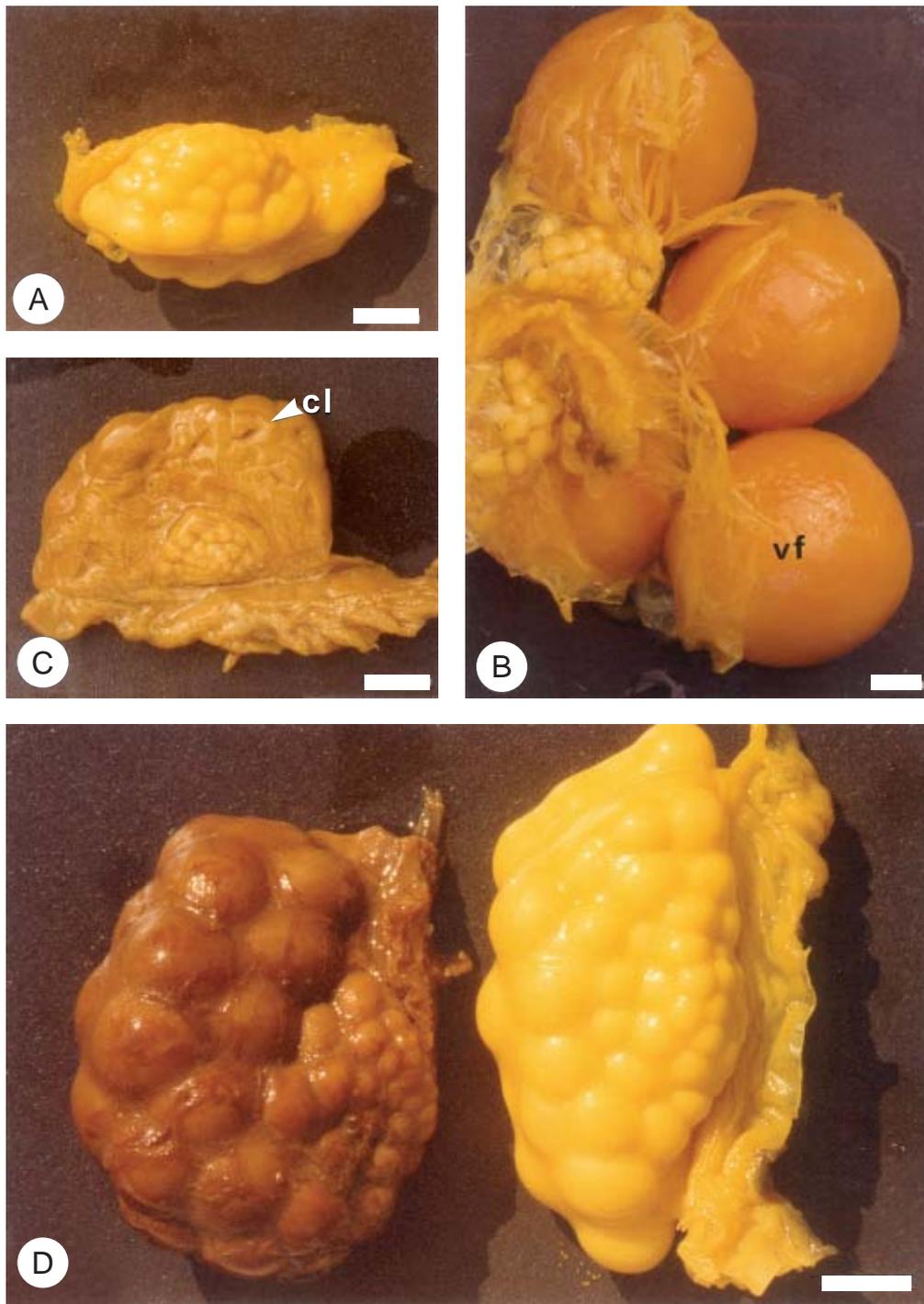
Ovaries in an individual selected at random from the sexually isolated females had a pigmentation darker than usual (Fig. 1D, left). They only contained atretic previtellogenic follicles with a thickened and entirely fibrous theca, hypertrophic and vacuolated granulosa cells, a discontinuous viteline membrane, and a disorganized oocyte cytoplasm invaded by presumably phagocytic cells (Fig. 2E).

## DISCUSSION

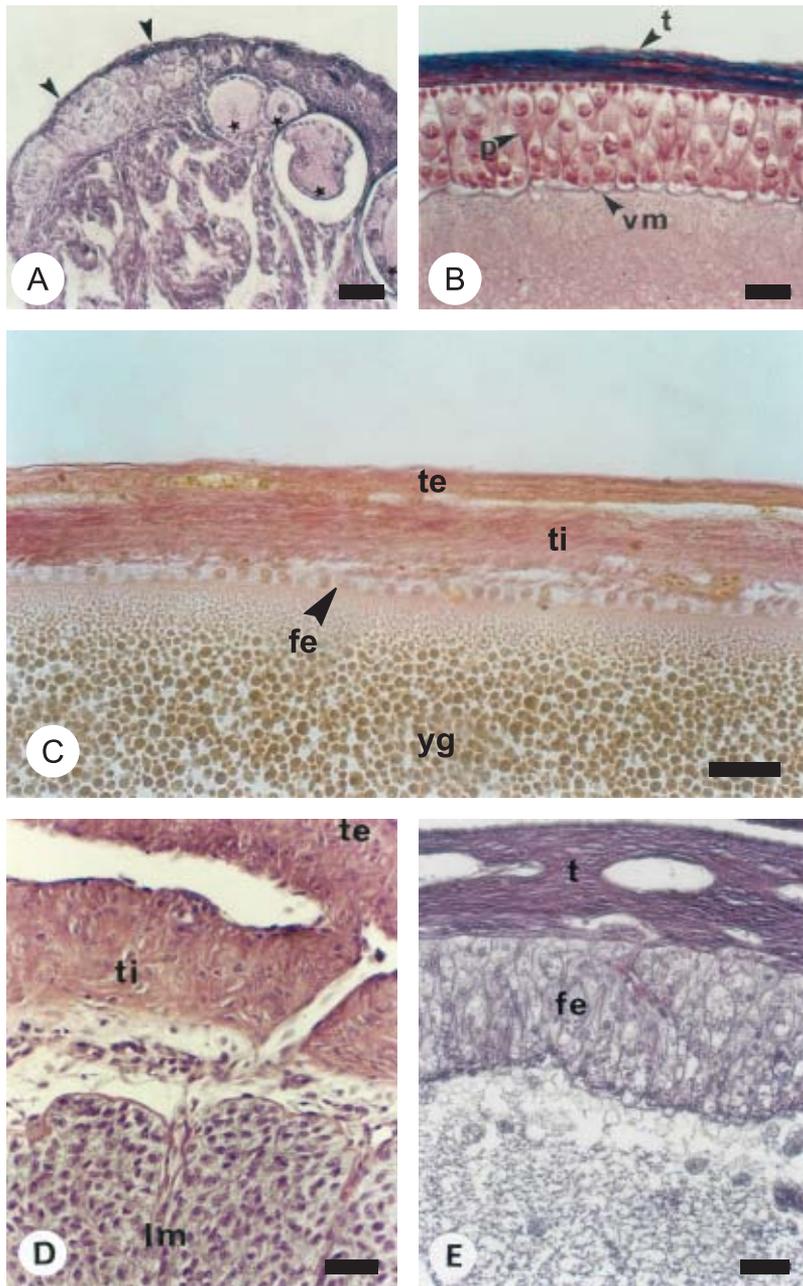
Seasonal activity of *Tupinambis merianae* in temperate and subtropical environments, with an hibernation period of nearly 6 months (Donadío and Gallardo, 1984; Mercolli and Yanosky, 1990; Noriega *et al.*, 1996), circumscribes the main reproductive functions (mating, egg laying, incubation and birth) to the spring and beginning summer.

Even more, male and female gonadal development appears to be asynchronous because at mating, the still immature female gonad (Noriega *et al.*, 1996 and present results) only contains previtellogenic follicles, with the oocyte surrounded by a multilayered granulosa and a cytoplasm filled with a dense fibrillar mesh (Varma, 1970; Uribe *et al.*, 1995; 1996; El Mouden *et al.*, 2001).

Abundance of dissociated gonadal cycles in reptiles, as that observed in *Tupinambis merianae*, was attributed to different gamete maturation requirements in both sexes (Saint Girons, 1984; 1985). Such a situation seems invariably associated to the presence of sperm storage devices in female genital ducts of various species (Cuellar, 1966; Smyth and Smith, 1968; Jones *et al.* 1997).



**Fig. 1A.** Previtellogenic ovary (September) showing follicles arranged by size. **1B.** Portion of a preovulatory ovary (October) showing four vitellogenic follicles (vf) and two groups of possibly previtellogenic follicles. **1C.** Postovulatory ovary (November) showing luteal bodies (cl) and a group of previtellogenic follicles. **1D.** Comparison between an ovary of a sexually isolated female (December) (left) and a normal previtellogenic ovary (September) (right). Notice darker pigmentation of the blocked ovary. Bars: 5 mm.



**Fig. 2A.** Germinal bed section in a postovulatory ovary (November). A thick germinal epithelium (arrow heads) containing oogonias is observed. In the underlying stroma, follicles surrounded by a single cell layer are found (stars). H-E. Bar: 80  $\mu$ m. **2B.** Previtellogenic follicle (September). Notice a dense fibrillar mesh in the oocyte cytoplasm. Pyriform cells (p) exhibit thin apices which seemingly contact the vitelline membrane (vm). Theca (t). Azan. Bar: 35  $\mu$ m. **2C.** Advanced vitellogenic follicle (October). Theca externa (te); theca interna (ti); follicular epithelium (fe); yolk granules (yg). van Gieson. Bar: 45  $\mu$ m. **2D.** Luteal body (November). Theca externa (te); theca interna (ti); luteal mass (lm). H-E. Bar: 35  $\mu$ m. **2E.** Atretic follicle (December). Theca (t); follicular epithelium (fe). H-E. Bar: 45  $\mu$ m.

An intense vitellogenic process, whose beginning appears closely related to the mating period, greatly expands the oocyte cytoplasm due to the accumulation of yolk granules, while reducing the granulosa to a single cell layer (Varma, 1970; Uribe *et al.*, 1995; 1996; El Mouden *et al.*, 2001).

Its proximity to hibernation (Noriega *et al.*, 1996) suggests an almost exclusive dependence on available body reserves. This supposition is reinforced by a close correspondence between gonadal cycle and fat bodies volume in *Tupinambis rufescens* (Fitzgerald *et al.*, 1993).

On the other hand, the remarkable brevity and uniformity of vitellogenesis, between 15 to 20 days (Noriega *et al.*, 1996 and present results), could be related to a particularly suitable nutritional state in captivity. Therefore, a bigger variability of the process under natural conditions could be expected if there exist complementary feeding demands.

It could also be assumed that post-uptial sequence of vitellogenesis found in *Tupinambis merianae* represents a particularly convenient mode for the oviparous reproduction: first, it avoids the need of a strict sexual coordination on account of the limited female gamete viability; and secondly, it assures the success of plastic and energy resources use through an anticipated sperm availability. Furthermore, ovarian cycle blockade as a consequence of sexual isolation, as indicated by the occurrence of females lacking abdominal expansion and oviposition, suggests the existence of signals promoting vitellogenesis. This possibility seems to be confirmed by the occurrence of a generalized previtellogenic follicular atresia in the ovaries of a sexually isolated individual, exhibiting the main features of the process (Saidapur, 1978).

Other examples in the literature (Mendoça and Crews, 1990; Shanbhag, 2003) would show that sexual regulation of gonadal recrudescence is a common

phenomenon in reptiles reproduction. As for the control mechanism, Mendoça and Crews (1990) have proposed the intervention of a neuroendocrine reflex, caused by the perception of a tactile stimulus occurred during the copulation.

With regard to luteal bodies formed in *Tupinambis merianae*, a typically oviparous lizard, they seem to be short-life structures formed by a rapid follicular wall shrinkage and later differentiation of endocrine (Saidapur, 1982). As in other oviparous species (Saidapur, 1982; Fox and Guillete, 1987), luteal bodies possibly control oviductal transport and egg shelling —via progesterone secretion—.

Present results suggest that *Tupinambis merianae* oviduct might play a central role in synchronizing gamete development and fertilization, acting as a vitellogenic signal receiver, storing sperm and possibly activating it in response to the ovulatory process. However, additional studies are required for a more detailed account of the species follicular development and control mechanisms involved.

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