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CALCIUM ATPASE LOCALIZATION IN THE UTERUS OF TWO SPECIES OF *PSEUDEMOIA* (LACERTILIA: SCINCIDAE) WITH COMPLEX PLACENTAE

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Abstract.—Loss of the eggshell in viviparous species represents the loss of a source of calcium for developing embryos. Calcium is a major requirement for developing embryos, raising the question of how calcium is transferred to the developing embryo in viviparous species. We characterized the calcium transport mechanism of viviparous lizards with complex placentae using indirect immunofluorescence to identify Ca^{2+}ATPase pumps in the uterus of two closely related species of skinks, *Pseudemoia spenceri* and *Pseudemoia entrecasteauxii*, throughout pregnancy. Although *Pseudemoia entrecasteauxii* is significantly more placentotrophic than *P. spenceri*, localization of Ca^{2+}ATPase pumps is broadly similar in both species. Shell glands are present in both species during vitellogenesis and early pregnancy; but they do not stain for Ca^{2+} ATPase pumps. From mid to late pregnancy, apical and basolateral immunofluorescent staining of Ca^{2+} ATPase pumps are present in the uterine epithelium in both the chorioallantoic (embryonic pole) and omphaloplacental (abembryonic pole) regions in both species. The glandular epithelial cells (shell glands) also stain in the uterus adjacent to the omphaloplacenta of *P. spenceri* from mid to late pregnancy but only during late pregnancy in *P. entrecasteauxii.* This prolonged expression of Ca^{2+}ATPase pumps throughout pregnancy may provide a means to supply calcium to the embryo when the demand is greatest.

Key Words.—Calcium ATPase; evolution; placentotrophy; *Pseudemoia entrecasteauxii*; *Pseudemoia spenceri*; viviparity.

INTRODUCTION

Although viviparity has evolved in many families of squamate reptiles (Blackburn 2000), complex placentae have only evolved in the lizard family Scincidae (Blackburn 1993; Stewart and Thompson 2000). Viviparous squamates display a range of placental complexities ranging from simple (lecithotrophic), where the embryo gets most of the nutrients required for development from the egg yolk, to complex (predominantly placentotrophic), where the size of the ovulated egg and yolk is reduced, resulting in the majority of nutrient uptake required by the embryo coming from the mother across a placenta (Blackburn et al. 1984; Blackburn 2000; Stewart and Thompson 2000; Thompson et al. 2000).

Reduction or elimination of the eggshell is among the major anatomical and physiological changes that must accompany the evolutionary transition from oviparity (egg laying) to viviparity (live birth; Thompson and Speake 2004, 2006). Developing embryos of oviparous species obtain a large proportion of their required calcium from the eggshell (Stewart et al. 2009b; Thompson et al. 2000) implying that there is insufficient calcium in the egg-yolk of reptiles to sustain development. Loss of the eggshell in viviparous species thus represents the loss of a source of calcium for the developing embryo, which raises the question of how calcium is transferred to the developing embryo in viviparous species, especially those with reduced yolks.

The uterus of reptiles is where egg-shelling occurs in oviparous species and where developing embryos are nourished and housed in viviparous species (Girling 2002). The uterus consists of three main tissue layers, the inner layer (endometrium), which includes the uterine epithelium that lines the uterine lumen, the stromal layer (lamina propria) that contains connective tissue, blood vessels and any shell glands, and the outer myometrium (longitudinal and circular muscle; Blackburn 1998; Girling 2002). The two major placental organs associated with maternal-embryonic exchanges in viviparous squamates are the chorioallantoic placenta, which forms at the embryonic pole and is the main respiratory organ, and the omphaloplacenta (yolk sac placenta), which occurs at the abembryonic pole and is involved with nutrient exchange (Blackburn 1993; Stewart and Blackburn 1988; Adams et al. 2005; Thompson et al. 2006).

Ca^{2+} ATPase pumps transport Ca^{2+} ions out of cells (Borke et al. 1989; Bar 2009) and are of major importance in the uterus of oviparous amniotes (e.g. birds and reptiles) during egg-shelling (Pike and Alvarado 1975; Watanabe et al. 1989; Herbert et al. 2006; Thompson et al. 2007; Parker et al. 2008; Bar 2009) for transporting a large quantity of calcium in a very short period of time. As part of our ongoing studies on the evolution of viviparity (Thompson et al. 2002), we

proposed a model for the evolution of calcium transport to the embryo during the evolution of viviparity, where there is a shift from transport across the shell glands in oviparous lizards, to a combination of shell glands and uterine epithelium in viviparous species with intermediate placental complexities (Herbert et al. 2006). At that time, we had few details of the distribution of Ca^{2+} ATPase pumps in the uterus of species with complex placentae throughout reproduction. The aim of this study was to test our model by describing the presence and distribution of Ca^{2+} pumps in the uterus of lizards with complex placentae and significant placentotrophy during pregnancy. We studied two species of skinks of the Australian genus *Pseudemoia* (*P. entrecasteauxii* and *P. spenceri*) because both are placentotrophic (Stewart and Thompson 1993, 1998; Thompson and Stewart 1994; Thompson et al. 1999a, b), although *Pseudemoia entrecasteauxii* is more placentotrophic than *P. spenceri* (Thompson et al. 2000). The genus *Pseudemoia* is part of the *Eugongylus* group of skinks, which forms the main focus of our studies.

MATERIALS AND METHODS

Animals and tissue harvest.—We collected gravid female *Pseudemoia spenceri* (n = 20) and *P. entrecasteauxii* (n = 22) from Kanangra Boyd National Park (33° 59' S, 150° 03' E) in eastern Australia in October 2005 to January 2006. They were returned to University of Sydney where they were housed until they reached the appropriate reproductive stage. We euthanized individual females by IP injection of sodium pentobarbitone (Nembutal, Merial) at different stages of pregnancy to give representative samples when they were vitellogenic, during early (stage 0–30), mid (stage 31–35) and late pregnancy (stage 36–40) and after parturition (two days and two weeks). We dissected both uteri from the females through a central ventral incision in the body (Girling 2002) and we removed ovulated eggs/embryos, leaving the uterus intact. We noted the condition of uterus, position and stage of eggs/embryos (non-reproductive, vitellogenic, newly ovulated and embryonic stage; based on Dufaure and Hubert 1961). When appropriate (after stage 30), we further separated the uterus into two regions, adjacent to the chorioallantoic placenta and adjacent to the omphaloplacenta. Tissue was laid flat onto a piece of wax, immediately covered in O.C.T. mounting medium (Tissue Tek, Torrance, California U.S.A) and placed into super-cooled isopentane (BDH Laboratory Supplies, Leeds, England) for at least 30 seconds. We removed the wax and stored the tissue in liquid nitrogen.

Tissue processing.—We determined the expression of calcium pump antigens in uterine tissue using indirect immunofluorescent microscopy. We cut sections (8 µm thick) using a Leica CM3050 cryostat (Leica, Heerbrugg, Switzerland) at –25° C, immediately transferred sections onto gelatin chrom alum coated glass slides, air dried them at room temperature for 1 h, and then fixed them for approximately 12 h in –20° C acetone. We randomly designated slides as experimental or controls. We air dried sections at room temperature and incubated them in a blocking solution of Fetal Calf Serum (FCS; 5%) in phosphate-buffered saline (PBS) prior to incubation with the primary antibody. This solution was also used for dilution of all primary and secondary antibodies. The monoclonal Ca^{2+} ATPase antibody (Clone 5F10. No. A7952. Sigma, St. Louis Missouri, USA) we used was raised in mice against human erythrocyte Ca^{2+} ATPase pumps (Borke et al. 1989) at a concentration of 6 µg/ml for 2 h at room temperature. After washing in PBS, we incubated sections in limited light for 30 min in a FITC-conjugated rabbit anti-mouse secondary antibody (Zymed, San Francisco, California, USA) at a concentration of 15 µg/ml. We again washed sections, mounted them in vectorshield mounting medium containing DAPI (Vector Laboratories, Burlingame, California, USA) and put on a cover slip. We ran negative controls concurrently with all experiments and we treated them exactly the same as experimental sections, but we replaced the primary antibody with FCS/PBS.All sections were viewed using a Diaplan microscope (Leitz, Wetzlar, Germany) set up for fluorescence microscopy. We captured digital images using a Leica DM 200 digital camera (Leica, Heerbrugg, Switzerland) and processed images with Adobe Photoshop 7.0 (Adobe Systems, San Jose, California, USA). The magnification of the images was calculated using a micrometer that was photographed with the sections.

RESULTS

Vitellogenic *Pseudemoia spenceri* females have shell glands in the lamina propria (stroma) of the uterus, but there is no Ca^{2+} ATPase staining in either the glandular epithelial cells (shell glands) or the uterine epithelium (Fig. 1A). In early pregnancy, there is basolateral staining for Ca^{2+} ATPase of the uterine epithelial cells and apical staining in the shell glands, which are small in size (Fig. 1B). Once the embryo has reached stage 35, the two placental regions have completely differentiated and the uterus in these regions has responded differently. The uterine epithelium adjacent to the chorioallantois shows some basolateral immunofluourescent staining and an increase in vascular density, but no shell glands are present (Fig. 1C). There are large shell glands in the uterus adjacent to the omphaloplacenta, with the glandular epithelium showing intense apical staining for Ca^{2+} ATPase, while the uterine epithelium stains basolaterally (Fig. 1D). This pattern of staining for Ca^{2+}

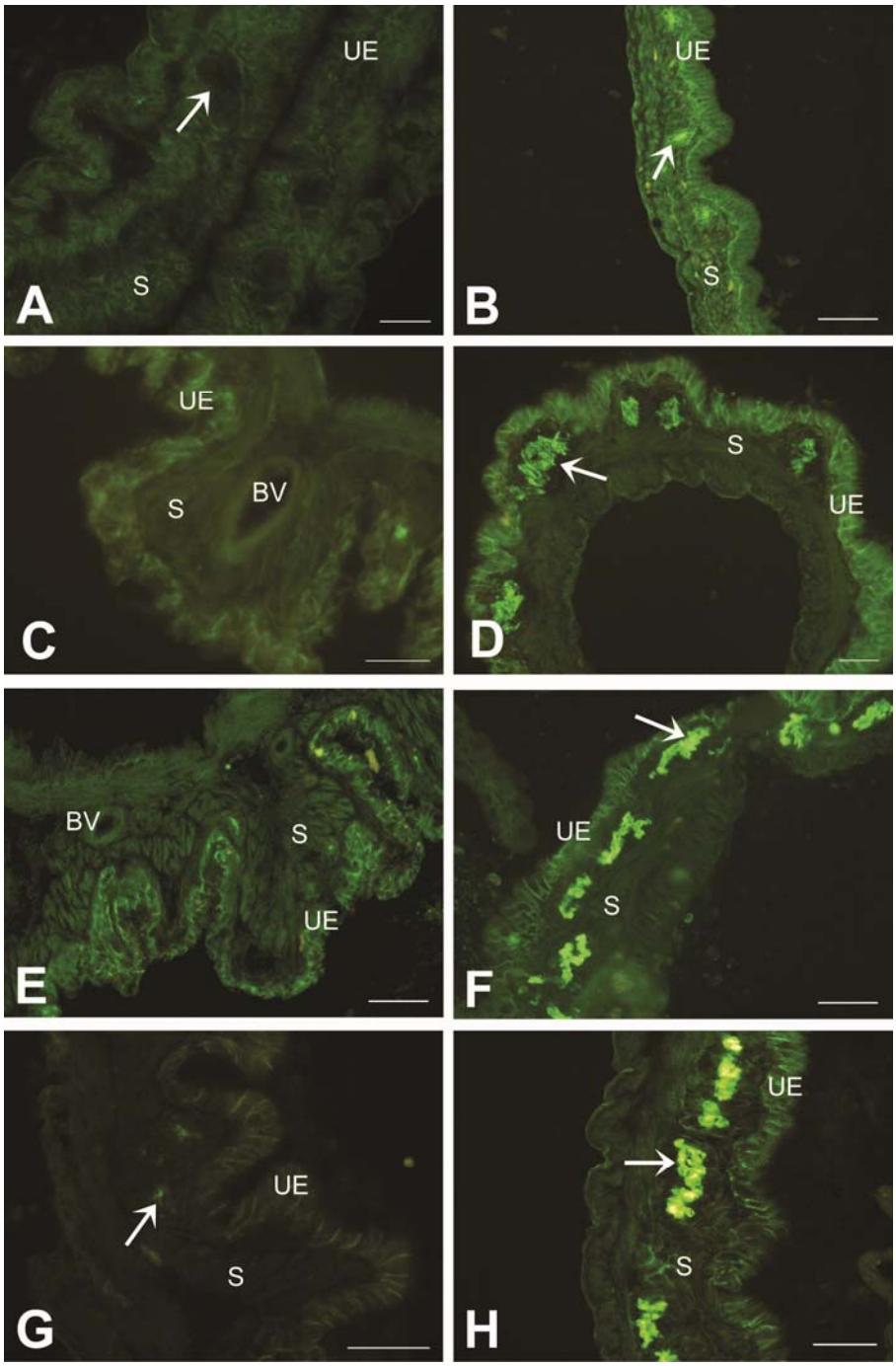

FIGURE 1. Immunofluorescent images of Ca^{2+}ATPase staining in uterine tissue of *Pseudemoia spenceri* (A-F) and *P. entrecasteauxii* (G-H). A. Uterus from *Pseudemoia spenceri* during vitellogenesis demonstrating no immunofluorescent staining in the shell glands (arrow) within the lamina propria (stroma, S) or in the uterine epithelium (UE). B. Uterus of *Pseudemoia spenceri* during early pregnancy (Stage 27) showing apical staining in the glandular epithelium of small shell glands (arrow) and basolateral staining in the uterine epithelial cells (UE). C–D and E–F. Uterus of *P. spenceri* during mid pregnancy (Stage 35) and late pregnancy (Stage 40), respectively. C and E. Uterus adjacent to the chorioallantois showing basolateral staining in the uterine epithelial (UE) cells and large blood vessels (BV). D and F. Uterus adjacent to the omphaloplacenta showing intense staining of the large shell glands (arrow) in the stroma (S) and basolateral staining of the uterine epithelial cells (UE). G. Uterus adjacent to the omphaloplacenta of *P. entrecasteauxii* during mid pregnancy (Stage 34) showing some basolateral staining of the uterine epithelium (UE) and a little immunofluorescent staining in the shell glands (arrow). H. Uterus adjacent to the omphaloplacenta of *P. entrecasteauxii* during late pregnancy (Stage 36) showing basolateral staining of the uterine epithelium (UE) and intense staining of the large shell glands (arrow) in the stroma (S). Scale bar represents 50 μm.

ATPase remains consistent in both regions from stage 35 to 40, just before birth (Figs 1D, 1E). Two days after parturition, the uterine epithelium shows slight basolateral staining for Ca^{2+} ATPase and glandular epithelial cells are still staining apically in a few small glands. However, neither the uterine or glandular epithelial cells are fluorescing for Ca^{2+} ATPase two weeks after parturition.

Pseudemoia entrecasteauxii is similar to *P. spenceri* for females that have recently ovulated eggs through to mid-pregnancy (Fig. 2). Small glands occur in the stoma, but there is no glandular epithelial staining for Ca^{2+} ATPase. There is, however, light basolateral staining for Ca^{2+} ATPase in the uterine epithelium. By mid-pregnancy (stage 32–34), the uterus has differentiated and, like *P. spenceri*, the uterus adjacent to the chorioallantois contains an increased number of blood vessels, with uterine epithelial cells staining basolaterally for Ca^{2+} ATPase and an absence of shell glands. The uterus adjacent to the chorioallantois remains the same until birth. The uterus adjacent to the omphaloplacenta contains shell glands in the stroma, but these glandular epithelial cells lack much immunofluorescent staining during mid-pregnancy (Fig. 1G). From stage 36 onwards, the shell glands become large with intense apical staining in the glandular epithelium for Ca^{2+} ATPase, as it does in *P. spenceri*. Similar basolateral staining occurs in the uterine epithelial cells (Fig. 1H). Two days after parturition, enlarged shell glands with intense apical staining in the glandular epithelium are still present in the stroma, but the number of glands is reduced. By two weeks after parturition, remaining glands are small and have little staining for Ca^{2+} ATPase. The uterine epithelium shows slight basolateral staining for Ca^{2+} ATPase two days after parturition, but this is lost by two weeks after parturition. Negative control images for all experimental runs showed no staining.

DISCUSSION

Calcium is important for embryonic growth and development (Tuan et al. 1991), but the amount of neonatal calcium that is transported across the placenta of viviparous lizard species, which lack a calcified eggshell, is highly variable (Stewart and Thompson 2000; Thompson et al. 2000; Ramirez-Pinilla et al. 2006). Calcium ATPase pumps provide an important mechanism of calcium supply across the wall of the uterus for egg-shelling in oviparous birds and lizards (Yamamoto et al. 1985; Wasserman et al. 1991; Thompson et al. 2007; Parker et al. 2008; Bar 2009) , and in viviparous species with intermediate placentae (Herbert et al. 2006). In contrast, placentotrophic species require the majority of their calcium to be transported directly across the uterus to the developing embryo via the placenta (Thompson et al. 1999b, 2000). The presence of Ca^{2+} ATPase pumps from mid to late pregnancy in the uterine and glandular epithelial cells (shell glands) of *Pseudemoia spenceri* and *P. entrecasteauxii* suggests that the pumps are involved in transporting calcium actively out of the epithelial cells and into the uterine lumen to the developing embryo in species with complex placentae as well.

Embryonic growth in skinks is slow for the first half of incubation as calcium and other nutrients are acquired slowly (Packard et al. 1985; Stewart et al. 2009a). However, during the second half of incubation (around

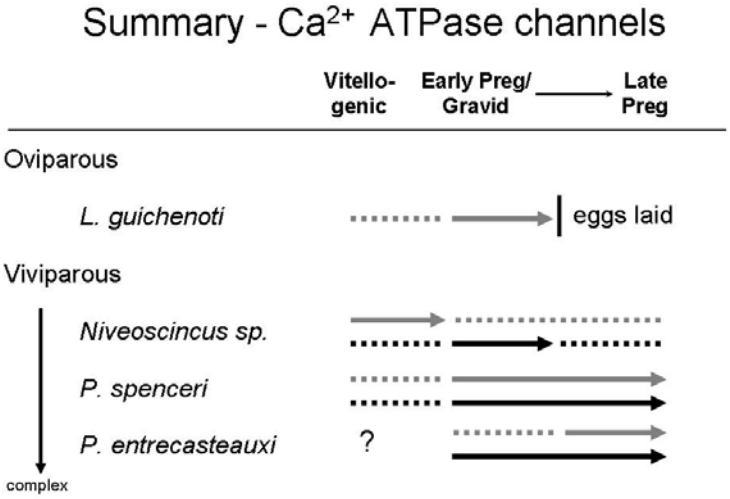

FIGURE. 2. Presence of Ca^{2+} ATPase pumps in oviparous *Lampropholis guichenoti*, and viviparous species (increasing in placental complexity) *Niveoscincus sp.* (*N. metallicus* and *N. ocellatus*), *Pseudemoia spenceri* and *P. entrecasteauxii.* Grey arrows indicate presence of Ca^{2+} ATPase pumps in the glandular epithelium (shell glands) and black arrows indicate presence of Ca^{2+} ATPase pumps in the uterine epithelium. Dashed lines indicate absence of Ca^{2+} ATPase pumps. The question mark indicates a gap in our sample.

stages 32–35; Thompson and Stewart 1997; Booth et al. 2000), embryos grow quickly and demand for calcium for embryonic development also accelerates at this time (Packard et al. 1985; Stewart et al. 2009a). The uterus of oviparous species provides calcium to form the eggshell early in embryonic development (prior to embryonic stage 30; Herbert et al. 2006; Thompson et al. 2007; Stewart et al. 2009a), but the embryo does not access it until late in development (Packard et al. 1985; Stewart et al. 2009a). In viviparous species with intermediate placentae (e.g., *Niveoscincus* sp.), the timing of provision of calcium is the same as it is in oviparous species, early in development before the embryo needs it (Herbert et al. 2006). The amount of calcium transported in viviparous species is likely to be less than in oviparous species because an eggshell is not required; the calcium that is transported must be stored for use by the embryo when it is required later in development (Herbert et al. 2006). The most likely site for storage of calcium is the yolk, although data for *Saiphos equalis* (Linville et al., 2010) does not support this hypothesis. Consequently, both oviparous and viviparous species with relatively simple placentae show expression of Ca^{2+} ATPase pumps when embryos are at early stages of development (Herbert et al. 2006).

The innovation with the evolution of complex placentae in both species of *Pseudemoia* is that Ca^{2+} ATPase pumps are expressed throughout pregnancy. The shell glands apparently do not function in any significant way, if at all, early in pregnancy, concomitant with the delay in provision of calcium across the placenta. The prolonged expression of Ca^{2+}ATPase pumps throughout pregnancy provides a direct means to supply calcium to the embryo at stages of growth when the demand is greatest and their presence increases towards the end of pregnancy when embryonic calcium demand is greatest.

Comparison of *Pseudemoia* species with *Eugongylus* group skinks with other reproductive modes illustrates a significant innovation with the evolution of a complex placenta; there is a change of location and timing of the transport of calcium via Ca^{2+} ATPase pumps from when eggs need to be shelled in oviparous species and those with relatively simple placentae, to later in pregnancy when the embryo needs calcium for ossification. Comparison with species in the genera *Chalcides* and *Mabuya* is required to determine whether this innovation is general in complex placentation or a phylogenetically constrained trait in the genus *Pseudemoia*. In some oviparous species, the embryos take the calcium they need from the eggshell at the same time (Packard et al. 1985; Stewart et al. 2009a) that there is maximum expression of Ca^{2+} ATPase pumps in the uterus of *Pseudemoia* species (i.e., late in development).

In conclusion, the evolution of viviparity results in a change in the mode of calcium provision to the embryo from an eggshell to possible storage in the yolk until it is required, but the evolution of a complex placenta in *Pseudemoia* provides a mechanism to delay the provision of calcium until it is most needed by the embryo. The shell glands in the omphaloplacental side of the uterus are the most likely route for provision of calcium. Whether the calcium is taken up directly by the embryo, or is stored in the yolk first is yet to be investigated. Future studies to determine the timing of placental secretion of calcium and the ontology of calcium mobilization by embryos in these two species is required to directly quantify where and when calcium is transported to embryos during pregnancy.

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LITERATURE CITED

Adams, S.M., J.M. Biazik, M.B. Thompson, and C.R. Murphy. 2005. Cyto-epitheliochorial placenta of the viviparous lizard *Pseudemoia entrecasteauxii*: a new placental morphotype. Journal of Morphology 264:264–276.

Bar, A. 2009. Calcium transport in strongly calcifying laying birds: Mechanisms and regulation. Comparative Biochemistry and Physiology 152A:447–469.

Blackburn, D.G. 1993. Chorioallantoic placentation in squamate reptiles: structure, function, development, and evolution. Journal of Experimental Zoology 266:414–430.

Blackburn, D.G. 1998. Structure, function, and evolution of the oviducts of squamate reptiles, with special reference to viviparity and placentation. Journal of Experimental Zoology 282:560–617.

Blackburn, D.G. 2000. Reptilian viviparity: past research, future directions, and appropriate models. Comparative Biochemistry and Physiology 127A:391–409.

Blackburn, D.G., L.J. Vitt, and C.A. Beuchat. 1984. Eutherian-like reproductive specializations in a viviparous reptile. Proceedings of the National Academy of Sciences of the United States of America 81:4860–4863.

Booth, D.T., M.B. Thompson, and S. Herring. 2000. How incubation temperature influences the physiology and growth of embryonic lizards. Journal of Comparative Physiology 170B:269–276.

Borke, J.L., A. Caride, A.K. Verma, L.K. Kelley, C.H. Smith, J.T. Penniston, and R. Kumar. 1989. Calcium pump epitopes in placental trophoblast basal plasma membranes. American Journal of Physiology 257:C341–C346.

Dufaure, J.P., and J. Hubert. 1961. Table de developpement du lezard vivipare *Lacerta (Zootoca) vivipara* Jacquin. Archives D'anatomie Microscopique et de Morphologie Expérimentale 50:309–328.

Girling, J.E. 2002. The reptilian oviduct: a review of structure and function and directions for future research. Journal of Experimental Zoology 293:141–170.

Herbert, J.F., L.A. Lindsay, C.R. Murphy, and M.B. Thompson. 2006. Calcium transport across the uterine epithelium of pregnant lizards. Herpetological Monographs 20:205–211.

Linville, B.J., J.R. Stewart, T.W. Ecay, J.F. Herbert, S.L. Parker, and M.B. Thompson. 2010. Placental calcium provision in a lizard with prolonged oviductal egg retention. Journal of Comparative Physiology 180:221-227.

Packard, M.J., G.C. Packard, J.D. Miller, M.E. Jones, and W.H.N. Gutzke. 1985. Calcium mobilization, water-balance, and growth in embryos of the Agamid lizard *Amphibolurus barbatus*. Journal of Experimental Zoology 235:349–357.

Parker, S.L., L.A. Lindsay, J.F. Herbert, C.R. Murphy, and M.B. Thompson. 2008. Expression and localization of Ca^{2+}-ATPase in the uterus during the reproductive cycle of King Quail (*Coturnix chinensis*) and Zebra Finch (*Poephila guttata*). Comparative Biochemistry and Physiology 149A:30–35.

Pike, J.W., and R.H. Alvarado. 1975. Ca^{2+} - Mg^{2+} - activated ATPase in the shell gland of Japanese Quail (*Coturnix coturnix japonica*). Comparative Biochemistry and Physiology. 51B:121–129.

Ramirez-Pinilla, M.P., G. De Perez, and J.F. Carreno-Escobar. 2006. Allantoplacental ultrastructure of an Andean population of *Mabuya* (Spuamata, Scincidae). Journal of Morphology 267:1227–1247.

Stewart, J.R., and D.G. Blackburn. 1988. Reptilian placentation: structural diversity and terminology. Copeia 4:839–852.

Stewart, J.R., T.W. Ecay, C.P. Garland, S.P. Fregoso, E.K. Price, J.F. Herbert, and M.B. Thompson. 2009a. Maternal provision and embryonic uptake of calcium in an oviparous and a placentotrophic viviparous Australian lizard (Lacertilia: Scincidae). Comparative Biochemistry and Physiology 153A:202–208.

Stewart, J.R., T.W. Ecay, and B. Heulin. 2009b. Calcium provision to oviparous and viviparous embryos of the reproductively bimodal lizard, *Lacerta (Zootoca) vivipara*. The Journal of Experimental Biology 212:2520–2524.

Stewart, J.R., and M.B. Thompson. 1993. A novel pattern of embryonic nutrition in a viviparous reptile. Journal of Experimental Biology 174:97–108.

Stewart, J.R., and M.B. Thompson. 1998. Placental ontogeny of Australian scincid lizards *Niveoscincus coventryi* and *Pseudemoia spenceri*. Journal of Experimental Zoology 282:535–559.

Stewart, J.R., and M.B. Thompson. 2000. Evolution of placentation among squamate reptiles: recent research and future directions. Comparative Biochemistry and Physiology 127:411–431.

Thompson, M.B., J.B. Biazik, S. Lui, S.M. Adams, and C.R. Murphy. 2006. Morphological and functional changes to the uterus of lizards with different placental complexities. Herpetological Monographs 20:178–185.

Thompson, M.B., L.A. Lindsay, J.F. Herbert, and C.R. Murphy. 2007. Calcium ATPase expression in the oviducts of the skink, *Lampropholis guichenoti*. Comparative Biochemistry and Physiology 147A:1090–1094.

Thompson, M.B., and B.K. Speake. 2004. Egg morphology and composition. Pp 45–74 *In* Reptilian Incubation: Environment, Evolution and Behaviour. Deeming, D.C. (Ed.). Nottingham University Press, Nottingham, United Kingdom.

Thompson, M.B., and B.K. Speake. 2006. A review of the evolution of viviparity in lizards: structure, function and physiology of the placenta. Journal of Comparative Physiology 176B:179–189.

Thompson, M.B., and J.R. Stewart. 1994. Egg and clutch size of the viviparous Australian skink, *Pseudemoia pagenstecheri* and the identity of species with type III allantoplacentae. Journal of Herpetology 28:519–521.

Thompson, M.B., and J.R. Stewart. 1997. Embryonic metabolism and growth in lizards of the genus *Eumeces*. Comparative Biochemistry and Physiology 118A:647–654.

Thompson, M.B., J.R. Stewart, and B.K. Speake. 2000. Comparison of nutrient transport across the placenta of lizards differing in placental complexity. Comparative Biochemistry and Physiology 127A:469–479.

Thompson, M.B., J.R. Stewart, B.K. Speake, M.J. Hosie, and C.R. Murphy. 2002. Evolution of viviparity: what can Australian lizards tell us? Comparative Biochemistry and Physiology 131B:631–643.

Thompson, M.B., J.R. Stewart, B.K. Speake, K.J. Russell, and R.J. McCartney. 1999a. Placental transfer of nutrients during gestation in the viviparous lizard, *Pseudemoia spenceri*. Journal of Comparative Physiology 169B:319–328.

Thompson, M.B., J.R. Stewart, B.K. Speake, K.J. Russell, R.J. McCartney, and P.F. Surai. 1999b. Placental nutrition in a viviparous lizard (*Pseudemoia pagenstecheri*) with a complex placenta. Journal of Zoology 248:295–305.

Tuan, R.S., T. Ono, R.E. Akins, and M. Koide. 1991. Experimental studies on cultured, shell-less fowl embryos: calcium transport, skeletal development, and cardio-vascular functions. Pp 419–433 *In* Egg Incubation: Its Effects on Embryonic Development in Birds and Reptiles. Deeming, D.C. and M.W.J. Ferguson (Eds). Cambridge University Press, Cambridge, United Kingdom.

Wasserman, R.H., C.A. Smith, C.M. Smith, M.E. Brindak, C.S. Fullmer, L. Krook, J.T. Penniston, and R. Kumar. 1991. Immunohistochemical localization of a calcium pump and calbindin-D-28k in the oviduct of the laying hen. Histochemistry 96:413–418.

Watanabe, E., S. Kobayashi, Y. Terashima, and H. Itoh. 1989. Adenosine triphosphatase in the uterus and duodenum of chicken hens during eggshell formation. Poultry Science 68:564–568.

Yamamoto, T., H. Ozawa, and H. Nagai. 1985. Histochemical studies of Ca^{2+}-ATPase, succinate and NAD^{+}-dependent isocitrate dehydrogenases in the shell gland of laying Japanese Quails: with special reference to calcium-transporting cells. Histochemistry 83:221–226.

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