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A COMPARSION OF PLACENTAL TISSUE IN THE SKINKS *EULAMPRUS TYMPANUM* AND *E. QUOYII*

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A comparison of placental tissue in the skinks *Eulamprus tympanum* and *E. quoyii*

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Abstract: The species *Eulamprus tympanum* and *Eulamprus quoyii* are viviparous skinks that are said to have a Type I placenta. This research compared histological characteristics of the oviduct during pregnancy in *E. tympanum* and *E. quoyii* and assessed whether structural features are consistent with a Type I placentation. A similarity was seen in both of these species and was not consistent with a Type I placenta. Extreme folding of the uterine tissue was observed in the early stages. An increase in glands around the later stages was observed while there was no change in blood vessel density over the course of pregnancy. Our research suggests that these species may not have a simple, Type I placenta.

Introduction

Eulamprus tympanum and *Eulamprus quoyii* are Australian skinks that are viviparous, meaning that they give birth to live young (Murphy et al., 2010). Both species have a simple Type I placenta that moves nutrients through the yolk sac to the embryo but does not need to change much in structure during development to meet the needs of the embryo (Hosie et al., 2003; Murphy et al., 2010). A simple placenta is defined as a placenta that has a relatively smooth uterine lining and has little intimate contact between the embryonic tissues and maternal tissues. During pregnancy, the placenta undergoes structural and functional changes associated with the changing metabolic demands of the growing embryo (Weekes, 1935; Blackburn,1933). One placental region is the chorioplacenta, which is rare to observe and appears only for a short time, if at all (Thompson & Speake, 2005). A second major placental region is called the omphaloplacenta, which is also known as the yolk sac placenta. Finally, the chorioallantoic placenta forms during the last stage of pregnancy (Stewart, 1992). This is the organ of respiratory exchange between the mother and embryo (Murphy et al., 2010). Changes at the molecular and cellular level of the placenta occur in forty stages during pregnancy; this process is known as the plasma membrane transformation in mammals (Murphy, 1998).

These structural and functional changes, which occur in *E. tympanum* and *E. quoyii* are critically important to support embryonic development in viviparous lizards (Thompson et al., 2002). During this study, we will begin comparing the morphological changes that occur in the placenta of *E. tympanum* and *E. quoyii*. We are comparing these two species because they are closely related, viviparous, and are considered to have a simple placenta. We believe these species undergo a plasma membrane transformation and may not have a simple placenta. This research will compare histological characteristics of the oviduct during pregnancy in *E. tympanum* and *E. quoyii* and assess whether structural features are consistent with a Type I placentation as documented by Weekes(1935).

Materials and methods

We obtained placental tissues from *E. tympanum* and *E. quoyii* at a range of stages throughout pregnancy. The staging scheme we use is based on Dufaure and Hubert (1961) where 0 is fertilization and 40 is birth. This staging scheme by describes the generalized temporal pattern of reptilian embryonic development. Each uterus was cut into separate uterine incubation chambers. One uterine chamber containing an intact embryo was fixed in 10% neutral buffered formalin for 24 hours and then stored in 70% ethanol. Once the uterine tissue had been fixed, while in its native shape and still stretched around the embryo, it was removed from the embryo by cutting longitudinally along the abembryonic side of the uterus. No further dissection of the uterine tissue was performed before it was embedded in paraffin. The tissue was sectioned transversely with regard to the long axis of the uterus. Paraffin-embedded uterine tissues were sectioned at six micrometers using a microtome. The sections were mounted on Fisher Superfrost Plus microscope slides and prepared for staining with hematoxylin and eosin. Stained tissue sections were examined with an Olympus BX51 microscope using a 40x lens. Oviductal sections obtained from females with embryos from embryo stages ranging from recent fertilization to near parturition were examined. We documented and quantified epithelial cell type (i.e. squamous, cuboidal, or columnar), number of glands, density of blood vessels, and presence of vacuoles. Approximately twelve tissues were randomly sampled from different blocks of each individual in order to find an average number of glands and an average density of blood vessels. In each sample, a 100mm range was used as a grid to determine where to count.

Results

Uterine morphology of *Eulamprus quoyii*

Uterine columnar epithelium of the early stages changed to cuboidal epithelium in the middle and later stages of *Eulamprus quoyii* (Fig. 1A,B). Squamous cells were present at almost every stage with more squamous being present around the middle of pregnancy. This can be explained by the need for oxygen intake by them embryo as development ensues. Deep folding between the maternal and embryonic tissues was noticed during the late stages of pregnancy (Fig. 1C) The average number of glands for *Eulamprus quoyii* was quantified during the stages shown in Table 1. We noticed an increase in glands around the late stages of pregnancy (Table 1). The average density of blood vessels did not change over the course of pregnancy (Table 1). Vacuoles were present in all of the stages and in almost all of the tissues of the sixty individuals we observed (Table 1).

Table 1. Findings for *Eulamprus quoyii*.

Eulamprus quoyii					
	Stage	Cell Types	Average Number of Glands per 100mm	Average Density of Blood Vessels per 100mm	Presence of Vacuoles
Eq04	Early	Sq Col Cub	2	3	Yes
Eq05	Early	Sq Cub	1	2	Yes
Eq 06	Early	Sq Col Cub	1	3	Yes
Eq08	<24	Sq Cub	2	4	Yes
Eq11	Early	Sq	2	3	Yes
Eq13	37	Sq Cub	3	2	Yes
Eq14	38	Sq Col	2	2	Yes
Eq15	37	Sq Cub	3	3	Yes
Eq17	36	Sq	3	4	Yes
Eq19	36	Sq Cub	2	3	Yes
Eq40	NA	Col Cub	1	4	Yes
Eq62	40	Sq Col	1	2	Yes
Eq65	Early 40	Sq	2	2	Yes
Eq68	39	Col Cub	2	3	Yes
Eq70	39	Col Cub	1	4	Yes
Eq77	Early	Sq Col Cub	3	2	Yes
Eq78	Early	Col Cub	1	3	Yes

Uterine morphology of *Eulamprus tympanum*

We noticed a trend continued in *Eulamprus tympanum*. Deep folds were noticed as pregnancy ensued to the late stage (Fig. 1D) Columnar epithelium transitioned to cuboidal epithelium as pregnancy ensued (Fig. 1E,F). The average number of glands increased in the later stages of pregnancy. The average density of blood vessels did not increase with development of the embryo. Vacuoles were present in every stage of pregnancy. Presently, we do not know what their function is.

Table 2. Findings for *Eulamprus tympanum*.

Eulamprus tympanum					
	Stage	Cell Types	Average Number of Glands per 100mm	Average Density of Blood Vessels per 100mm	Presence of Vacuoles
Et162	Early	Sq Col	1	3	Yes
Et164	29	Col	1	3	Yes
Et166	32	Sq Col	1	4	Yes
Et167	31	Sq Col	2	2	Yes
Et168	31	Sq Col Cub	2	2	Yes
Et170	36	Sq Col Cub	3	3	Yes
Et172	35	Sq Col Cub	4	4	Yes
Et173	36	Sq Col	3	3	Yes
Et175	40	Sq Col Cub	2	2	Yes
Et177	37	Col Cub	3	2	Yes
Et184	NA	Sq Col Cub	3	3	Yes
Et189	NA	Sq Col Cub	2	3	Yes
Et191	NA	Sq Col Cub	1	4	Yes
Et193	NA	Sq Col Cub	2	3	Yes

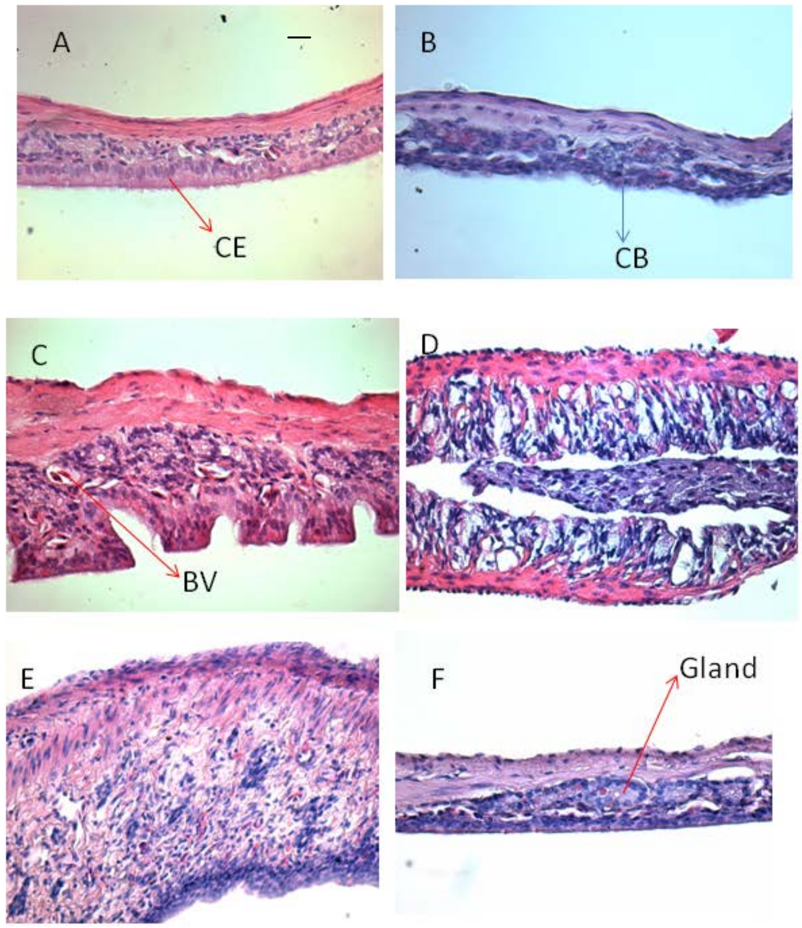

Figure 1. Haematoxylin and eaosin stained paraffin sections of the uterus of pregnant *Eulamprus quoyii* and *Eulamprus tympanum* (A) Early stage of pregnancy in *Eulamprus quoyii* with columnar epithelium still present. (B) Stage 37 embryo of *Eulamprus quoyii* with cuboidal now present. (C) Deep folds of the uterine epithelium in the embryonic segments of an early stage embryo of *Eulamprus quoyii*. (D) Deep folds of the uterine epithelium in the embryonic segments of an early stage embryo of *Eulamprus tympanum*. (E) An early stage of *Eulamprus tympanum*. (F) A late stage embryo of *Eulamprus tympanum*. Scale bar: 20µm. CE, Columnar epithelium; CB, cuboidal epithelium; BV, blood vessel; Gland.

Discussion

In the 1930's Claire Weekes began examining the placental changes in viviparous skinks. Essentially, Weekes was trying to describe placental morphology and introduced the four types of placenta (Weekes, 1930). The four types of placenta range from a simple, Type I placenta to the complex, Type IV placenta. The research into viviparous skink placenta morphology continued with Dufaure and Hubert who developed the scheme for the different stages of placental development (Dufaure & Hubert, 1961). *Eulamprus quoyii* and *Eulamprus tympanum* are said to have a Type I placenta (Weekes, 1935). This research brought into question whether these two related species had a Type I placenta or whether it was a more complex placenta. In order to determine whether it may be a more complex placenta, we needed to observe the morphological changes within the placenta at the different stages provided by Dufaure and Hubert. We also needed to quantify blood vessel density and the number of glands present throughout embryonic development. The presence of vacuoles, which could also be lipid vesicles, was observed throughout the stages as well. It is not clear whether they are vacuoles or vesicles.

Eulamprus quoyii and *Eulamprus tympanum* had striking similarities in placental morphology. Columnar epithelial tissue was present at the early stages, from approximately 0-20. As pregnancy ensued, we noticed that the columnar epithelium transitioned to columnar epithelium around the later stages. Also, we noticed that squamous epithelium became more numerous during the middle and late stages of pregnancy in order to meet the oxygen requirements for the developing embryo. For both species, we observed an increase in glands around the later stages of pregnancy as well. It is not presently known what these glandular functions are. It is presumed that they are secretory glands and may secrete hormones, glycoproteins, or ions (Herbert et al. 2006). Research completed previously on *Sphenomorous quoyii* determined that calcium was the main ion that was transported to the embryo through the placenta

(Thompson, 1982). We did not notice a change in blood vessel density over the course of embryonic development in either species. This could be explained by the blood vessels growing in diameter but not in number. Also, in histology it becomes hard to quantify blood vessels because they are so small (Murphy et al., 2011).

A similarity that is seen in both of these species but that is not consistent with a Type I placenta is the extreme folding of the placental tissue in the early stages. The species *Niveoscincus metallicus* is described as having an intermediate type placenta (Thompson et al.,1999). The folding of the uterine epithelium that is found in this species is also seen in *Eulamprus tympanum* and *Eulamprus quoyii*. Invaginations of the tissue increases intimacy between the maternal tissue and embryonic tissue. According to Blackburn and Flemmings, all reptilian membranes are beginning to become specialized and are evolving to find better ways to transport nutrients and water to the embryo (Blackburn and Flemming, 2009).

Previous research stated that *Eulamprus quoyii* has a Type III placenta because the vessel dense elliptical structure morphs with the placenta (Murphy et al., 2011). Previous research also suggest that *Eulamprus tympanum* had more than a Type I when compared to the plasma membrane transformation seen in mammals (Hosie et al., 2003) According to the research in this study, our results suggest that *Eulamprus tympanum* and *Eulamprus quoyii* may have more than a simple Type I. However, more research would need to be completed in order to be certain. The direction of evolutionary change in the genus *Eulamprus* cannot be determined according to previous research but it is important to study this genus because it has both oviparous and viviparous species (Murphy et al., 2012).

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