Gonad development in the corn snake (*Pantherophis guttatus*) - formation of the gonadal ridges and sexual differentiation of gonads

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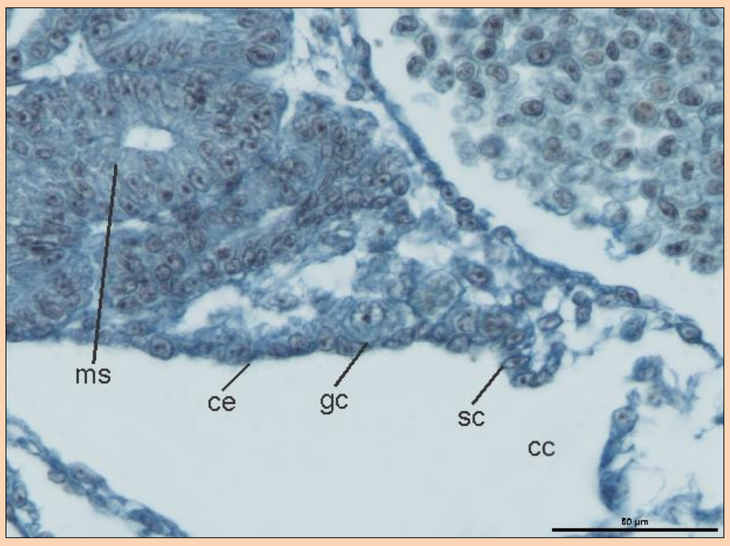
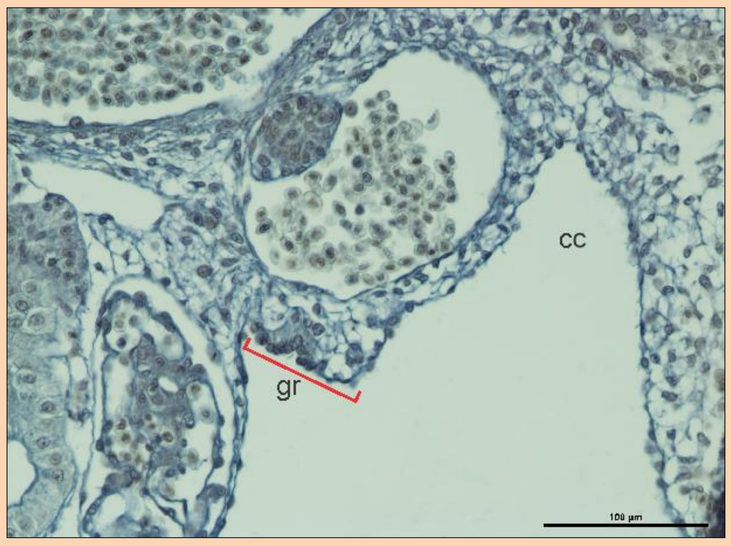
Introduction

The development of gonads has been extensively studied in two groups of amniotes: birds and mammals. However, in reptiles, gonadal development has received comprehensive analysis in only a few species, including one alligator and two turtle species. Many aspects of gonadogenesis in reptiles remain unclear, especially among squamates, such as lizards and snakes, which comprise 11,549 species (December 2022), accounting for 96.7% of currently known reptile species. In all vertebrates, including reptiles, gonads form as gonadal ridges on the ventro-medial surface of the embryonic kidneys, known as mesonephroi. These ridges are covered by coelomic epithelium, within which primordial germ cells complete their migration. Somatic cells accumulate in the central regions of these ridges, marking the onset of medulla formation. The primary objective of our study was to elucidate the early development of gonads, with a particular focus on the formation of gonadal ridges and the process of sexual differentiation in the corn snake (*Pantherophis guttatus*).

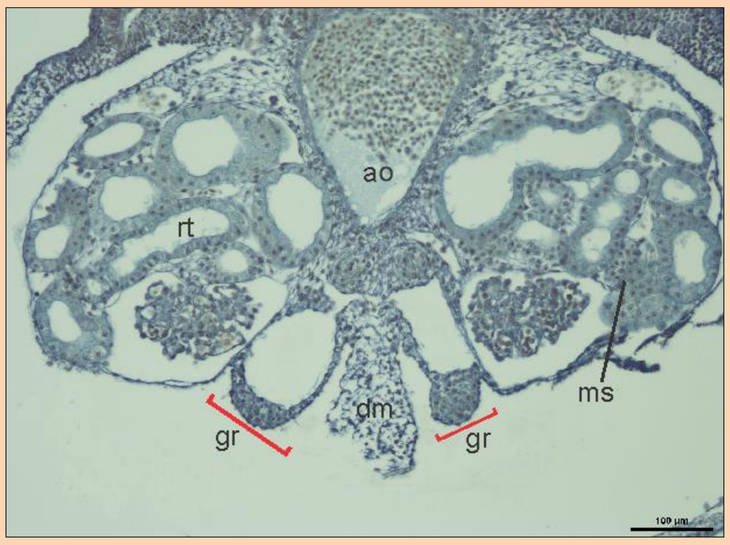
Gonadal ridges and undifferentiated gonads



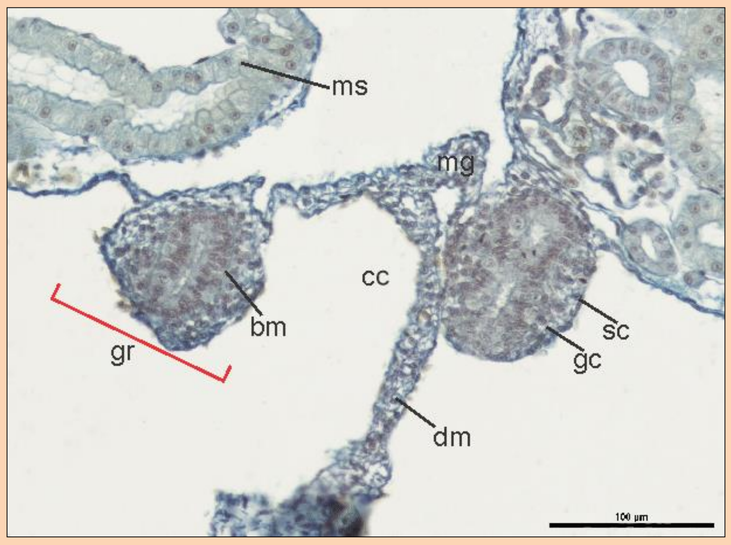
The undifferentiated gonad of the *P. guttatus* embryo at E1 (embryonic day 1)/S21 (stage 21).
(cc) coelomatic cavity, (gc) germ cells, (ms) mesonephros, (sc) somatic cells



The undifferentiated gonad of the *P. guttatus* embryo at E2/S22.
(cc) coelomatic cavity, (gr) gonadal ridge



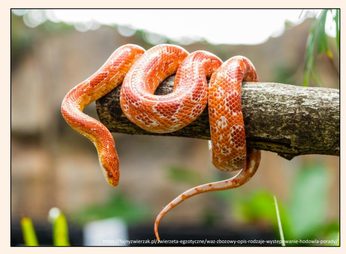
The undifferentiated gonad of the *P. guttatus* embryo at E8/S25.
(ao) aorta, (dm) dorsal mesentery, (gr) gonadal ridge, (ms) mesonephros, (rt) renal tubule

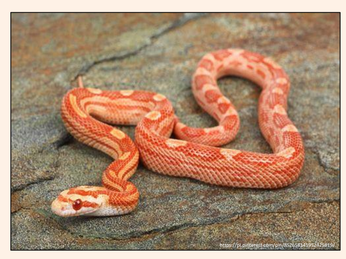


The undifferentiated gonad of the *P. guttatus* embryo at E9-10/S27.
(bm) basement membrane, (cc) coelomatic cavity, (dm) dorsal mesentery, (gc) germ cells, (gr) gonadal ridge, (mg) mesogonium, (ms) mesonephros, (sc) somatic cells

Materials and methods

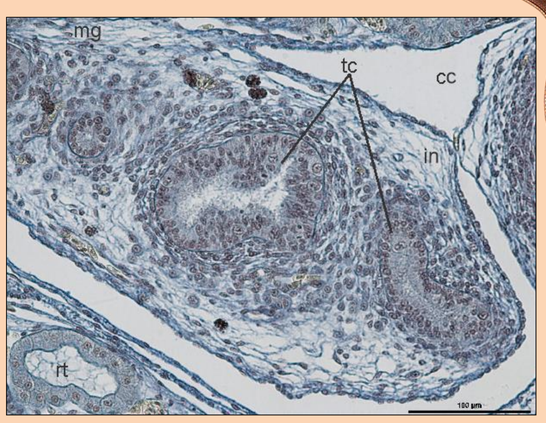
The eggs of the cron snake (*P. guttatus*) were obtained from private breeders in Kraków (Poland). The embryos were staged according to Zehr (1962). The gonads were dissected from embryos, fixed in Bouin's solution, embedded in paraffin, and stained according to Dubreuill trichrome (Kiernan 1990). This staining was used to visualize structure of embryonic gonads at stages from the beginning of gonadal ridge formation, sexual differentiation of gonads, until formation of the definitive structure of ovary and testis.





Differentiating testes

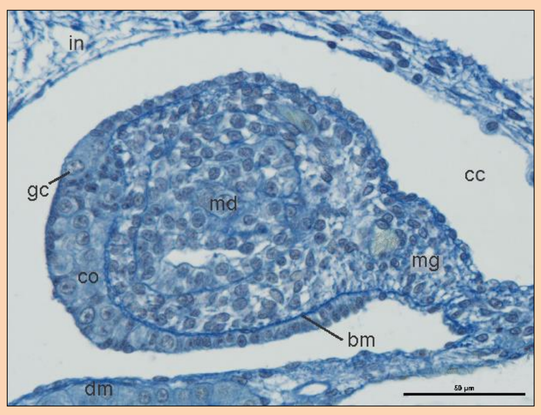
The developing testis of the *P. guttatus* embryo at E28/S33.
(in) interstitium, (mg) mesogonium, (rt) renal tubule, (tc) testis cords



The developing testis of the *P. guttatus* embryo at E52/S37.
(cc) coelomatic cavity, (mg) mesogonium, (in) interstitium, (rt) renal tubule, (tc) testis cords

Differentiating ovaries

The developing ovary of the *P. guttatus* embryo at E28/S33.
(bm) basement membrane, (cc) coelomatic cavity, (co) coelomatic cavity, (gc) germ cells,(md) gonadal medulla, (mg) mesogonium, (ms) mesonephros, (in) interstitium



The developing ovary of the *P. guttatus* embryo at E39/S36.
(bm) basement membrane, (cc) coelomatic cavity, (co) coelomatic cavity, (dm) dorsal mesentery, (gc) germ cells, (md) gonadal medulla, (mg) mesogonium, (in) interstitium

Results and Discussion

The analysis has revealed that gonadal ridges form on the ventro-medial surface of the mesonephros, in proximity to the cardinal vein. These gonadal ridges are covered by coelomic epithelium. Within this epithelium, primordial germ cells cease their migration. Somatic cells accumulate in the center of the gonad, marking the initiation of gonadal medulla formation. The development of undifferentiated gonads is succeeded by sexual differentiation into either testes or ovaries. Traditionally, the ovary was believed to develop from the gonadal cortex, and the testis from the medulla. However, our analysis has demonstrated that this perspective is oversimplified. While the majority of the testis volume does originate from the medulla, the male gonad is also covered by a thin tissue layer derived from the cortex. Similarly, the predominant portion of the ovary develops from the cortex, but a significant portion of the central ovarian tissue originates in the medulla. During ovarian differentiation, the cortex thickens, accompanied by an increase in the number of somatic and germ cells within this gonadal compartment. Germ cells diminish in the ovarian medulla. Conversely, during testis differentiation, the reverse process occurs as germ cells diminish in the thin cortex. The testis medulla grows and transforms into testis cords housing germ cells. Voluminous interstitium forms between these testis cords. Gonadal development in the corn snake shares similarities with other vertebrates; however, this study has identified some unique features. In *P. guttatus*, during sexual gonadal differentiation, cords develop within the medulla in both sexes, and lumina form within both testis and ovarian cords. Notably, these cords exhibit more pronounced development within the testes, where they house germ cells.

References

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- Zehr, DR. 1962. Stages in the normal development of the common garter snake, Thamnophis sirtalis sirtalis. Copeia 1962:322-329.

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