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Cover Page Footnote
We thank Chris Thigpen for technical assistance. We are also appreciative of the continuing support by Kelly Irwin, herpetologist, of the Arkansas Game and Fish Commission (AGFC). Turtle collection was authorized by a scientific collection permit from the AGFC, and we also thank Anthony Holt and Patrick Daniel for assistance in collecting the turtles.

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Germinal Epithelium Cytology during Spermatogenesis in the Alligator Snapping Turtle, *Macrochelys temminckii* (Reptilia: Chelydridae)

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Running Title: Germinal Epithelium Cytology in *Macrochelys temminckii*

Abstract

We investigated the cytology of the seminiferous epithelia of the Alligator Snapping Turtle (*Macrochelys temminckii*). Spermatogenic and regressed testes were assessed from 2 adult individuals collected in Arkansas in May and September of 1993. Specifically, we focused on the cellular phases of germ cell development and maturation. The germ cell morphology and developmental strategy within the germinal epithelium of *M. temminckii* appear similar to that of other genera of turtles previously studied. Interestingly, mitotic, meiotic, and spermiogeneic cells are nearly identical to that of other turtles studied based on light microscopy. There are also 6 recognizable steps to spermiogenesis, which is slightly different than the 7 steps of most turtles (step 7 absent). Though this study only uses 2 individuals (because of its endangered status), *M. temminckii* appears to start spermatogenesis in the spring, and the climax of spermiogenesis occurs in the fall similar to that of other temperate turtles studied to date based on light microscopy. Peculiar to both turtles in this study were the regular appearance of very large germ cells in the basal compartment of the germinal epithelium. Based on previous research and our histological analysis these enlarged spermatogonia exhibit hypertrophic characteristics typical of cells undergoing apoptosis.

Introduction

Most key features of amniote spermatogenesis and histological architecture of the testis are present in all reptiles (Volsøe 1944; Pudney 1995; Gribbins 2011). The spermatogenic process includes the following events: 1) a proliferative phase (spermatocytogenesis) in which large numbers of germ cells are successively generated by numerous mitotic divisions of spermatogonial cells, 2) a meiotic phase that produces haploid secondary spermatocytes, 3) a spermiogenic phase in which spermatids transform into motile sperm, and 4) a regressive or quiescent phase with little or no germinal cell activity (indicative of seasonally breeding).

Most spermatogenic studies in turtles have described their testicular cycles in terms of seasonal changes in seminiferous tubule size and activity often with little to no histological data. Limited information, however, exists on the specific cytological events of spermatogenesis at the light microscopic level in a few chelonians (Pudney 1995; Gribbins *et al*. 2003; Miller and Dinkelacker 2008).

In temperate zone chelydrid turtles, such as the Snapping Turtle (C*helydra serpentina*), testes are flaccid and have a minimal mass during the spring and early summer months, whereas the testes are large during late summer and early fall (Mahmoud and Cyrus 1992). Other than a brief report by Dobie (1971), who noted the year-round presence of sperm in reproductive tracts, no documentation of the testicular cycle or spermatogenic activity in the Alligator Snapping Turtle (*Macrochelys temminckii*) has been reported. Furthermore, there are only two studies to date that present cytological data on the specific germ cell morphologies as they progress through spermatogenesis in turtles (Gribbins *et al.* 2003; Lancaster *et al.* 2014).

The present study describes the cytology of developing germs cells in the testes of the Alligator Snapping Turtle using light microscopy at the height of spermatogenic activity in a September specimen and during the quiescent phase in May. We also document the presence of severely enlarged germ cells in the basal compartment, which are most common in a September specimen at the climax of spermiogenesis and spermiation. Although these cells in the present study appear to be much larger than previously reported in other reptiles, they are morphologically indicative of apoptosis as defined by recent studies in other turtles (Zang *et al*. 2007) and lizards (Comitato *et*

al. 2006).

Materials and Methods

Alligator Snapping Turtles were collected in Jackson and Ouachita counties on the 19th of May and on the 3rd of September, 1993, respectively. Both specimens were euthanized with an intra-pleuroperitoneal cavity injection of sodium pentobarbital. Samples of the right testis were extracted from both adult male Alligator Snapping Turtles prior to their deposition into the Arkansas State University Museum of Herpetology (ASUMZ 19010 and 19261; both turtles were of comparable size—the latter turtle measuring 507 mm in standard carapace length). Following testis fragment removal, each turtle was fixed using injections of 10% formalin. The whole turtles were then preserved in 70% ethanol.

For light microscopy (LM-plastic), we fixed testis fragments in 2% glutaraldehyde (GTA) for 2–4 hr. Segments were then dehydrated in a graded series of increasing ethanol solutions (50-100%), placed in a 50/50% acetone/plastic mixture for overnight infiltration, and were eventually placed in embedding molds using plastic resin, Mollenhauer's Epon-Araldite #2, as described by Dawes (1988). For thick sectioning of tissue blocks (approximately 1 µm in thickness) and staining, we used glass knives on an LKB Ultrotome (Type 4801A) with Ladd® multiple stain (LMS), respectively. For photomicroscopy, we used a Leica MC 120 HD camera atop a Leica DM 2000 LED compound light microscope.

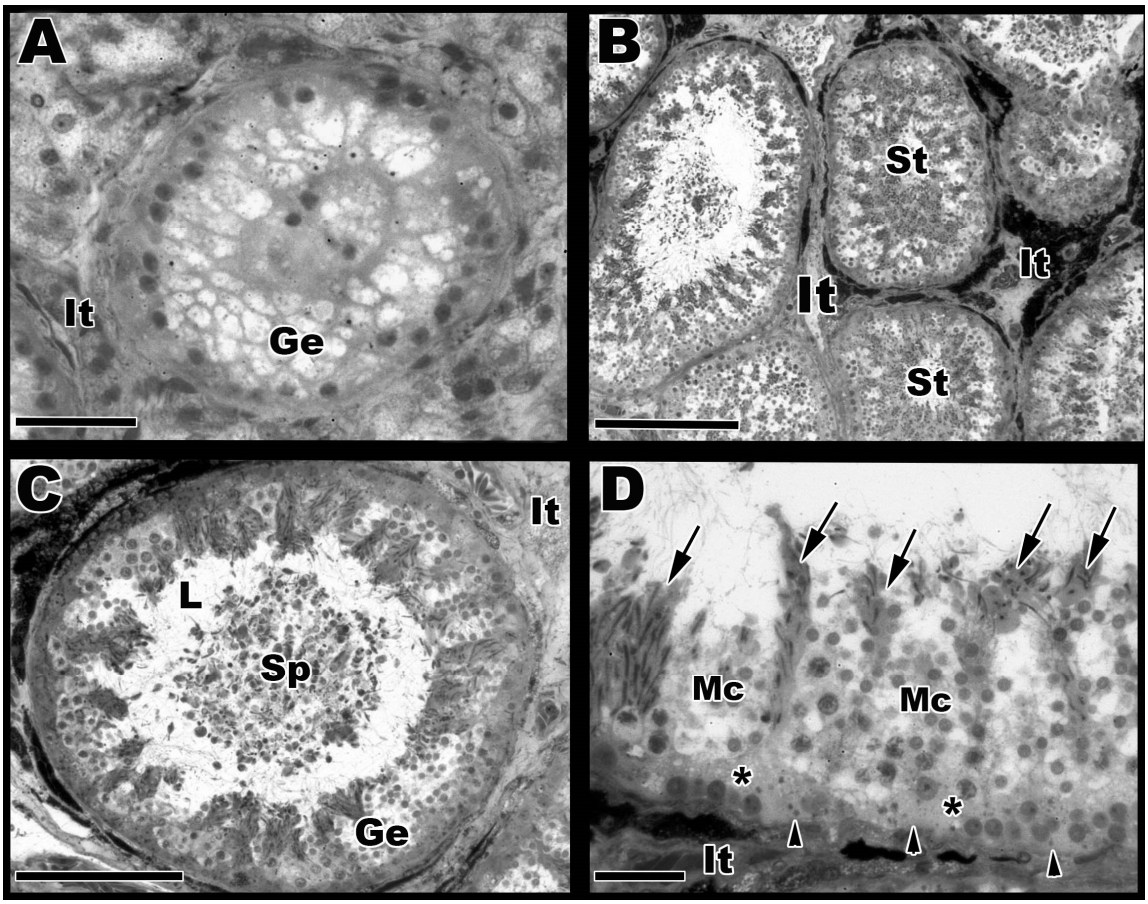

Figure 1. Light micrographs representing the organization of the testis in *Macrochelys temminckii* in May (A) and September (B, C, D) and. The Alligator Snapping Turtle exhibits the typical reptilian histological architecture with seminiferous tubules (St) interspersed with interstitial tissue (It) dominating the microscopic anatomy of the testis. The seminiferous tubules of the May testis are in quiescence with a heavily vacuolated germinal epithelium (Ge) with few germ cells, which are restricted to the basal compartment. In contrast, the September germinal epithelium shows spermatogenesis in full swing, with a thick epithelial lining exhibiting layers of different generations of developing germ cells. Sperm (Sp) are often located in the lumen (L) of each September seminiferous tubule along with shed Sertoli cell remnants. The thick epithelium is organized into columns (black arrows) containing cohorts of developing spermatids, intermediate meiotic cells (Mc), and basally located spermatogonia (*) resting on a prominent basement membrane (black arrowheads). Bars: A = 50 µm; B = 200 µm; C = 100 µm; D = 25 µm.

Results

The testis of the Alligator Snapping Turtle is comprised of seminiferous tubules that are lined with a continuous seminiferous epithelium, where germ cells develop in close association with Sertoli cells. The epithelium rests on a conspicuous basement membrane and the tubules are separated by interstitial space that is comprised of collagen-based connective tissue, blood vessels, and Leydig-like cells. The seminiferous epithelium in *Macrochelys temminckii* is quiescent in the month of May (Fig. 1A) within our samples, and the only dominant germ cells within the highly vacuolated epithelium are spermatogonia. The September seminiferous tubules (Fig. 1B, C, D), in contrast, are highly active in the process of spermatogenesis and multiple generations of germ cells with representative spermatogonia, spermatocytes, and spermatids, which are easily observed within the columns of seminiferous epithelia.

The most noteworthy germ cell morphology of the early and late seminiferous epithelia in *M. temminckii* is the presence of enlarged hypertrophic cells (Fig. 2A, C, Hp) near the basement membrane. These cells undergo a continual enlargement and can reach widths of over 30μm. These cells then undergo a complete breakdown of the cytoplasm and nucleus as seen in Fig, 2C. There are three types of spermatogonia found in the May seminiferous epithelium of the Alligator Snapping Turtle. The R type spermatogonia (Fig. 2A, SpR) have darkly staining nuclei and are inactive and not observed dividing within the epithelium. The type A and B spermatogonia (Fig, 2A, B, SpA, B) divide and then enter the start of meiosis within the basal compartment of the seminiferous tubules (Fig. 2A, B, Pl).

The September *Macrochelys temminckii* testis has seminiferous tubules that are dominated by developing generations of spermatids found in discernable columns of seminiferous epithelia (Fig. 1D). These fall turtles have started spermiation and tubular lumina often are occupied by spermatozoa (Fig. 1C). Meiotic spermatocytes (Fig. 3A–D, Pl, Pa, Zy, Di, Lp, M1, M2) are found near the basement membrane just above spermatogonia A and B, which have completed mitosis for the summer season. Interestingly, 4 to 7 generations of spermatids are observed within a cross section of a seminiferous tubule. These generations of maturing cell types are often in sequential generations, which appears to lead to waves of sperm release upon observation of multiple tubules in cross section.

Discussion

This qualitative study of spermatogenesis in spring and fall Alligator Snapping Turtles adds insight on germ cell development in turtles. The use of plastic embedded sections allowed acute visualization of the cellular events of spermiogenesis in these turtles, which has been noted as a problem in the past, particularly acrosome development (Gribbins *et al.* 2003). The acrosome vesicles are easily discernable in CS of the seminiferous tubules of the Alligator Snapping Turtle. Hypertrophic cells of great size are seen in *Macrochelys temminckii* basal compartments of the seminiferous epithelia. The incredible sizes of these

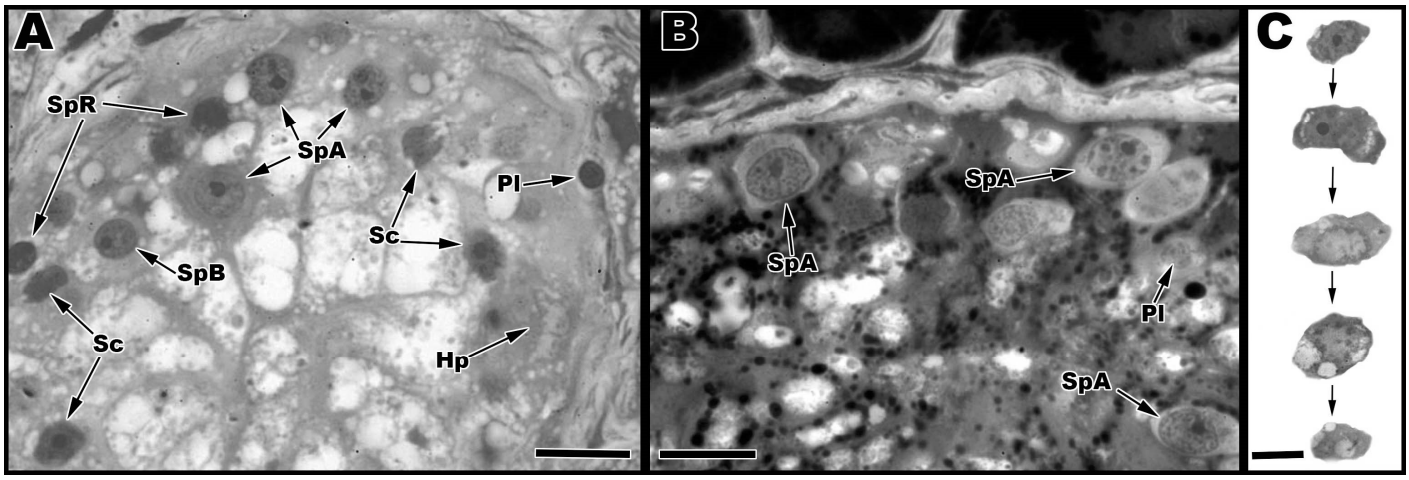

Figure 2. A and B (bars = 10 μm) light micrographs represent high magnification of the May germinal epithelium within the testis of *Macrochelys temminckii*. Note that only spermatogonia (SpR, SpA, SpB), Sertoli cell nucleus (Sc), and an occasional pre-leptotene (Pl) spermatocyte dominate the basal compartment of this epithelium. The only other cell type found in the seminiferous epithelium is sizeable hypertrophic cells (Hp) in various stages of enlargement or degradation (**C**) (bar = 5 μm).

cells are most likely an artifact of the infiltration and embedding process, which has been noted in basally located spermatogonia and apoptotic cells within other turtle testes (Hess 1990; Gribbins *et al*. 2003).

Nuclear elongation is enhanced in *M. temminckii* elongating spermatids and leads to linear sperm nuclei, which are bundled together within the seminiferous epithelium and most likely contributes to the column effect that is observed within the fully spermatogenic turtle in September. These spermatids in the elongation phase are very similar to avian spermatids (Sprando and Russell 1988, Kumar 1995) during late stage spermatogenesis, which has also been suggested in other species of turtles (Gribbins *et al*. 2003; Lancaster *et al*. 2014). The morphologies of spermatocytes and all three spermatogonia types in Alligator Snapping Turtles are similar to that of other turtles (e.g., *Chrysemys picta, Trachemys scripta,* and *Graptemys geographica*) as mentioned in Gribbins *et al*. (2003), Gribbins (2011), and Lancaster *et al*. (2014), respectively. Not only are these cell types similar in appearance, but also the mode and organization of the germ cells within the Alligator Snapping Turtle seminiferous epithelium corroborates what has been reported in these same species of turtles. Thus, it is reasonable to assume that *M. temminckii* follows the same postnuptial production of sperm that have been reported in other temperate turtles and snakes (Gibbons 1968; Moll and Legler 1971; Gribbins 2011). Also the arrangement of 4 to 7 generations of spermatids during late spermatogenesis in the Alligator Snapping Turtle, like that observed in other reptiles (Gribbins 2011; Gribbins and Rheubert 2015), suggests that these turtles follow a temporal germ cell development strategy rather than the spatial germ cell development strategy seen in mammals and birds (Rossen-Runge 1977; Russell *et al*. 1990).

The present cytological data on sperm development in the Alligator Snapping Turtle add needed information to what is known for spermatogenesis within Chelonia. Very few species have been studied to date, and though the data of the present study is from only two months of the year, our cytological results show similar trends to what is already known in turtles. These types of comparative histological data on the testis are important if inferences on the process of spermatogenesis within turtles are going to be robustly understood.

Acknowledgments

We thank Chris Thigpen for technical assistance. We are also appreciative of the continuing support by Kelly Irwin, herpetologist, of the Arkansas Game and Fish Commission (AGFC). Turtle collection was authorized by a scientific collection permit from the AGFC, and we also thank Anthony Holt and Patrick Daniel for assistance in collecting the turtles.

Literature Cited

Comitato R, T Esposito, G Cerbo, F Angelini, B Varriale, and **A Cordone.** 2006. Impairment of spermatogenesis and enhancement of testicular germ cells apoptosis induced by exogenous all-trans-retinoic acid in adult lizards *Podarcis sicula*. Journal of Experimental Zoology 305A(3):288-98.

Dawes CJ. 1988. Introduction to biological electron microscopy: theory and techniques. Ladd Research Industries, Inc. Burlington (VT). 315 p.

Dobie JL. 1971. Reproduction and growth in the alligator snapping turtle, *Macroclemmys temmincki* (Troost). Copeia 1971:645-58.

Gibbons JW. 1968. Reproductive potential, activity and cycles in the painted turtle, *Chrysemys picta*. Ecology 49:400-09.

Gribbins KM. 2011. Reptilian spermatogenesis: a histological and ultrastructural perspective. Spermatogenesis 1:1-20.

Gribbins KM and **JL Rheubert.** 2015. The architecture of the testis, spermatogenesis, and the spermatozoa. *In:* Siegel DS, JL Rheubert, and SE Trauth, editors. Reproductive biology and phylogeny of lizards and tuatara. CRC Press (Boca Raton FL). p 340-424.

Gribbins KM, DH Gist, and **JD Congdon.** 2003. Cytological evaluation of spermatogenesis and organization of the germinal epithelium in the male slider turtle, *Trachemys scripta*. Journal of Morphology 255:337-46.

Hess R. 1990. Quantative and qualitative characteristics of the stages and transition in the cycle of the rat seminiferous epithelium: light microscopic observations of perfusion-fixed and plastic-embedded testes. Biology of Reproduction 43:525-542.

Kumar M. 1995. Spermatogenesis in the house sparrow, *Passar domesticus*: histological observations. Pavo 33:1-4.

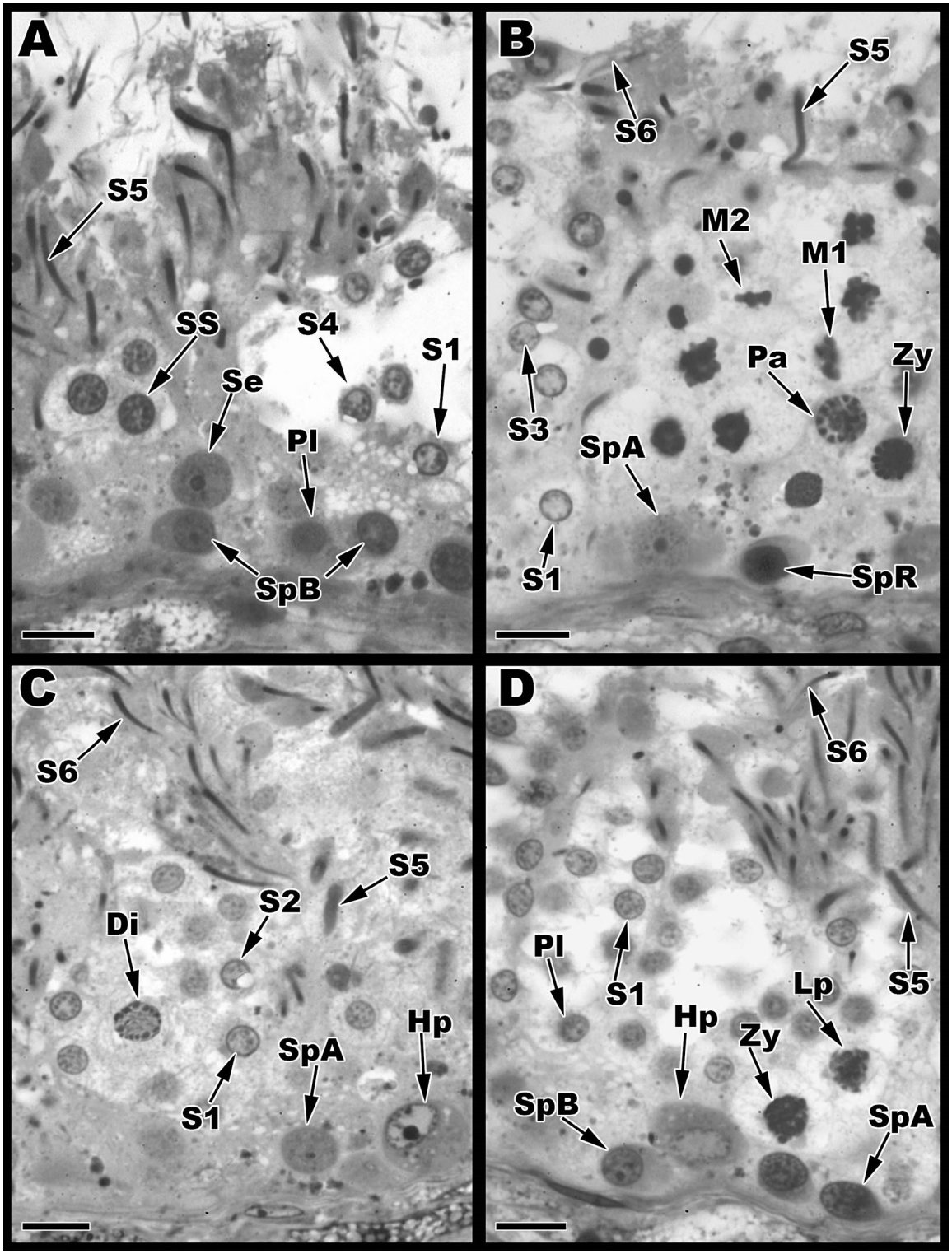

Figure 3. Light micrographs (A–D) representing various views of the September seminiferous epithelium at high magnification within the testis of *Macrochelys temminckii*. Notice that the fall testis of the Alligator Snapping Turtle is spermatogenically active with cell types of mitosis, meiosis, and spermiogenesis, and all are represented within the germinal epithelium. Se, Sertoli cell nucleus; SpR, resting spermatogonium; SpA, spermatogonium A; SpB, spermatogonium B; Hp, hypertrophic spermatogonium; Pl, pre-leptotene spermatocyte; Lp, leptotene spermatocyte; Pa, pachytene spermatocyte; Di, diplotene spermatocyte; M1, meiosis 1; SS, secondary spermatocyte; M2, meiosis 2; S1-S6, steps 1 through 6 spermatids. Bars = 10 µm.

Lancaster K, SE Trauth, and **KM Gribbins.** 2014. Testicular histology and germ cell cytology during spermatogenesis in the Mississippi Map Turtle, *Graptemys pseudogeographica kohnii* from northeastern Arkansas. Spermatogenesis 4:1-8.

Mahmoud IY and **RV Cyrus.** 1992. The testicular cycle of the common snapping turtle, *Chelydra serpentina*, in Wisconsin. Herpetologica 48:193-201.

Miller JD and **SA Dinkelacker.** 2008. Reproductive structures and strategies of turtles. *In:* Wyneken J, MH Godfrey, and V Bells, editors. Biology of turtles. CRC Press (Boca Raton FL). p 225-278.

Moll EO and **JM Legler.** 1971. The life history of a neotropical slider turtle, *Pseudemys scripta* (Schoepff) in Panama. Bulletin of the Los Angeles County Museum 11:1-102.

Pudney J. 1995. Spermatogenesis in nonmammalian vertebrates. Microscopy Research and Techniques 32:459-97.

Rossen-Runge EC. 1977. The process of spermatogenesis in animals. *In:* Abercrombie M, DR Newth, and JG Torrey, editors. Developmental and cell biology series 5. Cambridge University Press (Cambridge, UK). p 103-132.

Russell LD, Hikim SAP, Ettlin RA, and **ED Legg.** 1990. Histological and histopathological evaluation of the testis. Cache River Press, (Clearwater, FL).

Sprando RL and **LD Russell.** 1988. Spermiogenesis in the red-ear turtle and domestic fowl: A study of cytoplasmic events including cell volume changes and cytoplasmic elimination. Journal of Morphology 198: 95-118.

Volsøe, H. 1944. Structure and seasonal variation of the male reproductive organs of *Vipera berus* (L.). Spolia Zoologica Musei Hauniensis 5:1-157.

Zang L, H Xiang-Kuh, L Mei-Ying, B Hui-Jun, and **C Qui-Sheng.** 2007. Spermiogenesis in the soft-shelled turtle, *Pelodiscus sinensis*. Anatomical Record 290:1213-1222.