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Research Article

Sertoli Cell Junctions during Active Spermiogenesis in the African Sideneck Turtle (*Pelusios castaneus*): Implications for the Blood-Testis Barrier

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ABSTRACT

Testicular samples were collected at the peak of spermiogenesis from African sideneck turtles (n=10) (*Pelusios castaneus*) to describe the ultrastructural features of the Sertoli cell in relation to the Blood-Testis Barrier (BTB) in the turtle using light and electron microscopy. The nucleus of the Sertoli cell had irregular outline with numerous infoldings and dense granular nuclear chromatin. The cytoplasm of the Sertoli cell consisted of mitochondria, vesicles, vacuoles, lipid droplets as well as smooth and rough endoplasmic reticula. reframe the whole sentence Two prominent cell to cell contacts were identified around the Sertoli cell cytoplasm: Sertoli-Sertoli cell junctions and the Sertoli-germ cell junctions. The Sertoli-germ cell junctions were composed of zonula occludens (tight junction), zonula adherens (anchoring junction) as well as macula adherens (desmosomes) while the Sertoli-Sertoli cell junctions were composed of only tight junctions. The position and structure of the Sertoli cell junctions of the African sideneck turtle, were similar to those of the Asian soft-shelled turtles, can be traced to their role in the creation of the BTB as well as the nursing of germ cells. The present study, being the first report on the cytology of the Sertoli cell in any turtle of African origin, clearly revealed that there are structural differences between Sertoli-Sertoli and Sertoli-germ cell contacts in the African sideneck turtle.

Key words: Sertoli cell, Testis, African sideneck turtle, Spermatids, Nucleus

INTRODUCTION

The Sertoli cell rests on the basement membrane of the seminiferous tubule with its cytoplasm extending to the abluminal surface of the tubule. It nourishes and provides structural support to developing germ cells while being involved in the phagocytosis of degenerating germ cells (Young *et al*., 2006; Hai *et al*., 2014; Ahmed *et al*., 2017). The extensive cytoplasm of the Sertoli cell encloses all the cells of the spermatogenic series within the germinal epithelium. Hence, the cytoplasmic outline of the Sertoli cell is highly irregular and constantly changing to permit the progressive movement of developing spermatozoa towards the luminal surface of seminiferous tubules (Kellner and Schwanke, 2001). Division and maturation of germ cells within the seminiferous tubules advance through development in association with Sertoli cells (Ahmed *et al*., 2017). Thus, the role of Sertoli cells is crucial in the maintenance of normal spermatogenesis, being the only non-germinal element within the seminiferous tubules (Munoz *et al*., 2001; Ahmed *et al*., 2016).

Direct contact via cell junctions occurs between Sertoli and germ cells allowing for extensive interactions and communications between these cells throughout spermatogenesis both at the biochemical and molecular levels (Cheng *et al*., 2004). Specialized junctional complexes between neighboring Sertoli cells constitute the principal component of the Blood-Testis Barrier (BTB) that is responsible for maintaining the specialized physicochemical microenvironment required for completion of the meiotic process and spermiogenesis (Munoz *et al*., 2001). Studies from the past three decades have demonstrated that germ cells rely heavily on Sertoli cells for structural and nutritional support (Wiebe *et al*., 1987).

While studies on the Sertoli cell have been well documented in mammals (Ueno and Mori, 1990; Munoz *et al*., 2001; Johnson *et al*., 2008; Andrade *et al*., 2013), only a few studies have been reported on the reptilian Sertoli cell. Previous research reports on the Sertoli cells of turtles have been on the Asian soft-shelled turtles, *Pelodicus maackii*, (Park *et al*., 2015) and *Pelodicus sinensis*, (Ahmed *et al*., 2016, 2017). There is the paucity of information on the morphology of the Sertoli cells of

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African species of turtles. Existing related research reports on other turtles or reptiles are basically on the histology and ultrastructure of the testis (Al-Dokhi *et al*., 2004; Gribbins *et al*., 2010; Sever *et al*., 2013; Olukole *et al*., 2014, 2017). To date, not much is known about Sertoli-Sertoli cell and Sertoli-germ cell contacts of turtles, especially those of African origin.

This study was undertaken to describe the Sertoli-Sertoli and Sertoli-germ cell junctions in the African sideneck turtle (*Pelusios castaneus*), a freshwater turtle of the family *Pelomedusidae* (Kirkpatrick, 1995) and their implications for the BTB, using light and transmission electron microscopy in order to provide baseline information which may be useful in captive-breeding programmes. A hypothesis was therefore posed that there are structural differences between Sertoli-Sertoli and Sertoli-germ cell contacts in the African sideneck turtle.

MATERIALS AND METHODS

Experimental animals

Ten adult male African sideneck turtles (*Pelusios castaneus*) with an average bodyweight of 0.72kg, collected from river drainages in Ibadan, Nigeria were sampled in August and September, a period of peak spermiogenesis (Olukole *et al*., 2014). Carapacial and plastral characteristics of the turtle, as described by Kirkpatrick (1995), were used in the determination of their adulthood. The turtles were anaesthetized with an intramuscular injection of ketamine-HCl (Sigma, St. Louis, MO, USA) (25 mg/kg body-weight) and subsequently sacrificed by cervical decapitation. The testes were removed after separating the plastron from the carapace. All procedures were carried out according to the guidelines for the care and use of experimental animals (National Institute of Health (NIH), USA. The study was approved by the University of Ibadan Animal Care and Use Research Ethics Committee (UIACUREC: 12/13/05).

Light microscopy (LM)

Samples of the testes were fixed in Bouin's fluid and embedded in paraffin blocks. Sections 2-4 µm thick were stained with Haematoxylin and Eosin and Periodic Acid Schiff (PAS) (Rao and Shaad, 1985). The slides were then studied under a light microscope (Olympus BX63 with a DP72 camera).

Transmission electron microscopy (TEM)

Additional testicular tissues were fixed in glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) for 4 hours at 4 ^{0} C. The samples were then thoroughly washed in the same buffer, post-fixed in 1% osmium tetroxide, and subsequently dehydrated in a graded series of ethanol solutions. Tissues were then cleared with propylene oxide, infiltrated with a 1:1 solution of propylene oxide:epoxy resin, 1:2 solution of propylene oxide:epoxy resin, and then placed in 100% epoxy resin for 36 hour under vacuum. The samples were embedded in fresh epoxy resin and cured at 60 ^{0} C for 48 h. Semi-thin sections were stained with toluidine blue and observed under the light microscope (Olympus BX63 with a DP72 camera). Utra-thin sections (70-80 nm) were cut with a diamond knife on an ultramicrotome (Ultracut-Reichert, Austria). The sections were then double-stained with uranyl acetate and lead acetate. The copper grids were examined under a transmission electron microscope (Philips CM 10 TEM) operating at 80 kv. Representative micrographs of different sections of the testis were taken using a Gatan 785 Erlangshen digital camera (Gatan Inc., Warrendale, PA). Analysis and assembling of composite micrographs were carried out using Adobe Photoshop CS5 (Adobe Systems, San Jose, CA). Morphometric analyses of Sertoli cell were performed with the aid of GIMP 2 Software using five serial sections of each of ten semi-thin sections of testicular tissues stained with Toludine blue, totaling 50 serial sections per parameter of the Sertoli cell. The Sertoli cell parameters measured include length and width of nuclei as well as diameter of nucleoli.

RESULTS

Observations with the LM

The testicular tissue of the African sideneck turtle was composed of parenchyma consisted of numerous seminiferous tubules and interstitial tissue (Fig. 1). Seminiferous tubules were of various size and shape with circularly shaped ones making up about 70% of the testicular tissue. Each seminiferous tubule was also composed of a basement membrane lined with non-germinal elements. Sertoli cells and germ cells were arranged in successive layers, representing different stages of cell division (Fig. 2). Sertoli cell of the African sideneck turtle comprised broad bases resting on a basal lamina and the cytoplasmic processes extending to the adluminal surface of the seminiferous tubules (Figs. 2 and 3). The Sertoli cell nucleus, well outlined by PAS, was pear-shaped, possessing a prominent nucleolus (Fig. 3).

Observations with the TEM

The Sertoli cell nuclei of the African sideneck turtle located on the basal lamina of the seminiferous tubules, underneath primary spermatocytes or on same plane with spermatogonia (Fig. 4). TEM revealed that the Sertoli cell nucleus, measured between 10 to 12 µm in length, with a width ranging between 8 to 10 µm at the widest point. The Sertoli cell nucleolus ranged from 1 to 1.5 µm in diameter and was usually centrally located within the nucleus of the Sertoli cell (Fig. 4). The extensive apical cytoplasm of Sertoli cells contained numerous mitochondria and lipid droplets, rough and smooth endoplasmic reticula, free ribosomes, vesicles as well as vacuoles (Fig. 5 and 6). The Sertoli cell process not only enclosed microtubules on both sides of elongating spermatids but also extended into the adluminal surface of seminiferous tubules as sperm columns (Fig. 7).

Sertoli cells were engaged in two prominent cell-to-cell contacts, the Sertoli-germ cell and Sertoli-Sertoli contacts. The Sertoli-germ cell contact enclosed Sertoli cell nucleus and some organelles including mitochondria (Fig. 8). The Sertoli-germ cell contact was composed of zonula occludens (tight junction), zonula adherens (anchoring junction) as well as macula adherens (desmosomes) (Fig. 9). At the tight junction, the dense

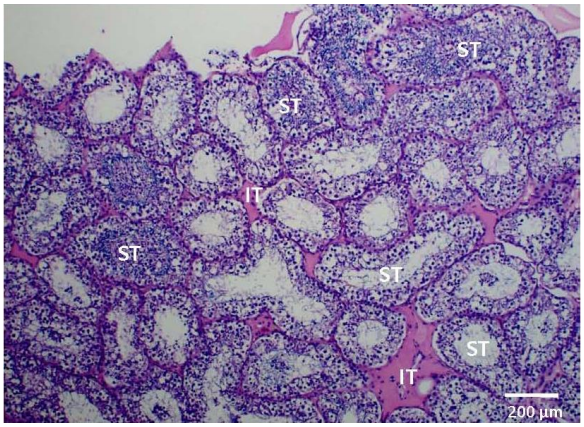

Fig. 1: LM of the testis of the African sideneck turtle showing seminiferous tubules (ST) of different shapes and sizes. IT: Interstitial tissues (H&E).

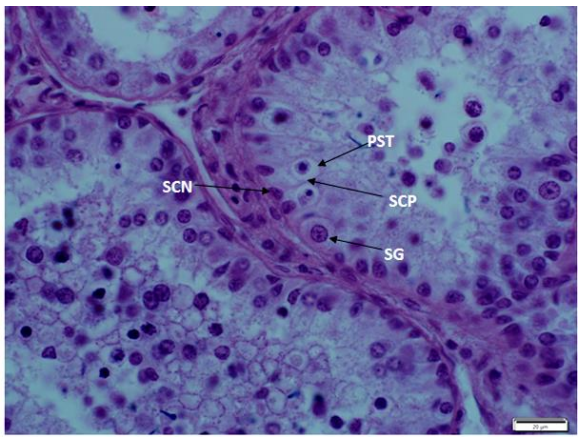

Fig. 2: LM of the testis of the African sideneck turtle.SCN: Sertoli cell nucleus. SCP: Sertoli cell process. SG: Spermatogonium. PST: Primary spermatocyte (H&E).

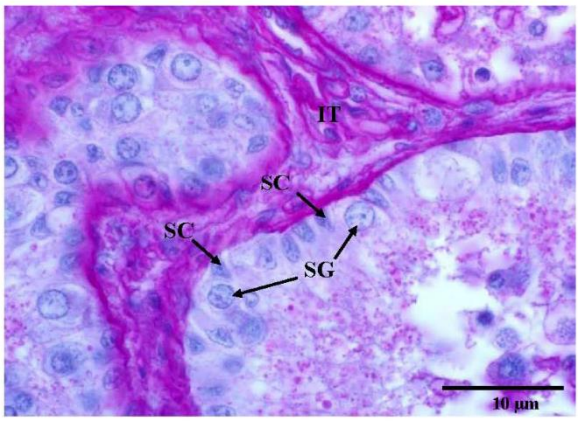

Fig. 3: LM of the testis of the African sideneck turtle. SC: Sertoli cell nucleus with nucleoli. SG: Spermatogonium. IT: Interstitial tissue. (PAS).

outer leaflets of adjoining membranes converge with the width of the membrane being about 0.07 µm. The anchoring junction lied below the zonula occludens linking it with the desmosomes. The space between outer leaflets of adjoining membranes was about 0.15 µm at the anchoring junction. The macula adherens lied below the anchoring junction and was composed of opposing membranes reinforced by dense plaques (Fig. 9). The width of the opposing membranes of the macula adherens

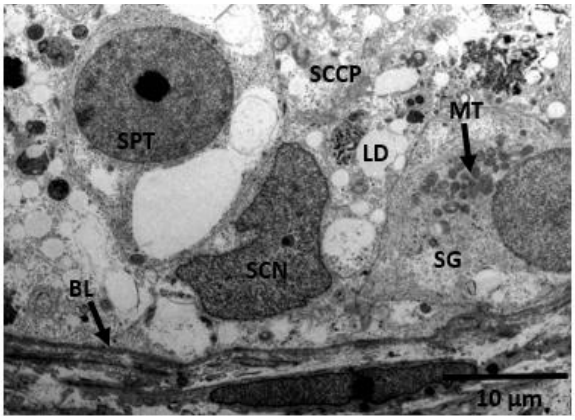

Fig. 4: TEM of the testis in the African sideneck turtle, showing the interaction between Sertoli and spermatogenic cells.SCN: Sertoli cell nucleus. SCCP: Sertoli cell cytoplasm. SG: Spermatogonium. SPT: Spermatocyte. BL: basal lamina. LD: Lipid droplet. MT: Mitochondria

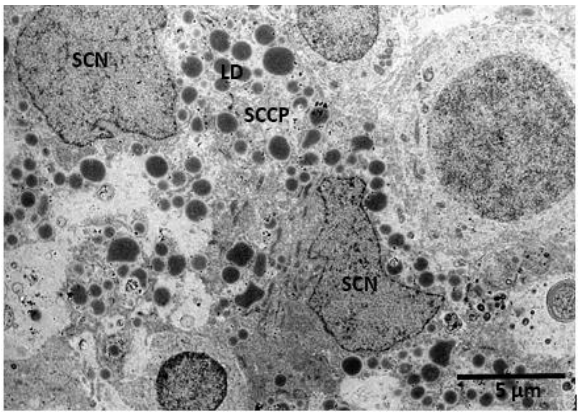

Fig. 5: TEM of Sertoli cell in the African sideneck turtle. SCN: Sertoli cell nucleus. SCCP: Sertoli cell cytoplasm. LD: Lipid droplets.

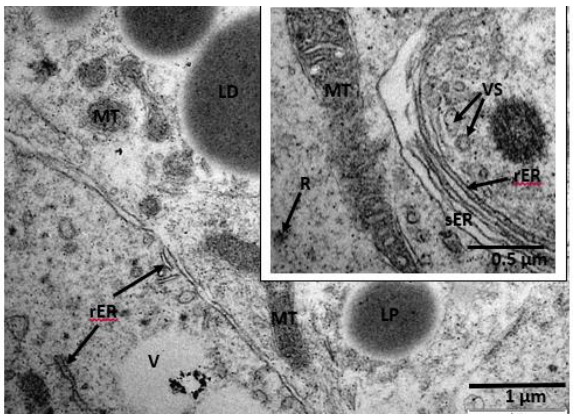

Fig. 6: TEM of the cytoplasm of a Sertoli cell. MT: Mitochondria. rER: Rough endoplasmic reticulum. LD: Lipid droplet. V: Vacuole. Inset. MT: Mitochondria. R: free ribosomes, rER: rough endoplasmic reticulum; sER: smooth endoplasmic reticulum; VS: Vesicles.

was about 0.2 µm. Unlike the Sertoli-germ cell contact, the Sertoli-Sertoli cell contact was composed of only tight junctions formed by the convergence of the outer leaflets of opposing Sertoli cell membranes in the basal region of the seminiferous epithelium (Figs. 10 and 11). The width of the outer leaflets of adjoining membranes of the Sertoli-Sertoli tight junction was about 0.05 µm.

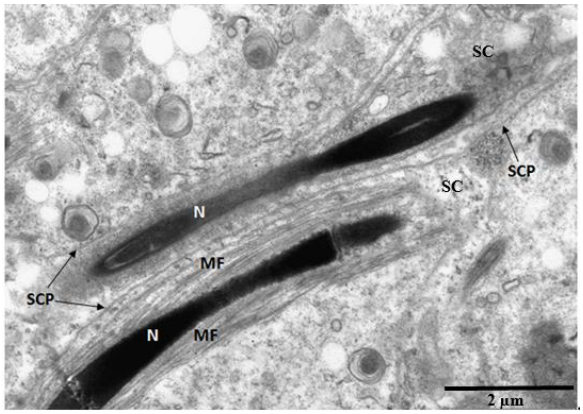

Fig. 7: TEM of the testis in the African sideneck turtle, showing a Sertoli cell process (SCP) enclosing the longitudinal sections of elongating spermatids. N: nucleus of spermatid. MF: microtubules of Sertoli cell. SC: sperm column.

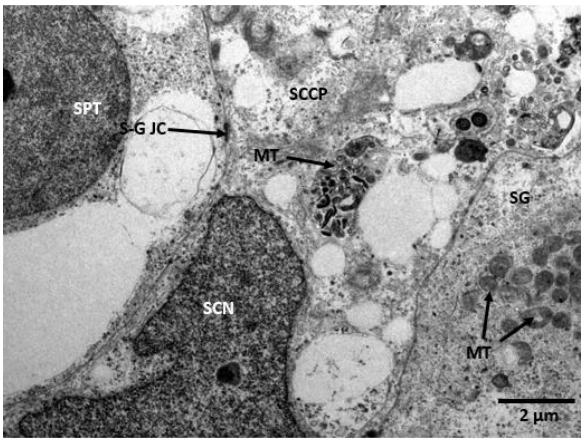

Fig. 8: TEM of the testis of the African sideneck turtle, showing Sertoli-germ cell junction (S-G JC). MT: Mitochondria. SG: Spermatogonium. ST: Spermatocyte. SCN: Sertoli cell nucleus. SCCP: Sertoli cell cytoplasm.

DISCUSSION

The histological and ultrastructural features of the testis of the African sideneck turtle is similar to previous reports on the reptilian testis (Al-Dokhi and Al-Wasel, 2001, 2002; Gribbins*et al*., 2003; Al-Dokhi*et al*., 2007; Araujo*et al*., 2013; Sever *et al*., 2013; Andrade *et al*., 2013). The germinal (spermatogonia, spermatocytes and spermatids) and non-germinal elements (Sertoli cells) of the seminiferous tubules observed in this study are similar in structure and organisation to those reported in the Chinese soft-shell turtle (*Pelodicus sinensis*) and other reptiles (Munoz *et al*., 2001; Johnson *et al*., 2008; Andrade *et al*., 2013, Ahmed, 2016). The basal position of Sertoli cell nuclei and its cytoplasmic processes extending to the adluminal compartment of the seminiferous tubules of the African sideneck turtle is similar to that reported in the turtle and reptile generally (Gribbins *et al*., 2003; Al-Dokhi *et al*., 2007; Araujo *et al*., 2013; Park *et al*., 2015; Ahmed *et al*., 2016, 2017). The Sertoli cell of mammals had been well reported in several species to have its nucleus with nucleoli, close to the basement membrane of seminiferous tubule as well as extensive cytoplasm that reaches the adluminal surface of seminiferous tubule (Morales *et al*., 1987; Bartke *et al*., 1994; França and Hess, 2005; Costa and Silva, 2010).

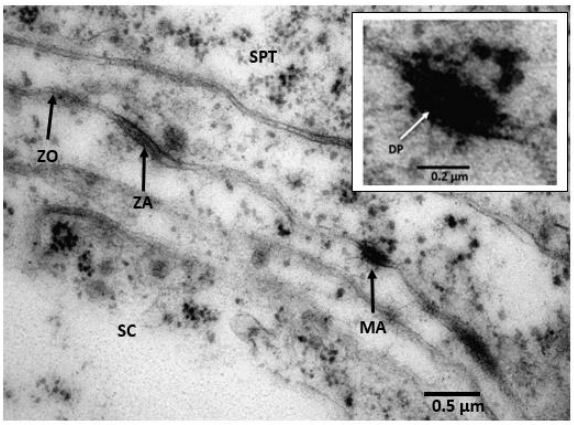

Fig. 9: TEM of the testis in the African sideneck turtle, showing the components of the Sertoli-germ cell contact. SC: Sertoli cell. SPT: Spermatocyte. ZO: Zonula occludens. ZA: Zonula adherens. MA: Macula adherens. Inset: The macula adherens, showing the dense plaques (DP).

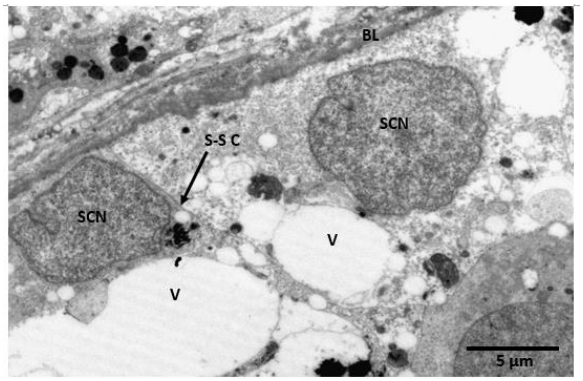

Fig. 10: TEM of the testis in the African sideneck turtle showing interaction between two Sertoli cells. SCN: Sertoli cell nucleus. S-S C: Sertoli-Sertoli cell contact. BL: Basal lamina. V: Vacuole.

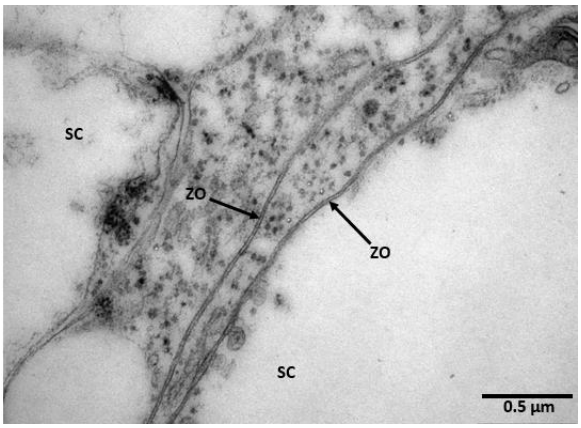

Fig. 11: TEM of the testis in the African sideneck turtle showing the Sertoli-Sertoli contact.SC: Sertoli cell. ZO: Zonula occludens.

The morphology of the Sertoli cell of the African sideneck turtle is similar to that of the Chinese soft-shell turtle (*Pelodicus sinensis*), in terms of its nuclear and nucleolar components as well as its cytoplasmic processes (Ahmed, 2016, 2017). The dimension and morphology of both the nucleus and nucleolus of the African sideneck turtle are also similar to those earlier reported in reptiles (Modesto and Anderson, 2004; Gribbins, 2011). Sertoli cell nuclei have been reported to exhibit a variety of

shapes, but they are usually oval- or pear-shaped with significant indentations in the nuclear membranes (Bacha and Bacha, 2000; Ahmed *et al*., 2016). The large distinctive nucleolus of the Sertoli cell nucleus observed in the African sideneck turtle had been reported to be the major characteristics of cells possessing high metabolic rates (Johnson, 1991; Johnson *et al*., 2008).

The organelle types found in the cytoplasm of Sertoli cells are similar to those reported for the Asian soft-shelled turtle: *Pelodicus maackii*, (Park *et al*., 2015) and *Pelodicus sinensis*, (Ahmed *et al*., 2016, 2017). The abundance of lipid droplets in the cytoplasm of Sertoli cells of the African sideneck turtle can be attributed to their role in spermatogenesis. Spermatogenesis is a complex process producing haploid spermatozoa, and the formation of lipid droplets within Sertoli cells is critical to maintaining normal spermatogenesis (Ahmed *et al*., 2017). The predominance of mitochondria and endoplasmic reticula in the cytoplasm of the Sertoli cell of African sideneck turtle suggests a marked capacity of Sertoli cells for synthesizing and metabolizing certain steroids. The presence of numerous mitochondria within the envelope created by Sertoli cell around the developing spermatid is also a clear indication that high metabolic activities go on within the seminiferous tubules during active spermiogenesis.

The enveloping by the African sideneck turtle Sertoli cell cytoplasmic processes of the germ cells, provides clear evidence of the supportive and nursing roles it plays to the germinal elements of the seminiferous tubules. These roles are brought about by the different interactions of Sertoli cells with the germs cells and are in consonance the findings of Park *et al*., 2015 and Ahmed *et al*., 2016 in the Korean soft-shell turtle, *Pelodicus maackii*, and the Chinese soft-shell turtle (*Pelodicus sinensis*), respectively. The extensive sperm columns formed by Sertoli cell processes from the basal lamina of the seminiferous tubule to its adluminal surface illustrate that Sertoli cells extend their cytoplasm to communicate directly with developing germ cells and thus provide structural support. Similar findings had been recorded in the Sertoli cells of the viscacha (*Lagostomus maximus maximus*) as well as in the Chinese soft-shell turtle (Munoz *et al*., 2001; Ahmed *et al*., 2016). The reptilian Sertoli cells play a principal role in the process of spermatogenesis starting from the division of spermatogonia up to the differentiation of mature spermatozoa (Al-Dokhi*et al*., 2004). The microtubules observed within the envelope formed by Sertoli cell processes around elongating spermatids support the findings of Ahmed *et al., (*2016) that Sertoli cytoskeleton in turtles is composed of microtubule, microfilament and intermediate filaments.

The Sertoli-Sertoli and Sertoli-germ cell junctions observed in this study can be traced to the role of the Sertoli cell in the creation of the BTB. The basal position of the Sertoli cell junctional complexes within the seminiferous tubules of the African sideneck turtle is consistent with previous report on the position of the BTB (Setchell, 2008). Studies have shown that the BTB anatomically divides the seminiferous epithelium into the basal and adluminal compartments so that spermiogenesis and spermiation take place in a specialized microenvironment at the apical compartment of the seminiferous epithelium composed of only Sertoli cells and developing germ cells (O'Donnell *et al*., 2011; Cheng and Mruk, 2011, Cheng *et al*., 2011). The presence of desmosomes and anchoring junctions in the Sertoli-germ cell contacts of the African sideneck turtle is in agreement with previous reports on the components of the BTB (Setchell, 2008; Mok*et al*., 2011). Both desmosomes and anchoring junctions have been reported to provide signaling function and communication between adjoining membranes of Sertoli cell contacts and thus maintain the immunological barrier during the epithelial cycle (Cheng *et al*., 2011).

Sertoli-Sertoli tight junctions have been reported to mediate paracellular sealing and thus restrict diffusion of fluid and small molecules through the BTB (Smith and Braun, 2012). This barrier creates a physiological milieu for spermatogenesis in the seminiferous tubules thereby protecting germ cells from auto-immune responses (Park *et al*., 2015). The periodic opening and closing of the BTB is required in the maintenance of spermatogenesis since it forms an immunological barrier that sequesters post-meiotic germ cell antigens from the immune system (Puri and Walker, 2013). The BTB has been described as one of the tightest blood-tissue barriers in mammals, restricting the diffusion of fluid and preventing the destruction of germ cells by the immune response (Wong and Cheng, 2005; Li *et al*., 2006; Setchell, 2008; Tsukita*et al*., 2008). Hence, a dysfunctional BTB will not only result in germ cell apoptosis but also inhibit germ cell differentiation as well as arrest spermiogenesis.

Conclusions

The structural difference between Sertoli-Sertoli and Sertoli-germ cell contacts observed in this study agrees with our hypothesis that there are structural differences between the two types of cell contact and thus confirm previous findings on Sertoli cell contacts in turtles (Park *et al*., 2015; Ahmed *et al*., 2016, 2017).

To the best of our knowledge, there is no report on the cytology of the Sertoli cell in any turtle of African origin. The Sertoli cell nuclear and cytoplasmic characteristics as well as junctional complexes of the African sideneck turtle could be traced to their roles in the creation of the BTB and the nursing of germ cells. However, seasonal and stage-dependent morphological changes of the Sertoli-Sertoli and Sertoli-germ cell contacts of the turtle remain to be investigated.

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