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Characterization of inter-Sertoli cell tight and gap junctions in the testis of turtle: Protect the developing germ cells from an immune response

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ABSTRACT

It is conceivable that early developing germ cells must across the basal to the luminal region of seminiferous tubules (STs) during spermatogenesis is associated with extensive restructuring of junctional complex. However, very limited information is documented about these junctional complexes in reptiles. In the present study we have determined the localization of inter-Sertoli cell tight junctions (TJ's), protein CLDN11 and gap junction protein Cx43 during spermatogenesis in the testis. In early spermatogenesis, weak immunoreactivity of CLDN11and focal localization of Cx43 was observed around the Sertoli cell in the luminal region, but completely delaminated from the basal compartment of STs. In late spermatogenesis, strong focal to linear localization of CLDN11and Cx43 was detected at the points of contact between two Sertoli cells and around the early stages of primary spermatocytes in the basal compartment of STs. In late spermatogenesis, localization of CLDN11and Cx43 was drastically reduced and seen only around Sertoli cells and spermatogonia near the basal lamina. However, transmission electron microscopy revealed that inter-Sertoli cell tight junctions were present within the basal compartment of STs, leaving the spermatogonia and early primary spermatocytes in the basal region during mid spermatogenesis. Gap junctions were observed between Sertoli cells, and Sertoli cells with spermatogonia and primary spermatocytes throughout spermatogenesis. Moreover, adherens and hemidesmosomes junctions were observed during spermatogenesis. The above findings collectively suggest that the intensity and localization of TJ's and gap junctions vary according to the spermatogenetic stages that might be protected the developing germ cells from own immune response.

1. Introduction

Inter-Sertoli tight junctions (TJ's), that create the blood-testis barrier (BTB), divide the seminiferous epithelium into the basal and the adluminal compartments within the mammals. For instance, Sertoli cell TJs mediate paracellular sealing near the basal lamina in the rat testis, this continuous circumferential seal restricts diffusion of fluid and other small molecules through the BTB. This obstruction creates a physiological milieu for spermatogenesis in the seminiferous tubules (STs) and protects germ cells from the immune response [1,2]. TJ's are comprised of three types of integral membrane proteins including claudins, occludins, JAMsandseveral associated peripheral proteins such as zonula occludens-1 (ZO1), −2, and −3 cingulins, and other junctional adhesion molecules [3,4].

The claudin 11 (CLDN11) is a key TJ protein among the claudins family that builds up the BTB in between Sertoli cells in the testis of mammalian and avian species [5–11]. In rodent testis, CLDN11 deficiency causes the functional and structural abnormality [12,13]. While, male CLDN11 null mice remain sterile, Sertoli cell detaches from the basement membrane, and their STs contain the aggregates of nucleated cells and Sertoli cells along with abnormal spermatogenesis, this illustrates the significance of CLDN11 in spermatogenesis [14,15]. Several studies about the TJ's have been well documented in mammals [16–19] and avian species [4,20,21], while, very limited information is available about the ultrastructure of TJ's within the reptiles.

Gap junctions are intercellular channels that attach the cell membranes of neighboring cells; this allows intercellular passage for the smaller size particles and regulating key processes during development and differentiation of various cells. Gap junctional pairing occurs between adjacent Sertoli cells, Sertoli cells with spermatogonia, and spermatocytes within the rat testis [22]. Each gap junction channel is formed by two hemichannels or connexons, and each connexon is

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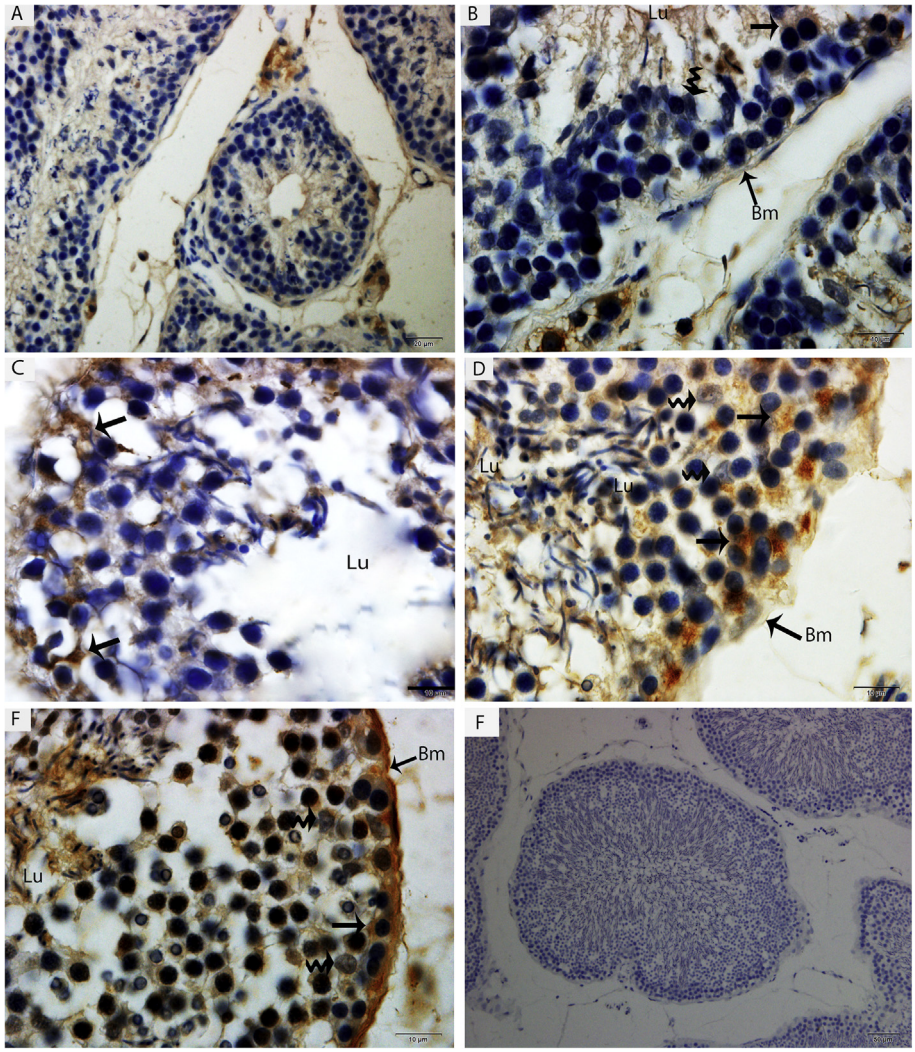

Fig. 1. Immunohistochemical localization of CLDN11 in *P. sinensis* testis. (A, B) During early spermatogenesis in May, (C, D) mid spermatogenesis in July and (E) late spermatogenesis in October. (F) Negative control. Curved arrow: Sertoli cell, (arrow): positive localization, Bm: basal membrane, Lu: lumen. Scale bar = 50 μm (F), 20 μm (A) and 10 μm (B, C,D, E).

composed by aggregation of protein subunits known as connexins [23]. While, connexin 43 (Cx 43) also known as GJA1 (gap junctional protein), is the most predominant testicular gap junction protein found in the rat and human testis [24,25]. Cx43 knockout mice were not viable postnatally due to cardiac abnormality, and their testes were found to be hypotrophic due to severe decreased number of germ cells within the STs and delayed maturation of Sertoli cells in adulthood. This knockout mouse models have demonstrated the significance of Cx43 during spermatogenesis [26,27]. However, the precise localization of the Cx43 has not been demonstrated in the reptile testis.

Spermatogenesis is dissociated from male mating behavior in several reptilian species. In China, Chinese soft-shelled turtles (*Pelodiscussinensis*) are widely distributed and known as one of the common species of reptiles [28,29]. Spermatogenesis in the soft-shelled turtle was noted to start in May and continuously progresses during summer and early autumn, while, spermiation being occurred by late October or early November. In the STs, majority of germ cells progressed in a single cohort through the various phases of spermatogenesis. This turtle is an ideal model to study various morphological changes in the testes, because its spermatogenesis is completed in longer time period (5–6 months) as compared to mammals [30]. Hence, we speculate that the localization of TJ's and gap junctions might vary during spermatogenesis in the testes of Chinese soft-shelled turtle. The current study investigated the characterization of inter-Sertoli cell tight junction and gap junction during various stages of spermatogenesis by immunohistochemistry, immunofluoresence and transmission electron microscopy.

2. Materials and methods

2.1. Animals

Fifteen mature male (3-4 years-old) *Pelodiscus sinensis,* soft-shelled turtles were purchased from an aqua farm in Nanjing, Jiangsu province of China in May (early spermatogenesis), July (mid spermatogenesis) and October (late spermatogenesis), five turtles during each time period. Then, all turtles were rendered comatose using intraperitoneally administered sodium pentobarbital (20 mg/animal) and were then sacrificed by cervical dislocation. The testes were collected immediately and fixed to performed different techniques (details below). Sample preparation was conducted according to accepted international standards and was approved by the Ethics Committee for Animal Care and Use by the Science and Technology Agency of Jiangsu Province (SYXK (SU) 2010-0009).