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Influence of low temperature on structure and dynamics of spermatogenesis during culture of *Oreochromis niloticus*

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ABSTRACT

Understanding the influence of different temperature conditions on the spermatogenesis is important for improvement of the fish aquaculture. This study evaluated the influence of low temperature on structural and quantitative dynamics of the spermatogenesis in *Oreochromis niloticus*. Adult males were cultivated with room temperature water (20.28–22.46 °C) and testes were collected for histological, ultrastructural and morphometric analyses. This species has unrestricted lobular testis with cystic spermatogenesis and type I spermiogenesis that results in a single anacrosomal aquasperm. Seminiferous lobules and spermatogenic cells had a radial arrangement toward the spermatic duct. Superior and central portions of testes had a greater lobular area than the inferior portion in all samplings. Spermatogonia (9.3%) were distributed in the inferior portion of testes, spermatocytes (25.3%) and spermatids (34.4%) in the central portion, while spermatozoids (39.4%) and secretory cells (4.6%) in the superior portion. Throughout the study, correlation between water temperature and lobular area characteristics was significant only in the inferior portion of testes ($r^2 = 0.95$), although the lobular area in the other testicular portions increased when the water temperature increased by 2 °C. Correlation between the water temperature and spermatogenic cells was significant for undifferentiated spermatogonia ($r^2 = 0.54$) and number of spermatids ($r^2 = 0.67$). It is concluded that low cultivation temperatures may positively influence the generation of primary spermatogonia in the inferior periphery of *O. niloticus* testes. In addition, males maintain reservoirs of germ cells at low temperatures and the radial zonation of spermatogenesis has an important role in the renewal and production of germ cells.

1. Introduction

The Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) (Perciformes: Cichlidae) is one of the most cultivated freshwater fish species in world aquaculture (Food and Agriculture Organization of the United Nations (FAO), 2016). This species has great breeding potential due to its hardiness, rapid growth rate, adaptation to different conditions and the good organoleptic characteristics of its flesh (Bhujel, 2000; Little and Hulata, 2000). In *O. niloticus* males develop quickly and reach a larger body size in comparison to females, thus, have a greater commercial importance because of carcass yield (Mair et al., 1997). Tilapia males are

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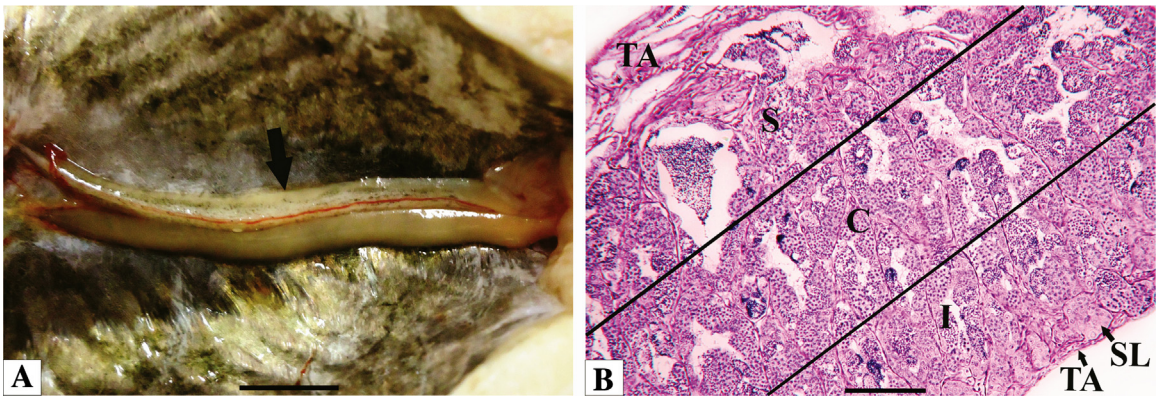

Fig. 1. Macroscopic (A) and microscopic (B) characteristics of the analysed testes in *O. niloticus*. (A) Filiform testes (arrow) in maturation at the beginning of the experiment (day 0). (B) Superior (S), central (C) e inferior (I) portions examined in transverse plane of the testes. TA, tunica albuginea; SL, seminiferous lobules. Scale bars: 1 cm (A), 200 μm (B).

also excellent models for the study of reproductive biology because the cystic spermatogenesis arrangement that allows a clear morphometric analysis of the spermatogenic cells (Matta et al., 2002; Vilela et al., 2003).

During spermatogenesis, three distinct phases usually occur: spermatocytogenesis, meiosis, and spermiogenesis (Papah et al., 2013). In spermatocytogenesis, primary spermatogonia undergo a series of mitotic divisions to produce spermatocytes, which go through two consecutive meiotic divisions that give rise to spermatids (Schulz et al., 2010). In spermiogenesis, the spermatids undergo drastic changes such as chromatin condensation, cytoplasmic reduction, and development of the flagellum, resulting in spermatozoa (Siqueira-Silva et al., 2012). After these stages are completed spermiation is complete and spermatozoa are released into the lumen of the spermatic duct (Fishelson, 2003). The morphological organization of the testes differs among groups of fish, and the dynamics of spermatogenesis also reflects different reproductive strategies (Burns et al., 2009; Melo et al., 2011).

The evaluation of environmental factors in the reproduction of fish provides important information for the management of the species in aquaculture (Schulz et al., 2006). Water temperature is one of the important environmental variables that regulate diverse reproductive parameters in teleosts, especially gonadal development, maturation and spawning (Fraser et al., 2002; Arantes et al., 2011; Domingos et al., 2012). Temperature is also an important spermatogenesis modulator in fish, because duration, function and effectiveness of the spermatogenic process are correlated to temperature (Koger et al., 1999; Shimizu, 2003; Dadras et al., 2016). Specifically, *O. niloticus* males are able to reproduce throughout the year if the water temperature is about 25 °C or greater (Lacerda et al., 2006). Although the reproduction of *O. niloticus* is affected by low temperatures, few studies address this relationship quantitatively under cultivation conditions (Vilela et al., 2003; Alvarenga and França, 2009). According to the results of previous research, lesser water temperatures (20 °C) result in increased proliferation of spermatogonia, Sertoli and Leydig cells in Nile tilapia, whereas greater temperatures (30–35 °C) resulted in a more rapid germ cell differentiation.

Studies investigating the influence of different temperature conditions on the reproductive system of Nile tilapia males are important for management and the improvement of the cultivation of this species. Thus, the present study aims to evaluate the structural and quantitative dynamics of spermatogenesis of *O. niloticus* during culture at low temperatures.

2. Materials and methods

2.1. Fish sampling

The experiment was conducted from June to August 2011 in the Aquaculture Laboratory of the School of Veterinary Medicine, Federal University of Minas Gerais (19°52′16.42″S 43°58′13.90″W) in Belo Horizonte, state of Minas Gerais, Brazil. A group of *O. niloticus* adult males and females was kept for 1 month in 5 m^3 tanks with continuous aeration by an air diffuser, photoperiod of 12 h:12 h light:dark, and heaters with thermostatic control were used to maintain the appropriate water temperature for gonadal development of the species (mean ± SD temperature: 27.33 ± 0.15 °C, dissolved oxygen: 4.72 ± 0.21 mg l^{-1}, pH: 7.04 ± 0.12, conductivity: 0.28 ± 0.05 mS cm^{-1}, total dissolved solids: 0.17 ± 0.03 g/l, salinity: 0.13 ± 0.02 ppt). Fish were fed *ad libitum* twice daily with commercial feed (32% crude protein, 6–8 mm extruded), and the remains were collected after 30 min. After macroscopic selection for sex gender, 40 adult *O. niloticus* males were transferred to hapas de 1 m^3 (eight animals per happa) in a 5 m^3 tank with cultivation conditions similar to those previously described, except for the room temperature water (temperature: 21.32 ± 1.28 °C, dissolved oxygen: 6.96 ± 0.38 mg l^{-1}, pH: 7.31 ± 0.17, conductivity: 0.23 ± 0.07 mS cm^{-1}, total dissolved solids: 0.16 ± 0.05 g/l, salinity: 0.11 ± 0.04 ppt), which was unfavourable for reproduction of this species (Vilela et al., 2003). The study period corresponded to the winter season in a region with tropical highland climate according to the Köppen classification (Alvares et al., 2013). The physicochemical variables