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Apoptosis-mediated seasonal testicular regression in the Japanese Jungle crow (*Corvus macrorhynchos*)

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Abstract

The present study investigated effects of apoptosis observed during seasonal testicular regression in Japanese Jungle Crows. The study was conducted during January to June 2008, 2009. Testes from adults captured during non-breeding (January), prebreeding (February to mid-March), main-breeding (late March to early May), transition (mid-May to late May), and post-breeding (June) seasons were analyzed. Apoptosis was assessed by *in situ* terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) assay. Paired-testis volume increased 95-fold from the non-breeding to the main-breeding season ($P < 0.05$), and subsequently decreased 26-fold from the main breeding to the post-breeding season ($P < 0.05$). Testicular activity was evaluated from the total germ cell count and sperm index, which increased 42- and 5-fold, respectively, in the main-breeding season, and subsequently decreased 33- and 5-fold in the post-breeding season. In testes, TUNEL-positive germ cells were at low levels in the non-breeding season, absent in the prebreeding and the main-breeding seasons, and highest in mid-May ($P < 0.05$). In contrast, TUNEL-positive Sertoli cells occurred only in late-April. In addition, TUNEL-positive fibroblast-like cells were observed in the outer zone of the tunica albuginea in the post-breeding season. Collectively, these data suggested that the seasonal rise in the testicular competence occurred slowly in Japanese Jungle Crows; however, testis function was terminated rapidly after the breeding season. Furthermore, we concluded, similar to other avian species, Sertoli cell apoptosis followed by massive germ cell death was responsible for rapid testicular regression in Jungle Crows.

Keywords: Apoptosis; Breeding; Crow; Testicular regression; Testis; TUNEL

1. Introduction

In adult birds and mammals, spermatogenesis is characterized by germ cell proliferation, differentiation and maturation into spermatozoa in the seminiferous tubules. To achieve this precise homeostasis, of each germ cell type in the adults, germ cell renewal, proliferation export and apoptosis must be finely balanced [1–3]. This appears to occur at the cost of substantial germ cell wastage in mammals [2,4]. In addition, apoptosis might be the responsible for seasonal testis regression both in mammals and birds [5–8]. However, mechanisms of germ cell apoptosis during testicular activity remain unclear.

Photoperiod is considered a main temporal factor in many wild avian species, including crows, for seasonal reproduction in temperate zones [9,10]. Changes in gonadal functions are mediated in response to photoperiod through the hypothalamic-pituitary-gonadal axis in birds, as well as in other species of amniotes. The photoperiod of long day length can initially stimulate

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testicular development, as well as cause testicular regression. The modulation from short to long day lengths stimulates the onset of seasonal reproductive activities in the European Starling (Sturnus vulgaris) [7]. In photosensitive avian species, long day lengths initially induce testicular development in adult males. Long-term exposure to long-day photoperiod, however, inhibits the hypothalamo-pituitary-gonadal response to stimulatory long days and results in the onset of photorefractoriness. Inhibition of the hypothalamo-pituitary-gonadal action gives rise to a marked increase in prolactin concentrations and a marked decrease in GnRH. Subsequently, gonadotropins-LH and FSH concentrations drop to undetectable values [8,11]. These hormonal changes, along with elevated prolactin concentrations, appeared to be responsible for rapid atrophy in both testicular volume and function [12].

The Jungle Crow (*Corvus macrorhynchos*) is a very widespread Asian species of crow. The habitat range is extensive and stretches from the northeastern Asian seaboard to Afghanistan and the eastern Islamic Republic of Iran in the west, and south to India down to the Malaysian peninsula in the southeast. They prefer environments with an abundant supply of large garbage or carcasses in cities and coastal areas [13]. Kuroda [14] divided the breeding cycle into prebreeding (February–mid-March), main-breeding (late March–May), post-breeding (June–August), and non-breeding (September–January) seasons. The prebreeding or early breeding signs of the Jungle crow begin with selection of a nest-site and nesting is completed within the last week of March [14,15]. In many seasonally breeding species, the sexually active testis enlarges many-fold in the main breeding season, due to proliferation of spermatogenic cells and expansion of the seminiferous tubules [16]. In contrast, during transition from the main breeding to the non-breeding state, a reduction in testis size coincides with reduced spermatogenesis, lowered testosterone production, and ultimately a loss of reproductive activity. Despite substantial changes in testicular size and reproductive activity during the transition from the breeding to the non-breeding seasons, the time course of the morphologic changes and cellular types involved during seasonal testicular regression have not described in the Japanese Jungle Crow.

Apoptosis is a mechanism that regulates normal tissue development and homeostasis [17]. Apoptotic activation is initiated by extra- or intracellular pathways and leads to a cascade of signaling events that ultimately results in DNA fragmentation, cell shrinkage, and disassembly of the nuclear envelope. Neighboring cells then phagocytose the dying cell, generally without triggering a systemic immune response [18]. Apoptosis is the pre-eminent form of cell death that occurs during seasonal testicular regression in most vertebrates studied to date, wherein cell death limits the renewal of the germ cell population [7]. However, both germ cells and spermatogenesis-supporting Sertoli cells have been observed undergoing apoptotic cell death in the European Starling [7]. These birds undergo testicular regression in response to photoperiod more rapidly than that observed in many mammals, and Sertoli cell death is believed to serve as a mechanism to allow expeditious testis atrophy to occur [7]. Recent studies on testicular regression in the form of apoptosis were done both naturally and experimentally by manipulating photoperiods and analyzing hormone profiles in several breeding mammals, including horses (Equus caballus) [6], silver foxes (Vulpes vulpes) [19], Djungarian hamsters (Phodopus sungorus) [20], Syrian hamsters (Mesocricetus auratus) [21] and mice (Peromyscus leucopus) [2,22,23]. In contrast, only a few experimentally induced testicular apoptotic studies have been done in birds, including the European Starling [7] and the American Crow (Corvus brachyrhynchos) [1]. Moreover, the degree to which testis size is reduced after the breeding season was much more extensive (90–95% compared with 50–70% regression) in most birds than it is in most rodents e.g., white-footed mouse (Peromyscus leucopus) [23]. Data on the seasonal testicular regression mediated by germ cell apoptosis in birds are rare, except reports on the American Crow [1]. The effects of germ cell apoptosis on testicular regression and the apoptotic cell types are unknown in the Japanese Jungle Crow. Thus, the objectives of the present study were to quantify seasonal apoptosis of germ cells and Sertoli cells during the seasonal breeding cycle, and the contribution of apoptotic cell death to seasonal testicular regression in the Japanese Jungle Crow. We expected that analysis of apoptotic germ cell death will provide a greater understanding of the mechanisms and timing of germ cell apoptosis during the seasonal breeding cycle in this species.

2. Materials and methods

2.1. Collection of birds

Adult Jungle Crows used in this investigation were obtained from the Ueno Zoo of Tokyo, Niza City of Saitama Prefecture and Moka City of Tochigi Prefecture, Japan from January to June in both 2008 and 2009. The catching of crows was permitted by Niza City (Permit nos. 01 and 02) and Tochigi Prefecture (Permit