International Journal of Advanced Research in Biological Sciences
www.ijarbs.com

Research Article

# Regulation of ovarian follicular Atresia through apoptotic process in Japanese quail (*Coturnix coturnix japonica*)

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## Abstract

Apoptosis is a form of physiological cell death, essential for normal embryonic development and adult tissue homeostasis and so it is often referred as a programmed cell death. A majority of primordial and developing ovarian follicles in both avian and mammalian species are eliminated on a continuous basis throughout the female's reproductive life via follicle atresia. The objective of this study was to determine whether the follicular atresia of the ovarian follicle in Japanese quail (*Coturnix coturnix japonica*) is regulated by the apoptotic process. Initiation of atresia in avian species is initially restricted to slowly growing white follicles. Additionally, atretic follicles were easily distinguishable from the healthy counterparts of the same developmental state based on the presence of surface hemorrhages and/or collapsed and deformed condition. The ovaries were collected from the slaughtered meat quails meant for sale. The follicles were dissected and pooled according to the size and the morphological characters showing healthy/atretic changes. Total DNA was prepared from each pool of the follicles and processed for ethidium bromide staining in 2% agarose gel using standard protocol. Histological studies were also carried out to find the atretic follicles in the ovary. The ethidium bromide staining showed the presence of typical apoptotic DNA ladder pattern in the atretic follicles as a result of internucleosomal cleavage of cellular DNA. The histology study indicated the presence of characteristic of apoptotic cells in the follicles. The present findings suggest that the ovarian follicular atresia in Japanese quail is regulated by the process of apoptosis.

**Keywords:** Japanese Quails, ovarian follicle, apoptosis, histology

## Introduction

The Japanese quail is the smallest avian species highly preferred as farming bird under intensive system of management for meat, egg and have become an important experimental animal for scientific research. They belongs to the order Galiformes, family Phasidae, genus *Coturnix*, and species *japonica* (Rezvannejad et al. 2013). Many of the molecules involved in mammalian apopotic signalling and follicular development have also been shown to function in the ovaries of chickens and quail. Ovarian follicles that initially begin to grow but fail to reach the fully differentiated stage would become atretic. Based upon the etiomology of the word (greek; a = not, tresia = perforated), follicular atresia sternly refers to the failure of a ovarian follicle to ovulate. Once the primordial follicle pool is established, depletion of most of the remaining oocytes occurs indirectly as a result of atretic degeneration of follicles where atresia occurs prior to selection into the preovulatory hierarchy. The death and resorption of ovarian follicles occurred via apoptotic process (Tilly et al., 1991). Atresia occurred with high pre-hierarchical follicles and under the normal physiological conditions and it is generally absent among preovulatory hierarchical follicles. The high rate

of atresia in pre-hierarchical follicles correlates with the susceptibility of the granulose cell layer to undergo apoptosis. It has been proposed that a transition from atresia-susceptibility to atresia-resistance is initiated coincident with follicle selection into the preovulatory hierarchy (Johnson, 2003).

Role of atresia in hen is well studied (Gilbert et al., 1983) through dye method where, atresia occurred in the smaller follicles long before they reach ovulation size in domestic hen. The incidence of atresia in the small yolky follicles has been recorded for some seasonally breeding birds [Erpino, 1969, 1973; Kern, 1972). Atresia commonly occurred at the time of breeding in those birds, increased during the advance of the breeding season to reach a peak at the time of nesting. Apoptotic cells are recognized by a characteristic fragmentation of their DNA which is generated by endonucleolytic cleavage of genomic DNA into oligonucleotides that are multiples of approximately 180-200 bases, which is the striking biochemical halmark in apoptosis. This fragmentation of DNA gives a typical ladder pattern on gel electrophoresis. The aim of the present study was to find out the whether atresia takes place through the apoptotic process in both the prehierarchal and hierarchal follicles of Japanese quail (*Coturnix coturnix japonica*) by observing DNA laddering pattern and histological examination follicles.

## Materials and Methods

The present experiment complies with all relevant institutional and national animal welfare guidelines and policies.

Japanese quail (n = 320) hens maintained at Quail farm in Central Agricultural Research Institute, Andaman and Nicobar Island, were used for the study. Only productive healthy birds within the age group 6 to 12 week were used. The birds were maintained under standard management conditions with optimal feed, water and light. The ovaries were collected from post slaughtered birds meant for sale of meat.

Follicles were dissected from ovaries of the sexually matured Japanese quail that were slaughtered for meat purpose. The follicles were pooled according to their hierarchal (Fig 1) status: a) F1- 15-22 mm follicles that were destined for imminent ovulation, b) F2- 10-15 mm yellow follicles, c) F3- <10 mm rapidly growing yellow follicles, d) WF- slowing growing healthy white follicles, e) AF-atretic follicles which is easily distinguishable from their healthy counterparts of the same developmental state based on the presence of surface hemorrhages and/or a collapsed and deformed condition (Gilbert et al., 1983).

Total DNA was isolated from individual follicles as described (Janz and Van Der Kraak, 1997) and it was processed for ethidium bromide staining. DNA/lane was resolved through 2%agarose gels, stained with ethidium bromide and visualized under UV light. The molecular size of the DNA fragments was estimated by comparison of migration distance to a 100-bp DNA ladder.

The ovaries collected from the post slaughtered birds were fixed in the 10% neutral buffered formal saline and embedded in paraffin wax. Five micron thick sections were made and stained with haematoxylin-eosin for histological examination by following standard procedure (Banks, 1974).

## Results and Discussion

The hierarchal position of the follicles in Japanese quail showed different hierarchical status as shown in Figure 1. In most of the birds, ovary showed F1, F2, F3, small yellow follicles and numerous white follicles which indicate multiple hierarchies. The present observation is similar to many studies which showed that an abundance of LYF commonly results in multiple hierarchy arrangement. However, certain birds showed less number of yellow follicles, white follicles and variation in hierarchial status and few birds showed completely regressed ovaries with a lack of yellow follicles. This variation in ovarian follicular activity and hierarchical status could be attributed to genetic, nutritional and/or management conditions.

The DNA pattern of F1, F2, F3 follicles and WF from Japanese quail showed a clear and sharp band, whereas DNA prepared from the atretic follicles displayed a ladder pattern as a result of internucleosomal fragmentation of DNA into 180 – 200 bp multiples, is shown in Figure 2. In the white follicles there was no clear fragmentation but still there was dispersed nature of the DNA indicating the low concentration of DNA that might have undergone complete atresia. The exact mechanism by which apoptotic internucleosomal DNA fragmentation occurs is a complex process and remains unclear. A variety of caspase substrates are involved in the regulation of DNA structure, repair and replication (Nicholson and Thornberry, 1997). It was found that CAD (Caspase-activated DNase) and DNAS1L3 (deoxyribonuclease I-like 3) cooperate to process chromatin degradation during apoptosis (Nicholson and

Thornberry, 1997). DNAS1L3 achieves such function by translocating from the ER to the nucleus. As this study is carried out using total DNA of the individual follicle it was not able to identify the type of cell (i.e., granulosa vs. theca cells) undergoing apoptosis. In quails, incidence of atresia was high among prehierarchical follicles (<9mm in diameter). On the contrary, under normal physiological conditions it is absent among preovulatory hierarchical follicles (Gilbert et al., 1983). The results of the present study are similar to the observation in mature leghorn chicken (Tilly et al., 1991) and rat ovary (Angela and John, 1994).

Histological observation of atretic follicles were characterized by the dispersed nature of the granulosa cell, as observed in Figure 3 and this could be due to estradiol and IGF-I which is involved in controlling apoptosis in granulosa cells during follicular atresia (Yuan Song et al., 2004). Atretic follicles were recognized by several histologic characteristics: disruption, pyknosis, and thinning of the granulosa cell layer with some degree of hypertrophy of the theca cell layer. Pyknotic cells was clearly observed in the granulosa cells as this clearly indicates the sign of apoptosis as pyknosis is the irreversible condensation of chromatin in the nucleus of a cell undergoing apoptosis [Kroemer et.al., 2009; Ramalingam, 1996). The cytoplasm with yolk granules and the nucleus were found to be absorbed partially. The thecal layer was distinct and was not able to differentiate as theca interna and theca externa. These were found in the smaller and the medium sized follicle and classified under the category of invasive atretic follicles. As the regression advance, the cytoplasm with yolk granules and the nucleus were found to be absorbed partially or fully. The present study demonstrates that follicular atresia is regulated by apoptosis. Further studies are needed with autoradiographic identification of DNA cleavage and the gene expression study would help to know about the genomic and endocrine regulation of apoptotic mechanism involved in avian ovarian follicular atresia.

**Figure 1**: F1, F2, F3- Hierarchal position of large yellow follicles, SYF -small yellow follicles, LWF- large white follicles, SWF- Small white follicles.


**Figure. 2** Internucleosomal fragmentation of DNA into 182bp multiples of various classes of follicles of *Japanese quail* (lane AF, WF, F3, F2, F1). by 2% Agarose gel electrophoresis containing 0.5% ethidium bromide. First lane is 100-bp ladder that was used as a marker.

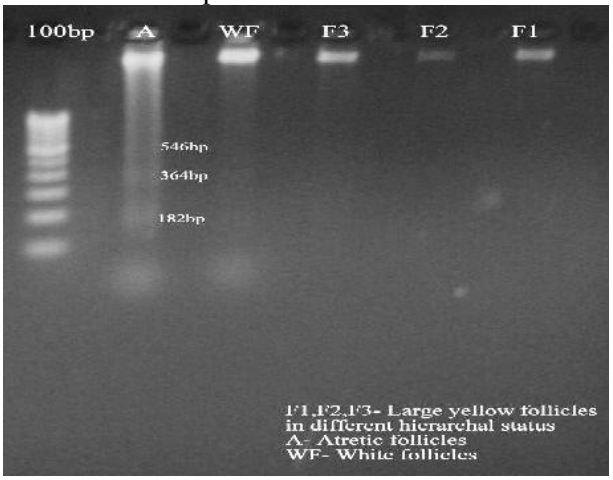


**Figure 3**: H&E stain, paraffin section, 100 µm showing various stages of developing follicle in japanese quail

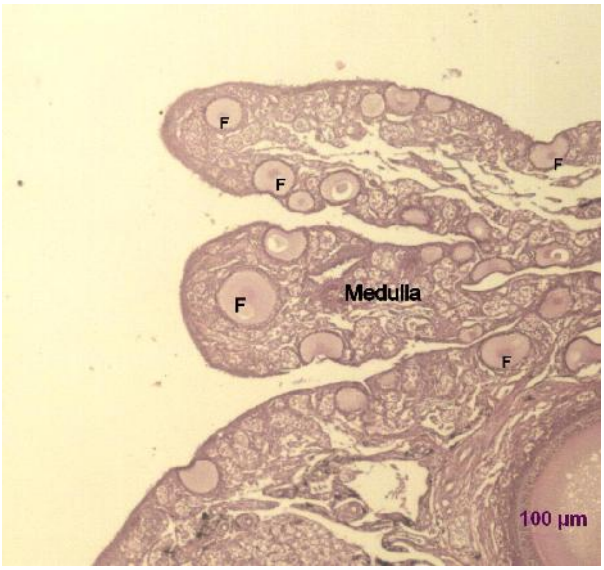
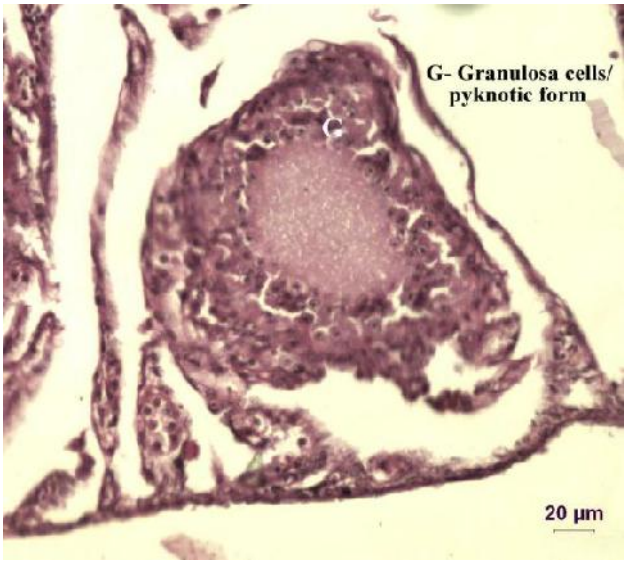



**Figure 4:** H&E stain, paraffin section, 100 µm showing Atretic follicle (bursting type) in japanese quail




## Conclusions

Atresia is regulated by apoptotic mechanism in the ovary of the Japanese quail. Histological examination revealed that ovarian follicles could be classified into primary, developing, mature, atretic and post ovulatory follicle based on the structural detail in Japanese quail. This study paves way in finding the mechanism involved in ovarian follicle development in avians and to find out various management and breeding options to regulate follicular growth. Japanese quail could be used as an experimental model where it is easy to maintain and it is similar molecules that are involved in mammalian apopotic signalling and follicular development.

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