RESEARCH ARTICLE

Ovarian Follicles Morphology and Morphometry of Adult Kadaknath and Vanaraja Chicken

Vijay Kumar, Sanjay Kumar Bharti, Avnish Kumar Gautam*, Manoj Kumar Sinha

Abstract

The present experiment was conducted on gross anatomical and histological observation of ovary in twelve laying Kadaknath and Vanaraja Chicken. Under gross observation the shape of the left ovary was elongated triangular in the form of bunch of grapes in Kadaknath and Vanaraja chicken. Histological observation of the ovary showed that the division of the ovary in to cortex and medulla was evident in both the breeds. The surface epithelium was made up of single layer of cuboidal cells with spherical to oval nuclei. However, squamous epithelium was also noticed at some places. Developing primordial and primary follicles were seen in the outer cortical zone just beneath the germinal epithelium in both the breeds. Proliferation was noticed in germinal epithelium at several places and cells were seen migrating from here in to the cortical area. This layer was surrounded by flattened/stromal cells and these cells formed almost continuous layers.

Key words: Chicken, Kadaknath, Morphology, Morphometry, Ovarian Follicles, Vanaraja.

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Introduction

In the worldwide poultry industry, egg-laying production efficiency is a significant economic trait. Rural poultry farming is of great importance in a state like Bihar (India) as it not only generates income levels, employment opportunities to marginal landless farmers including women but also brings about desired socio-economic change in rural areas which are crucial for rural development and rural prosperity. Kadaknath poultry birds are unique, medium sized indigenous breed of fowl popularly found in Jhabua and Dhar districts of Madhya Pradesh, popularly known as Kala masi because the bird is black inside and outside including skin, feathers, legs, meat, blood, etc. These birds are poor in egg production potential, but their black flesh is very delicious and popular among tribal people. Sometimes this flesh is being used for the treatment of many diseases by tribal, which needs proper scientific intervention (Thakur *et al.,* 2006). Vanaraja birds, a dual purpose variety for free range farming in rural and tribal areas are developed by ICAR-Directorate of Poultry Research, Hyderabad. The females of Vanaraja lays about 130-150 eggs in a laying year. The bird is hardy and has better immunocompetence due to which it is successfully adaptable under backyard farming system (Niranjan *et al.,* 2008). Due to its multicoloured plumage, brown eggs and meat taste similarity with desi birds, it is well accepted by the rural people including tribals. The laying performance of chickens is determined by the growth, development and function of chicken ovarian follicles (Johnson, 2015). The laying of eggs starts with an orderly development of follicles in the chicken ovaries until the eggs are produced. Therefore, without the progressive stages of ovarian follicles' growth and development, the chickens' egg production performance will be reduced (Wang *et al.,* 2017; Li *et al.,* 2019). Hence, the present study was undertaken to reveal the hierarchy of ovarian follicles in Kadaknath and Vanaraja chicken.

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Materials and Methods

The present study was conducted in Histology laboratory of Department of Veterinary Anatomy, Bihar Veterinary College, Patna (India). A total of 24 adult female Kadaknath (12) and Vanaraja (12) chicken were purchased from local market and Indian Council of Agricultural Research, North Eastern Region Zone, Patna, and Institutional Livestock Farm Complex of the College in Patna. Birds were euthanized by injecting overdose of sodium pentobarbital IP at the dose rate of 120 mg/kg and the organs of interest were harvested carefully (Gourdon, 2016; Gautam *et al.,* 2020). After the collection of ovary, gross morphology and morphometry were performed with scale and digital Vernier calliper.

For histological study, the organs of interest were fixed in 10% neutral buffer formalin for 48-72 h at room temperature.

After fixation the tissues were washed in running tap water for overnight, dehydrated in ascending grades of ethyl alcohol, cleared in two change of xylene followed by paraffin impregnation in a thermostatically controlled oven to prepare paraffin blocks. Sections were cut into 5-6 µm thickness and stained with Haematoxylin and Eosin staining for general histo-architecture (Luna, 1968), Gomori Reticular stain for reticular fibres (Bancroft and Stevens, 1996), Verhoeffs staining for elastic fibres (Luna, 1968) and Masson trichrome staining for collagen fibres (Luna, 1968) were used.

Results and Discussion

In current study, the mature ovaries of Vanaraja and Kadaknath chicken gave typical appearance of a bunch of grapes, and were located just ventral to the ventral surface of the anterior lobe of left kidney (Fig. 1, 2). These findings were in accordance with observation of Dyce *et al*. (1987) in chicken, Mahajan *et al.* (2021) in White Leghorn and Kadaknath hens and Akinloye (2014) in Japanese quail. In both breed the mature ovary was situated at the upper part of the abdominal cavity just below the left last two ribs as earlier reported by Ghosh (2003) in domestic fowl. The left ovary of Vanaraja and Kadaknath chicken had yellowish, rounded mass called follicles, each containing an ovum or yolk. The mature ovary showed numbers of follicles of various sizes which were attached each other with small stalk (Fig. 1, 2). Similar findings were reported in adult chicken by King and McLelland (1975), King (1977) and Dyce *et al*. (1987).

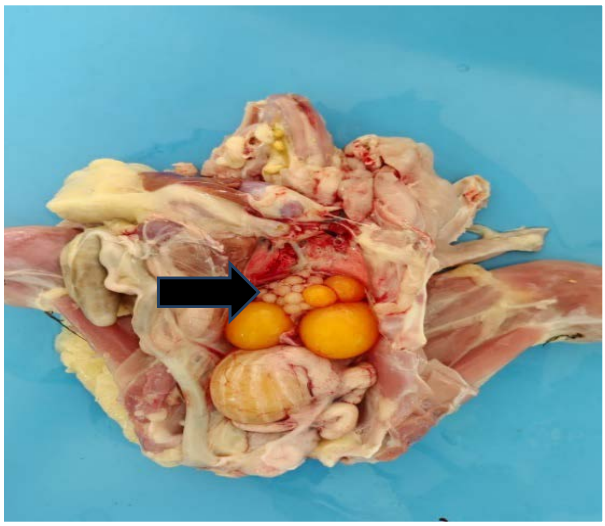

Fig.1: Gross photograph showing in situ female reproductive parts of adult Vanaraja chicken.

Fig.2: Gross photograph showing in situ female reproductive parts of adult Kadaknath chicken.

Fig.3 Gross photograph showing in situ ovary (OV), spleen (S), Provneticulus (P) and caecum (C) of adult Vanaraja chicken

Fig. 4: Gross photograph showing of diameter of mature follicles in Vanaraja chicken

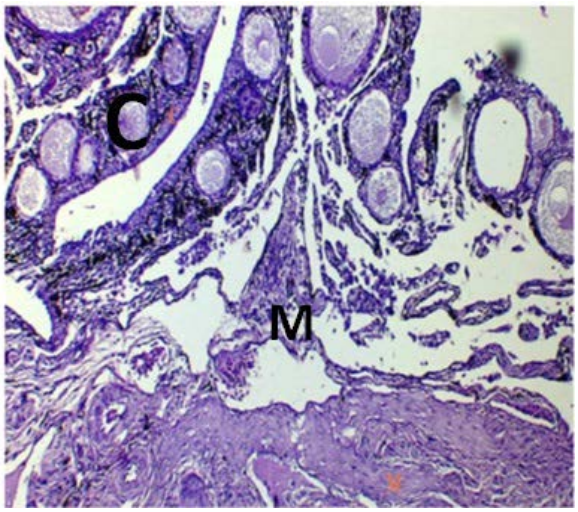

Fig. 5: Photomicrograph showing cortex (C) and medullary region (M) of ovary in Kadaknath chicken (H & E, X 100)

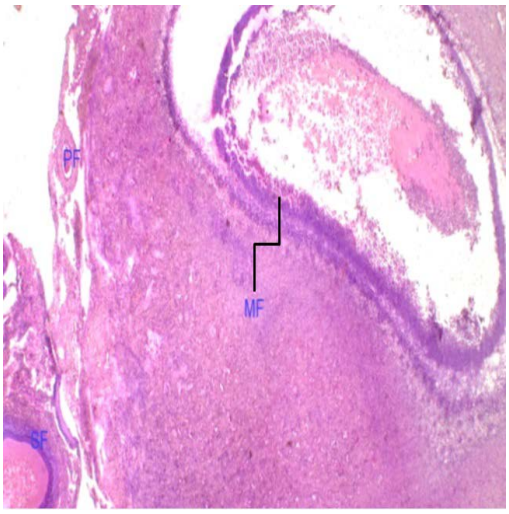

Fig. 6: Photomicrograph showing primary follicle (PF), secondary follicle (SF) and mature follicle (MF) in Vanaraja chicken (H & E X400)

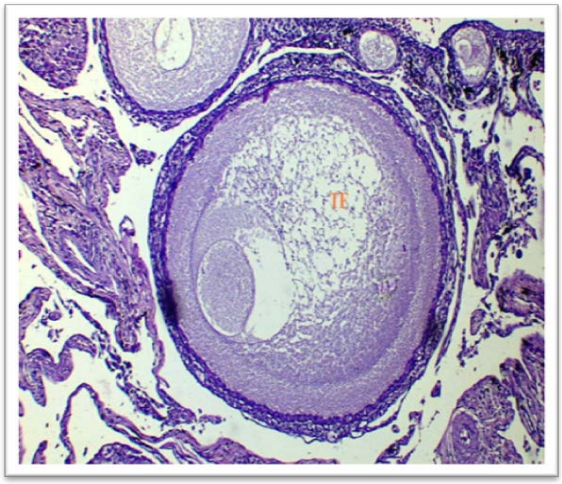

Fig. 7: Photomicrograph showing different stages of primordial follicles, tertiary follicles, eccentrically placed nucleus of ovary in Kadaknath chicken (H & E X 100)

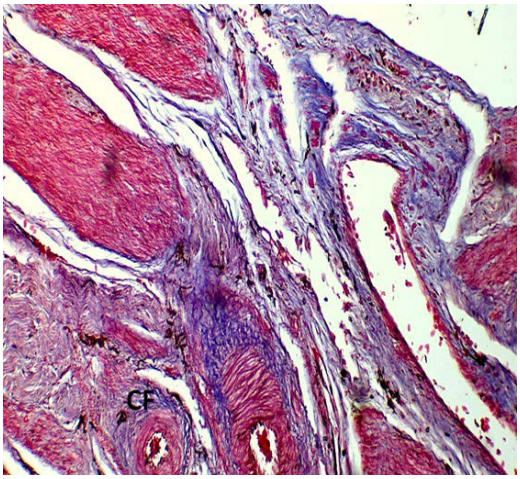

Fig. 8: Photomicrograph showing the collagen fibers (CF) in cortex region of ovary in Vanaraja chicken (Masson trichomes X400)

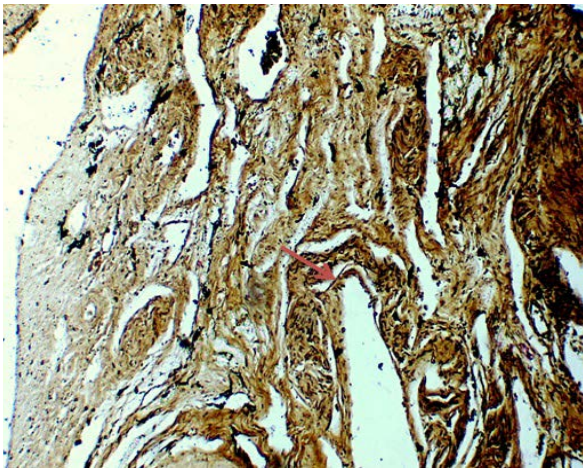

Fig. 9: Photomicrograph showing reticular fibers (arrow mark) in ovary of Kadaknath chicken (Gomori stain for reticulam X100)

Most of the follicles were rounded to oval in shape and remained in contact with the gizzard ventrally and proventriculus right laterally, spleen on the mid line, right lobe of liver right laterally and caudal part of ileum and caeca caudally (Fig. 3) and reached the adrenal gland at its cranial border with aorta at medial aspect.

The average diameter of mature follicles of ovary was 21.88±0.23 mm and 20.90±23.83 mm in adult Vanaraja and Kadaknath chicken, respectively. Its range varied from 20.03 mm to 23.05 mm in Vanaraja chicken, whereas in Kadaknath chicken it ranged from 19.83 mm to 21.97 mm. (Fig. 4). Significant difference was observed in the diameter of mature follicles of Vanaraja and Kadaknath chicken.

Histological Observations

The histomorphological study on the ovary of adult female Vanaraja and Kadaknath chicken revealed that the ovary

consisted of outer parenchymatous cortex and inner vascular medulla and division between cortical and medullary layers was indistinct as earlier reported by Komarek and Prochazkova (1970) and King (1977) in fowl, Alshammary *et al.* (2017) in geese, Dhyaa and Al-Saffar (2015) in duck and Parida *et al.* (2000) in Japanese quail. In the present study, cells of the cortex were more basophilic, while in medulla it was more eosinophilic in both breed of birds. Rao and Vijayaragvan (2000) and King (1977) reported that with the onset of sexual maturity the distinction between cortex and medulla was virtually lost.

Ovarian Cortex

Ovarian cortex of both Vanaraja and Kadaknath breed of chicken consisted of numerous ovarian follicles of different size within the whole cortex area. A germinal layer of cuboidal epithelial cells covered the cortex as well as a very thin layer of dense connective fibres, which formed the tunica albuginea. Below the tunica albuginea stroma of loose connective tissue were evident in both breed. In current study ovarian follicles were observed in different stages of development throughout the stroma of cortex and differentiated into primordial, primary, secondary and tertiary follicles (Fig. 5). Similar findings were reported in domestic duck by Hodge (1974) and Rao and Vijayaragvan (2000).

Primordial Follicles

In both the breed of chicken the primordial follicles, the quiescent oocytes were enclosed by a single layer of squamous type of follicular cells. These follicles were observed near the tunica albuginea. The primordial follicles were differentiated from primary follicle by their size and volume as the size was more in primary follicle and also had homogeneous cytoplasm with fine granules (Fig. 5).

Primary and Secondary Follicles

The oocytes in primary follicles were surrounded by low or high cuboidal epithelium. The follicles and oocytes were larger than that of primordial follicle in both breed of chicken.

In both the breed of chicken, the secondary follicles were observed throughout the central part of cortex and characterized by larger follicles, which had well developed oocytes with clear cytoplasm and fine granules with centrally placed or eccentrically placed nucleus. Secondary follicles of ovary were larger than the primary follicles with large oocyte and covered by many layers of follicular cells (Fig. 6).

Tertiary Follicles

The tertiary and matured follicles of both breed of chicken contained growing oocytes covered by many layers which were the theca externa, theca interna, membrane granulosa and peri vitteline membrane with rounded prominent nucleus. The theca externa was chiefly composed of dense connective tissue that had groups of luteal cells which were also observed in cortical stroma (Fig. 7).

The theca interna of the follicular wall consisted mainly of collagen fibers and less amount of reticular and elastic fibers (Fig. 8). Numerous flattened fibroblast lied among the connective tissue fibers and smooth muscle fibers were observed at theca externa. The theca externa of tertiary follicles surrounded the theca interna which consisted of compact layer of spindle shaped smooth muscle cells. There was a distinct basement membrane which separated the theca interna from membrane granulosa. The stratum granulosum was made up of many layers of polyhedral epithelioid cells.

Medulla

The ovarian medulla of adult indigenous chicken consisted of numerous blood and lymph vessels with nerve fibres being meshed in a network of collagen fibers and reticular fibers with very few elastic fibres (Fig. 9). The blood vessels in medulla were comparatively larger than those present in the ovarian cortex. Similar findings were observed by Hodge (1974) and Dellmann and Carithers (1996).

Ovarian Stroma

The ovarian stroma of adult Vanaraja and Kadaknath chicken was formed by a network of connective tissue fibres consisting of collagen, reticular and elastic fibres (Fig. 8, 9). It also consisted of different follicles, fibroblasts, smooth muscle fibers, nerve fibers, blood and lymph vessels.

Conclusion

The ovarian morphology of Vanaraja and Kadaknath chickens reveals a well-organized structure, with distinct follicular development from primordial to tertiary stages. Both breeds exhibit similar ovarian characteristics, such as the presence of multiple follicular layers, well-developed oocytes, and complex stroma consisting of connective tissue, blood vessels, and nerve fibers. These findings provide a detailed understanding of the ovarian anatomy in indigenous chicken breeds, contributing to the knowledge of their reproductive physiology.

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References

Akinloye, A.K. (2014). Gross morphometric study on the reproductive system of Japanese quail (*Coturnixcoturnix japonica*). *African Journals Research Tropical Veterinarian, 32*(3), 87-96

Alshammary, H.K.A., Jabar, A.I., & Nasser, R.A. (2017). Geese ovary and oviduct from an anatomical and histological point of view. *Research Journal of Pharmaceutical, Biological and Chemical Sciences, 8*(6), 207-219.

Bancroft, J.D., & Stevens, A. (1996). *Theory and Practice of Histological Techniques*. 4th edn., Churchill Livingstone, New York.

Dellmann, H.D., & Carithers, J.R. (1996). *Cytology and Microscopic Anatomy*. 5th edn. Lea and Fibiger, Box 3024, USA, pp. 275-277.

Dhyaa, A.A., & Al-Saffar, F.J. (2015). The post-hatching development of the female genital system in Indigenous Mallard Duck (*Anas platyrhynchos*) *The Iraqi Journal of Veterinary Medicine, 39*(2), 17-25.

Dyce, K.M., Sack, W.O., & Wensing, J.G. (1987). *A Textbook of Veterinary Anatomy*. 2nd edn. W.B. Saunders Company, Philadelphia, pp. 791-792.

Gautam, A.K., Ray, S., Das, P., Mandal, A.K., Tudu, N.K., & Singh, D. (2020). Macroscopic study on the cerebrum of post-hatch broiler chicken with reference to age. *Journal of Entomology and Zoology Studies, 8*(3), 1926-1929.

Ghosh, R.K. (2003). *Primary Veterinary Anatomy*. 3rd edn., Current Book International, Kolkata, Mumbai, Chennai, India, pp. 214-215.

Gourdon, J. (2016). Avian Euthanasia-Standard Operating Procedure: In: *Comparative Medicine and Animal Resource Centre*, McGill.

Hodge, R.D. (1974). *The Histology of the Fowl*. Academic Press, London, New York, San Francisco, pp. 326-347.

Johnson, A.L. (2015). Ovarian follicle selection and granulosa cell differentiation. *Poultry Science, 94,* 781-785.

King, A.S. (1977). Aves urogenital system. In: *Sisson and Grossman's the Anatomy of the Domestic Animals*. Robert Getty (eds.), 5th edn. Vol. 2, W.B. Saunders Co., Philadelphia, p. 1937.

King, A.S., & McLelland, J. (1975). *Outline of Avian Anatomy*. Bailler Tindall, London, p. 65.

Komarek, V., & Prochazkova, E. (1970). Growth and differentiation of ovarian follicles in the post-natal development of chicken. *Acta Veterinsrica Brno, 29,* 11-16.

Li J., Luo W., Huang T., & Gong Y. (2019). Growth differentiation factor 9 promotes follicle-stimulating hormone induced progesterone production in chicken follicular granulosa cells. *General and Comparative Endocrinology, 276,* 69-76.

Luna, L.G. (1968). *Manuals of Histological Staining Methods of Armed Forces Institute of Pathology*. 3rd edn., McGraw Hill Book Co., London.

Mahajan, T., Joshi, S., Thanvi, P.K., & Saini, M. (2021). Gross and morphometrical studies on the female reproductive tract of White Leghorn and Kadaknath hens. *Journal of Animal Research, 11*(6), 1071-1076.

Parida, S., Ramesh Geeta., Siva Kumar, M., & Vijaragavan, C. (2000). Histological observations on the infundibulum of Japanese quail (*Coturnix coturnixjapanica*). *Indian Journal of Veterinary Anatomy, 12*(1), 62-68.

Rao, C.T.S., & Vijayaragavan, C. (2000). Micro-anatomical studies on the transitional and host gland of the domestic duck (*Anas boschasdomesticus*). *Indian Journal of Veterinary Anatomy, 12*(1), 81-85.

Thakur, M.S., Parmar, S.N.S., & Pillai, P.V.A. (2006). Studies on growth performance in Kadaknath breed of poultry. *Livestock Research for Rural Development, 18,* 1-93.

Wang, Y., Chen, Q., Liu, Z., Guo, X., Du, Y., Yuan, Z., Guo, M., Kang, L., Sun, Y., & Jiang, Y. (2017). Transcriptome analysis on single small yellow follicles reveals that Wnt4 is involved in chicken follicle selection. *Frontiers in Endocrinology, 8,* 317.