

A rare case of Triorchidism in indigenous Fowl

Shubham Sharma, Praggaya Priya Lakra*, M.K. Gupta and Amit Kumar Mahto

Department of Veterinary Pathology, College of Veterinary Science and A.H, Birsa Agricultural University, Ranchi, Jharkhand

Address for correspondence

Dr. Praggaya Priya Lakra, Assistant Professor cum Junior Scientist; Department of Veterinary Pathology, College of Veterinary Science and A.H, Birsa Agricultural University, Ranchi, Jharkhand; E-mail: praggaya.lakra@gmail.com

Received: 21.9.25; Accepted: 14.11.25

ABSTRACT

A case of triorchidism in an indigenous fowl was recorded in Ranchi, Jharkhand. Triorchidism is an exceptionally rare congenital abnormality, which could be ascribed to faulty embryological development with developmental cause that are distinct from testicular asymmetry. During postmortem examination of this fowl, three testes were observed. The third testis which was smaller as compared to the right and left testes was attached to the proximal end of the left testes with which it shared the same epididymis. The left, right and third testes weighed 4.2983 gm, 2.2193 gm and 0.8475 gm, respectively, whereas the length and width of the left, right and third testes were 1.2x2.9cm, 1.2x2.0cm and 1.0x1.2cm, respectively. The testes revealed, white fluid on cutting. This appears to be the first case of triorchidism in poultry from Jharkhand, India.

Keywords: Congenital, first, indigenous fowl, triorchidism

INTRODUCTION

Triorchidism is a condition described to the incidence of having three testes in an individual and has been reported earlier in poultry. Supernumerary right² and left³ testes have been reported in domestic chicken earlier. These authors also reported that the small round supernumerary testis was softer in consistency, and normal in color and showed spermatogenic activity on histological examination. Hocking systematically examined the testes of 378 male domestic chickens and found three cases of triorchidism, each of which comprised of two left testes⁴. Both right and left testis could be affected by this phenomenon. Triorchidism is different from testicular asymmetry, which is widespread in birds and has been found to correlate with age and secondary sexual characteristics⁵. The phylogenetic distribution of triorchidism in vertebrates is poorly known due to dearth of information.

METHODOLOGY

An adult male indigenous fowl was brought for postmortem examination to the Department of Veterinary Pathology, College of Veterinary Science & A.H, Ranchi, Jharkhand. Critical postmortem examination revealed presence of three testes (Fig 1) in this bird. It was noted that the third testis was smaller than the right and left testes and was attached to the proximal end of the left testis and shared the epididymis with the latter. The weight and length of all right, left and the third testes were determined using Metler's analytical balance and meter rule respectively. The left, right and third testes weighed 4.2983gm, 2.2193gm and 0.8475gm, respectively while the length and width of the left, right and third testes were 1.2x2.9 cm, 1.2x2.0 cm and 1.0x1.2 cm, respectively. When the third testis was cut open, a milky white fluid oozed out similar was the observation when the right and left testes were cut open. The right and left testes were bean-shaped whereas the third testis was slightly elongated in shape. The three testes were nearly identical in color and firmness (Fig 1, 2). The testicular tissues were then fixed in 10% formalin for histopathology. The tissue was routinely processed, sectioned and stained with Hematoxylin and Eosin (H&E) stain⁶.

How to cite this article : Sharma S., Lakra P.P., Gupta M.K., and Mehta A.K. 2026. A rare case of Triorchidism in indigenous Fowl. Indian J. Vet. Pathol., 50(1) : 86-89.

RESULT

Histopathological examination (Fig. 2) of right testis (T1) revealed presence of seminiferous tubules of variable sizes, which showed intact germinal layer, however in most of the tubules, there was detachment of spermatid and other developing stage of spermatogonia from germinal epithelium. In few places, the developing stages of spermatozoa showed continuity from germinal epithelium. In most of the seminiferous tubules, degenerative changes were observed. Spermatozoa were mostly in degenerative stages characterized by hypocellularity. Lumen of seminiferous tubules showed significant number of spermatozoa. No infiltrative changes in parenchyma or interstitium was seen. Calcification was also not observed.



Fig. 2. Gross: Triorchid testis found in desi fowl

In the left testis (T2), the epididymal lumen showed necrosis and calcification. Loss of columnar lining epithelial cells of duct was observed with lesser number of spermatogonia.

Hypocellularity was also marked. Seminiferous tubules were lesser in diameter and loss of germinal epithelial cells was evident along with calcified zone and necrosis.

In the smaller left testis (attached to proximal end of left testis, T3) there was loss of germinal cells. In most of the places, the seminiferous tubules revealed loss of germinal cells with only thin basement membrane or sparsely distributed germinal cells. In few seminiferous tubules, there was presence of spermatogonia but in most of the area spermatids were seen without formation of spermatogonia. Hypocellularity was significant.

| Testis | T1 | T2 | T3 |
|---|----|----|----|
| <p>Seminiferous tubule size</p> <p>Hypocellularity in T3</p> <p>H&E x400</p> | | | |
| <p>Germinal Layer</p> <p>1. Intact germinal layer in T1, T2</p> <p>H&E x1000</p> <p>2. Loss of germinal layer in T3</p> <p>H&E x1000</p> | | | |
| <p>Spermatozoa</p> <p>Lumen showed high Presence of spermatozoa in T1&T2 compared to T3.</p> <p>H&E x1000</p> | | | |

Fig.2. Histopathology

McFarland¹, who examined 2,000 male Japanese Quails and found one case of triorchidism in which the right testis was divided into two nearly co-equal sections, both of which were undergoing normal spermatogenesis.

DISCUSSION

In chicken, testis development is governed by the Z-chromosome-linked DMRT1 gene, which directly or indirectly activates the male factors, HEMGN, SOX9 and AMH. Recent single cell RNA-seq has defined cell lineage specification during chicken testis development, while comparative studies point to deep conservation of avian testis formation⁷.

Normally, the reproductive tract of poultry consists of a testis, epididymis and highly convoluted ductus deferens running alongside ureter. Poultry testes are paired organ, bean-shaped, light-yellow structures located within the abdominal cavity; along the backbone; near the cranial pole of the kidneys. Each testis is connected to a vas deferens, which transports sperm to the cloaca. Each testis is an aggregate of anastomosing seminiferous tubules, with associated interstitial space enveloped by connective tissue capsule. Testicular capsule is an important component of three layers: tunica mucosa, tunica albuginea and tunica vascularis. Tunica albuginea represents main tissue layer and comprises cellular elements that alternate with thick bundles of collagen fiber. Capsule is thinner in birds. Normally, the testis weighs from 0.4 - 28g. After 28 weeks, weight of testis significantly decreases, reaching 15g by week 42⁸. Gonadal differentiation in chick embryo occurs at 6.5 to 7 days of incubation. Embryonic testis is characterized by a germinal epithelium that recedes with time, a thicker capsule, absence of secondary or cortical sex cord as well as the presence of primary sex cord surrounded by stroma. Biochemically, gonadal differentiation is evident in terms of increased cyclic nucleotide concentration, increased protein synthesis and the pattern of sex steroid synthesis⁹. Once the testis has formed, the Mullerian duct ceases to develop and undergoes regression under the effect of AMH (*Anti Mullerian Hormone*). The gene encoding AMH is expressed in both male and female but higher in male during sexual differentiation¹⁰.

There are various factors which can lead to pathology/ supernumerary testes during developmental stages. Any disruption in mesodermal cells during gonadal development could lead to formation of accessory gonadal structure¹¹. Gonadal development is regulated by FSH (follicle stimulating hormone) & Testosterone. Genes like DMRT1 (*Doublesex and mab-3 Related Transcription factor 1*), SOX9 (*Sex-determining region Y-box9*) and PITX2 (*Paired haemodomain transcription Factor 2*) play crucial role in testicular differentiation along with regulating the left-right symmetry of the gonads during development¹².

The mutations in FGF9 (*Fibroblast growth factor 9*) and SF1 (*Steroidogenic factor 1*) lead to atypical gonadal development which can result in extra testicular lobules. Sertoli cells express SOX9 and DMRT1 as markers which are involved in spermatogenesis whose improper differentiation can result in development of additional testicular lobule¹³. Anomalies in basement membrane and tubular structure due to dysregulation in extracellular matrix protein facilitate atypical testicular formation in birds.

DMRT1 plays a central role in testicular development. DMRT1 is present on both ZZ chromosome in males but only on Z of ZW chromosome in female¹⁴. It is confirmed that DMRT1 is master Z-linked genetic trigger for testis formation in chicken^{15,16}. It encodes for zinc finger like transcription factor with DNA binding DM domain^{15,17}. Through the analysis of 5' regulatory region, it is proposed that DMRT1 first plays role in germ cell development and then is recruited to somatic cell of gonads, where it plays role in Sertoli cell specifications¹⁸. DMRT1 is required for SOX9, HEMGN (Hemogen) and AMH expression, while inhibiting the FOXL2/ Aromatase female pathway.

Testis formation in poultry is also attributed to activation of SOX9 by DMRT1. SOX9 is central hub gene required for initiation of pre-Sertoli cell development in gonadal medulla. In chicken, SOX9 in male is upregulated during testis formations^{17,19,20}. Downregulated SOX9 expression following DMRT1 knockdown in male gonads (ZZ) is ectopically activated when DMRT1 is mis expressed in ZW^{15,17,18}.

Another target FGF9, is required for proper Sertoli cell development and testis formation. DMRT1 protein is therefore likely to act as transcriptional activator and transcriptional repressor in embryonic avian gonads. Misexpression of the gene in the gonad induces SOX9, HEMGN, AMH and represses aromatase expression. HEMGN plays role in chicken testis development. Signals from the Sertoli cell lineage must drive interstitial cell development, and formation of the squamous surface epithelium, and induction of germ cell mitotic arrest though the exact nature of those signals are unknown. The resulting organ is a structurally and functionally integrated unit, supporting gain to genesis and male sex hormone production.

A similar case of triorchidism was reported in an indigenous breed of fowl in India²¹.

CONCLUSION

Avian testis development represents an ideal model for understanding the molecular genetics of vertebrate gonadal sex differentiation. Much of our knowledge in this area has come from studies on the chicken embryo. Thus, the cause of triorchidism affiliates to

all those factors or genes which play significant roles in the development of testes during embryonic stages. Congenital anomaly due to abnormal development might be the possible cause for triorchidism in this case.

Financial support & sponsorship: None

Conflicts of Interest: None

Use of Artificial Intelligence (AI) Assisted technology for manuscript preparation: The authors confirm that there was no use of AI – Assisted Technology for assisting in the writing of the manuscript and no images were manipulated using AI

REFERENCES

- McFarland LZ. 1965. A triorchid Japanese quail. *Poult Sci.* **44**:306–307. <https://doi.org/10.3382/ps.0440306>
- Katiyar AK, Shrivastava AB, Awadhya RP, Vegad JL. 1986. Supernumerary testes in a domestic fowl. *Vet Rec.* **118**:306–307.
- Shrivastava AB, Katiyar AK, Awadhya RP, Vegad JL. 1988. Triorchidism in a domestic fowl. *Vet Rec.* **123**(4):110.
- Hocking PM. 1992. Bilateral testicular asymmetry and supernumerary testes in the domestic fowl (*Gallus domesticus*). *Br Poult Sci.* **33**:455–460.
- King AS, McLelland J. 1975. Outlines of avian anatomy. 1st edn. London: Bailliere Tindall.
- Bancroft JD, Gamble M. 2008. Theory and practice of histopathological techniques. 6th edn. Philadelphia: Churchill Livingstone, Elsevier.
- Møller AP. 1994. Directional selection on directional asymmetry: Testes size and secondary sexual characters in birds. *Proc R Soc Lond B Biol Sci.* **258**:147–151. <https://doi.org/10.1098/rspb.1994.0157>
- Teng C. 1982. Ontogeny of cyclic nucleotides in embryonic chick gonads. *Biol Neonate.* **41**:123–131.
- Smith CA, Smith MJ, Sinclair AH. 1999. Gene expression during gonadogenesis in the chicken embryo. *Gene.* **234**:395–402. [https://doi.org/10.1016/S0378-1119\(99\)00225-6](https://doi.org/10.1016/S0378-1119(99)00225-6)
- Spranger R, Gunst M, Kuhn M. 2002. Polyorchidism: A strange anomaly with unsuspected properties. *J Urol.* **168**(1):198. <https://doi.org/10.1097/01.ju.0000021184.81676.7e>
- Ioannidis J, Taylor G, Zhao D, Liu L, Idoko-Akoh A, Gong D, Lovell-Badge R, Guioli S, McGrew MJ, Clinton M. 2021. Primary sex determination in birds depends on DMRT1 dosage, but gonadal sex does not determine adult secondary sex characteristics. *Proc Natl Acad Sci U S A.* **118**(10):e2020909118. <https://doi.org/10.1073/pnas.2020909118>
- Lee HJ, Seo M, Choi HJ, Rengaraj D, Jung KM, Park JS, Lee KY, Kim YM, Park KJ, Han ST. 2021. DMRT1 gene disruption induces incomplete gonad feminization in chicken. *FASEB J.* **35**:e21876. <https://doi.org/10.1096/fj.202100129R>
- Smith CA, Roeszler KN, Ohnesorg T, Cummins DM, Farlie PG, Doran T, Sinclair A. 2009. The avian Z-linked gene DMRT1 is required for male sex determination in the chicken. *Nature.* **461**:267–271. <https://doi.org/10.1038/nature08298>
- Takada S, Ota J, Kansaku N, Yamashita H, Izumi T, Ishikawa M, Wada T, Kaneda R, Choi YL, Koinuma K. 2006. Nucleotide sequence and embryonic expression of quail and duck Sox9 genes. *Gen Comp Endocrinol.* **145**:208–213. <https://doi.org/10.1016/j.ygcen.2005.08.009>
- Kent J, Wheatley SC, Andrews JE, Sinclair A, Koopman P. 1996. A male-specific role for SOX9 in vertebrate sex determination. *Development.* **122**:2813–2822.
- Da Silva SM, Hacker A, Harley V, Goodfellow P, Swain A, Lovell-Badge R. 1996. Sox9 expression during gonadal development implies a conserved role for the gene in testis differentiation in mammals and birds. *Nat Genet.* **14**:62–68. <https://doi.org/10.1038/ng0996-62>
- Lambeth LS, Raymond C, Roeszler KN, Kuroiwa A, Nakata T, Zarkower D, Smith CA. 2014. Over-expression of DMRT1 induces the male pathway in embryonic chicken gonads. *Dev Biol.* **389**(2):160–172. <https://doi.org/10.1016/j.ydbio.2014.02.012>
- Estermann MA, Major AT, Smith CA. 2021. Genetic regulation of avian testis development. *Genes.* **12**(9):1459. <https://doi.org/10.3390/genes12091459>
- Vizcarra JA, Kirby JD. 2015. Sturkie's avian pathology: Reproduction in male birds. In: Sturkie's Avian Physiology. 6th edn. London: Academic Press. p. 669.
- Akazome Y, Abe T, Mori T. 2002. Differentiation of chicken gonad as an endocrine organ: Expression of LH receptor, FSH receptor, cytochrome P450c17 and aromatase genes. *Reproduction.* **123**(5):721–728. <https://doi.org/10.1530/rep.0.1230721>
- Shrivastava, AB, Shrivastava, HC, Goyal, RA. 1988. A case of triorchidism in a cock. *Indian Journal of Animal Sciences,* **58**(8): 984-985.