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Age related histological and histochemical studies on the testis of Aseel and Vanaraja breeds of poultry

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Aseel is one of the important indigenous breeds of poultry having its breeding tract in southern part of Chhattisgarh and Vanaraja is dual purpose breed of poultry, developed at ICAR Project Directorate on Poultry, Hyderabad to improve the livelihood of tribal peoples. There is paucity of detailed information on histological and histochemical aspects of testis in Aseel and Vanaraja breeds of poultry. Hence, the present experiment was designed in these breeds to explore the histological and histochemical studies of testis in relation to age of birds.

The experiment was conducted on 40 apparently healthy male birds irrespective of their sex, belonging to two different age groups of Aseel and Vanaraja poultry. The birds were procured from Government Poultry Farms, Jagdalpur and Durg of Chhattisgarh. Based on age, these birds were divided into two groups (grower and adult). Each group was having 20 birds (10 Aseel and 10 Vanaraja). Representative samples of testis were collected from the identical sites and fixed in 10% buffered formalin or Bouin's fluid for 24–48 h. The fixed tissue samples were processed in aclohol-xylene sequence, embedded and blocked in paraffin wax (Singh and Sulochana 1997). The paraffin sections of the tissues fixed in neutral buffered formalin were stained with haematoxylin and eosin for normal histological structure, Van Gieson's for collagen fibres, Gomori's for reticular fibres, Verhoeff's for elastic fibres, PAS for carbohydrates and AB-PAS for mucopolysaccharides. After recording different histological

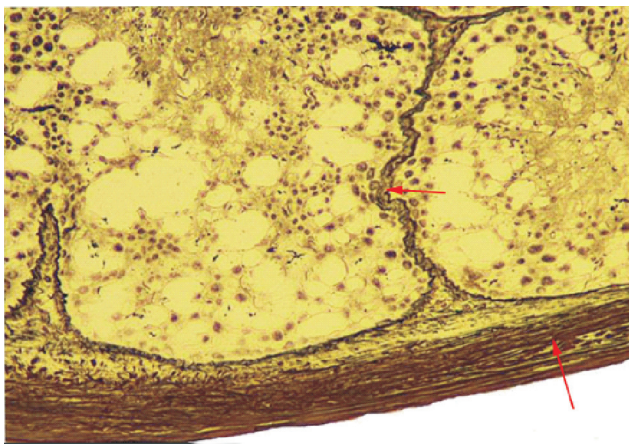

Fig. 1. Photomicrograph of testis of Aseel (group 2) showing reticular fibers (arrow) in the tunica albuginea and peritubular tissue (Gomori's, 400X).

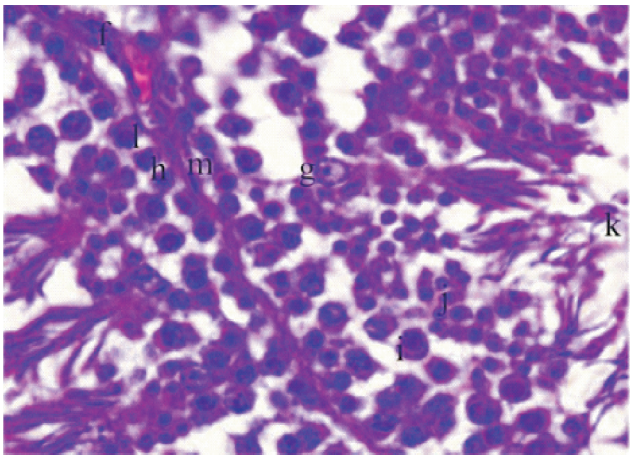

Fig. 2. Photomicrograph of testis of Vanaraja (group 2) showing f. interstitial cells, g. Sertoli cell, h. spermatogonia, i. primary spermatocyte, j. spermatid, k. spermatozoa. I. fibroblast, m. myoid cell (H&E, 1000X).

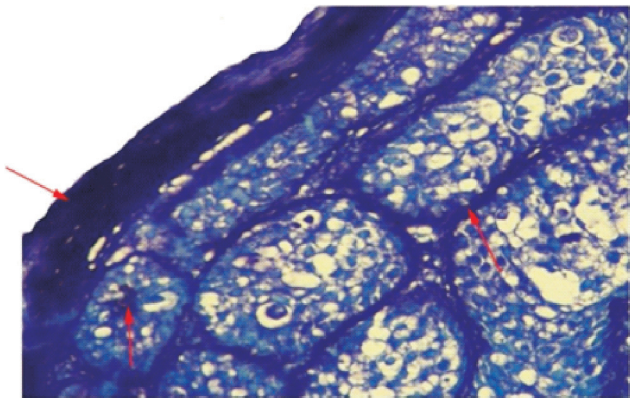

Fig. 3. Photomicrograph of testis of Aseel (group 1) showing AB-PAS activity (arrow) in tunica albuginea, basement membrane and germinal cells (AB-PAS, 400X).

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parameters of the testicles, the data was analyzed statistically and interpreted (Snedecor and Cochran 1994).

The testicles were encapsulated by tunica albuginea, which were made up of mainly collagen fibers with few elastic and reticular fibers. The fibers were finer in growers and their density was less in Aseel as compared to Vanaraja (Fig. 1). The thickness of capsule was higher in adults than growers in both breeds. Thin fibrocellular septae descended from the tunica albuginea, which continued as inter tubular connective tissue septae in the parenchyma of the testis. However, division of the testicular parenchyma into lobules was absent in adult birds. Multiple layers of myoid cells and elongated fibroblasts were seen in the peritubular tissue (Fig. 2) as reported in Japanese quail (Kannan *et al.* 1996),

Table 1. Histomorphometrical observations of testis group 1

Segments of testis		Breed	Group–1 Grower													
			Thickness of capsule		Maximum diameter of seminiferous tubules		Minimum diameter of seminiferous tubules		Number of seminiferous tubules per field		Diameter of primary spermatocytes		Number of sustentacular cells per tubule		Number of interstitial cells per field	
			Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Right	Cranial	Aseel	19.52	0.98	60.62	3.03	34.16	1.71	67**	2.50	6.1	0.31	6	0.3	10**	0.48
		Vanaraja	23.88**	1.19	100.80**	5.04	47.32**	2.37	30	1.45	7.3**	0.37	9**	0.45	6	0.30
	Middle	Aseel	23.04	1.15	71.49	3.57	37.63	1.88	50**	3.35	8.4	0.42	9	0.4	13**	0.65
		Vanaraja	27.12**	1.36	118.14**	5.91	53.42**	2.67	29	1.50	9.5**	0.48	12**	0.58	9	0.45
	Caudal	Aseel	19.72	0.99	63.74	3.19	34.58	1.73	58**	2.90	6.8	0.34	7	0.35	12**	0.57
		Vanaraja	24.06**	1.2	106.18**	5.31	47.88**	2.39	29	1.45	7.6**	0.38	10**	0.48	8	0.37
Left	Cranial	Aseel	19.61	0.98	61.18	3.06	34.30	1.72	68**	2.55	6.5	0.33	7	0.35	11**	0.55
		Vanaraja	24.04**	1.2	101.50**	5.08	47.67**	2.38	32	1.55	7.5**	0.38	10**	0.5	7	0.35
	Middle	Aseel	24.32	1.22	72.47	3.62	38.54	1.93	51**	3.40	8.6	0.43	10	0.49	14**	0.70
		Vanaraja	28.23**	1.41	119.47**	5.97	54.54**	2.73	31	1.60	9.7**	0.49	14**	0.7	10	0.50
	Caudal	Aseel	20.32	1.02	64.51	3.23	35.01	1.75	59**	2.95	6.9	0.35	8	0.4	12**	0.60
		Vanaraja	25.32**	1.27	107.95**	5.40	48.72**	2.44	31	1.55	7.9**	0.4	12**	0.6	8	0.40

Mean values with (*) superscripts show significant difference (P< 0.05). Mean values with (**) superscripts show significant difference (P< 0.01).

Table 2. Histomorphometrical observations of testis group 2

Segments of testis		Breed	Group 2 adult													
			Thickness of capsule		Maximum diameter of seminiferous tubules		Minimum diameter of seminiferous tubules		Number of seminiferous tubules per field		Diameter of primary spermatocytes		Number of sustentacular cells per tubule		Number of interstitial cells per field	
			Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Right	Cranial	Aseel	34.84	1.74	149.94	7.50	49.21	2.46	24**	1.00	3.1	0.16	10	0.50	6**	0.28
		Vanaraja	39.12**	1.96	210.14**	10.51	77.21**	3.86	18	0.80	4.3**	0.22	15**	0.75	3	0.15
	Middle	Aseel	40.68	2.03	190.54	9.53	59.51	2.98	20**	1.20	3.5	0.17	15	0.7	9**	0.45
		Vanaraja	45.16**	2.26	257.81**	12.89	91.14**	4.56	16	0.90	5.1**	0.26	21**	1.05	6	0.29
	Caudal	Aseel	36.04	1.8	158.98	7.95	51.91	2.60	21**	1.05	3.4	0.17	12	0.60	7**	0.3
		Vanaraja	40.84**	2.04	225.75**	11.29	79.71**	3.99	17	0.85	4.4**	0.22	18**	0.90	5	0.25
Left	Cranial	Aseel	35.84	1.79	164.15	8.21	57.61	2.88	25**	1.05	3.5	0.18	11	0.55	7**	0.32
		Vanaraja	39.68**	1.98	224.56**	11.23	87.51**	4.38	20	0.85	4.5**	0.23	17**	0.85	4	0.20
	Middle	Aseel	41.48	2.07	207.13	10.36	63.07	3.15	21**	1.25	3.7	0.19	16	0.80	10**	0.50
		Vanaraja	46.32**	2.32	259.14**	12.96	102.69**	5.13	17	1.00	5.2**	0.26	22**	1.10	7	0.35
	Caudal	Aseel	37.52	1.88	175.29	8.76	52.78	2.64	22**	1.10	3.6	0.18	14	0.70	8**	0.40
		Vanaraja	42.21**	2.11	233.07**	11.65	91.07**	4.55	18	0.90	4.5**	0.23	19**	0.95	6	0.30

Mean values with (*) superscripts show significant difference (P< 0.05). Mean values with (**) superscripts show significant difference (P< 0.01).

in quail (Marilena Longo 2007) and in white rooster (Razi *et al.* 2010). Parenchyma of the testis was mostly occupied by sections of the seminiferous tubules of different shapes and dimensions. The maximum and minimum diameter of seminiferous tubules was significantly lower in growers and adults of Aseel than Vanaraja (Tables 1, 2).

The seminiferous tubules were lined by mainly two types of cells, spermatogonial and Sertoli cells. The spermatogonial cells were spermatogonia, primary spermatocytes, secondary spermatocytes and spermatids. The diameter of primary spermatocytes was significantly higher in growers than adults due to initial stage of spermatogenesis, where mitotic division is slow. It was significantly higher in middle than cranial and caudal parts. Similarly it was significantly lower in growers and adults of Aseel than Vanaraja (Tables 1, 2). It indicated the presence of more germplasm in Vanaraja.

The Sertoli cells extended through the entire thickness of the germinal epithelium from the basal lamina (Fig. 2). The number of Sertoli cells was significantly higher in middle than cranial and caudal parts. It was significantly lower in growers and adults of Aseel than Vanaraja (Tables 1, 2). It was indicative of faster spermatogenesis in Vanaraja, in which the spermatogenic cells need more nutritional support through Sertoli cells.

The interstitial tissue was consisted of sparse loose connective tissue. Capillaries, arterioles, venules, lymphatics, nerve-cells and Leydig cells were observed in the interstitial tissue (Fig. 2). It corroborated the findings of Kannan *et al*. (1996) in Japanese quail, Marilena Longo (2007) in quail and Razi *et al.* (2010) in white rooster. The number of interstitial cells might be attributed to development of secondary sexual characters at grower stage. The nuclei of which were larger as compared to adults. It was significantly higher in middle than cranial and caudal parts. It was significantly higher in growers and adults of Aseel than Vanaraja (Tables 1, 2). It supported the aggressive behavioural characteristics of birds of Aseel breed.

PAS reaction was intense in the basement membrane and moderate in the germinal cells of the seminiferous tubules. The reaction was weak in the Leydig cells and Sertoli cells. It supported the findings of Tingari and Lake (1972). Less PAS activity was seen in Aseel as compared to Vanaraja. Tunica albuginea, intertubular connective tissue, basement membrane of seminiferous tubules and the germinal cells showed acid mucopolysaccharides. Less strong acid mucopolysaccharides could be demonstrated at similar location in Aseel as compared to Vanaraja (Fig. 3). All parameters were indicative of presence of reproductively more active testis in Vanaraja than Aseel birds.

SUMMARY

Birds (10) of 2 age group, viz. 5 months (grower) and 13 months (adult) of Aseel and Vanaraja breeds of poultry were used. The thickness of tunica albuginea, maximum and minimum diameter of seminiferous tubules, diameter of primary spermatocytes and number of sustentacular cells or Sertoli cells per seminiferous tubule were significantly higher in growers and adults of Vanaraja than Aseel whereas, number of interstitial cells per field were significantly higher in growers and adults of Aseel than Vanaraja. The density of connective tissue fibers, PAS activity and AB-PAS activity was more in both groups of Vanaraja than Aseel.

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