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Research Paper

Gross anatomical changes at various developmental stages in the testis of Gurez sheep (*Ovis aries*)

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ABSTRACT

The present study was designed with the objective to give a detailed account of the gross anatomical changes in testis of Gurez sheep at different developmental stages. A total of 39 samples, which included 8 prenatal and 31 post-natal testes, were collected from slaughter houses of Gurez valley. The approximate age of embryos/foetuses and the post-natal animals was estimated using standard methods. The results revealed that at early gestation period the organogesis of testis had already initiated. The shape of testis changed from elongated oval to oval shape at complete maturity. Initially the testes were intra-abdominal upto 70 days of gestation period. The testicular descent, along with the development of spermatic cord, was observed around 120 days of gestation. The testes were closely related with the mesonephric kidney, which completely regressed at the late gestation stage, a period which coincided with the development of epididymis from the mesonephric duct. The blood vessels of the spermatic cord changed from cylindrical to cone shape with the advancement of age. The cremaster muscle enclosed the spermatic cord from all aspects except the caudal surface. The average testicular weight of a sexually mature Gurez ram was 120 grams, a value that is significantly much less than what has been reported in most of the other sheep breeds. While moving from pre-pubertal to pubertal stage, a 7.5 fold increase in the weight of testis was observed. The reports of the current study and the literature cited clearly indicate that the rate of growth in the testes of Gurez sheep was less when compared with the other sheep breeds of the same age group.

Key words: *Gurez sheep, testis, gross anatomy, pampiniform plexus*

Among the various indigenous breeds of sheep, Gurez is a registered, as well as, the largest breed of the union territory of Jammu and Kashmir. This breed, limited to Gurez valley is reared by 'Dardi' Tribe of the Gurez valley (Rather *et al*., 2021). The breed, besides being well adapted to harsh climatic conditions of the valley, has high fecundity and is also characteristically polycerous in nature (Ganai *et al*., 2010; Kour *et al*., 2018). Uncontrolled cross breeding with Kashmir Marino and a lack of proper conservation policy pose challenging threats to the integrity of this breed. It therefore highlights the need to record the scientific knowledge of this breed pertaining to various structural and functional aspects.

Reproduction is one of the defining factors in the development of the livestock industry. The sexual characteristics and the reproductive power of a male individual are strongly defined by the status of its testis. Physical examinations like palpation of testis provide insufficient evidence regarding the status of testis (Tibary *et al*., 2018). The testicular biometry and the fertility potential are positively correlated to each other (Boukhliq *et al*., 2018). The optimal reproductive capacity and normal male phenotype formation depend upon the proper development of testis (Dufour *et al*., 2002 and Dehkordi *et al*., 2008). The postnatal growth in general is determined by the events that proceed during the prenatal period (Allyson *et al*., 2001).

Anatomical studies are reported on the testis of buffalo (Kaur *et al*., 2011), cattle (Gofur *et al*., 2008) and horses (Shukla and Rajput, 2019). Most of the studies on the sheep testis have been carried out either on the exotic breeds (Parchami *et al*., 2008; Alsadoon *et al., 2*019), or the indigenous breeds of tropical region (Kishore *et al*., 2011; Basavaiah *et al.,* 2021). To our knowledge, there is no literature available on the anatomy of reproductive system of Gurez sheep in general and testis in particular. Keeping in mind these facts the present study has therefore been designed with the objective to give a detailed account of the gross anatomical changes in testis of Gurez sheep at different developmental stages.

MATERIALS AND METHODS

A total of 39 samples (78 testes), which included 16 prenatal and 62 post-natal testes, were collected from slaughter houses of Gurez valley. The approximate age of embryos/fetuses was estimated by Richardson's formula (Richardson, 1980) i.e X= 2.1(Y+17), where X denoted the developmental age in days and Y denoted Crown Rump length (CRL) in cm. The details of prenatal animals are given in Table 1. For approximate age estimation in post-natal animals, the pattern of dentition was analyzed (Sastry *et al.,* 1982).

Prenatal study: To expose the testis, ventral abdominal incision was given and the topographic anatomy was recorded. At late gestation the testis were exposed by approaching the scrotum from its lateral aspect.

Post-natal study: The testes were dissected out from rest of the genital organs immediately after slaughter. The gross anatomical features of testes were then recorded. The testicular weight and volume of both the left and right testis were recorded and analysed using standard statistical methods (Snedecor and Cochran, 1994). For in-situ demonstration of testis and its associated structures, one neonatal Gurez lamb, procured during a post-mortem case study, was utilised for the present study. To expose the testis, scrotal sac was approached caudally and a midline incision was given. The area around the inguinal area was also cleared off.

Table.1: Grouping of prenatal Gurez sheep testis based upon Crown Rump Length

Group	Age	No. of samples	CRL (cm)
Pre-natal	Upto 50 days of gestational age	03	3.5 (43.05 days)
			4.0 (44.10 days)
			4.3 (44.73 days)
	51-100 days of gestational age	03	9.80 (56.28 days)
			11.57 (59.99 days)
			16.00 (69.30 days)
	101-150 days of gestational age	02	37.4 (114.24 days)
			39.6 (118.86 days)

RESULTS AND DISCUSSION

Prenatal development: *Early gestation:* The testicular differentiation was evident grossly at 43.05 days (CRL-3.5 cm). The sheep embryo at this age showed a clearly visible genital tubercle (Fig.1a). Similar findings have been reported in goat foetuses (Banankhojasteh *et al*., 2006). Upon dissection, the testes were grossly visible and positioned ventromedial to the Mesonephric kidney (Parchami and Dehkordi, 2008). The testes were having an elongated oval shape (Fig.1b). At 44.10 days (CRL-4.0 cm), the testes appeared oval to bean shape. At this stage the other organs like heart and stomach were also in the development phase (Fig.1c).

At 44.73 (CRL-4.3 cm) days, no significant change was observed when compared with the previous age. Farooqui *et al*. (2011) in goat foetuses reported that the shape of testis (40-50 days of age) varied from oblong to oval shape. At all three ages the testes were intra-abdominal in position, in agreement with the reports of Farooqui *et al*. (2011).

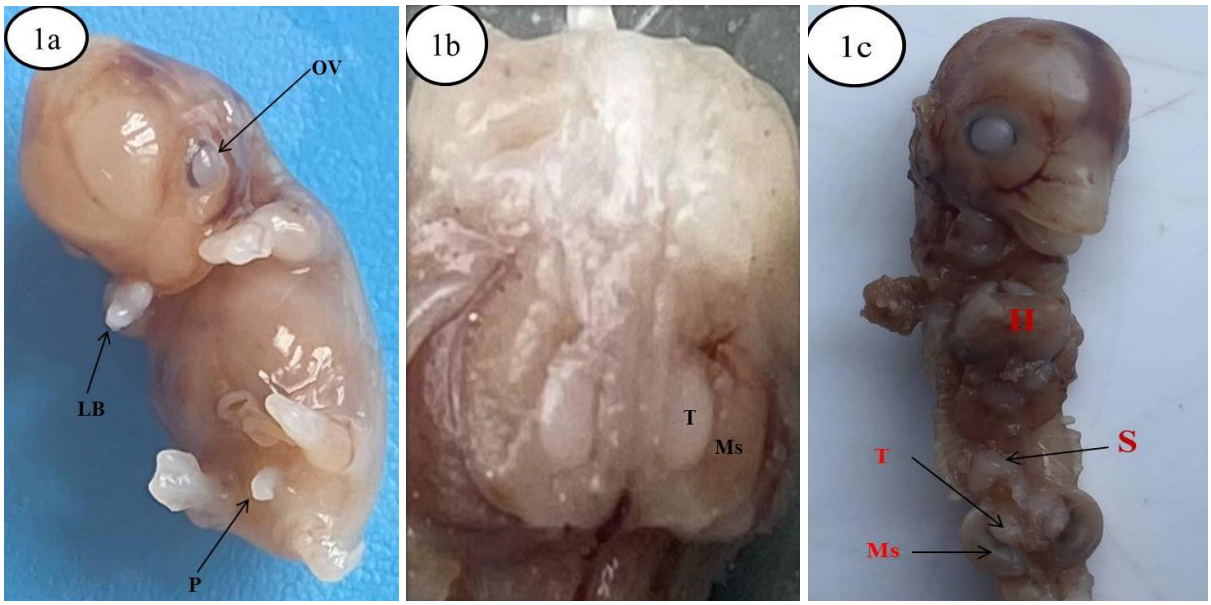

Fig.1a & 1b: (CRL- 3.50 cm), Fig.1c (CRL- 4.0 cm); Ventral view showing limb buds (LB) phallus (P) optic vesicle (OV), -mesonephros (Ms), testis (T), heart (H) and stomach (S)

Mid gestation: Grossly, at 56.28 (CRL- 9.80), the testes, still associated with mesonephros, were having a typical oval shape. Cranially and dorsally the metanephric kidney was closely associated with the testis (Fig.2a). At 59.99 (CRL 11.57) days, the testes were pushed further caudally, with more prominent metanephros and an intact mesonephros (Fig.2b). The testis at this stage was positioned at the caudal pole of the metanephros (Basavaiah *et al*., 2021). Similar findings have been reported by Farooqui *et al*. (2011) in goat foetuses of around 60 days of age. However, unlike the reports of Hejazi *et al*. (2011) in sheep foetuses of 60 days of age, the mesonephros of the Gurez sheep foetuses of same age group was still intact. At 69.3 (CRL-16 cm) days, the testes were pushed further caudally and clearly separated from metanephric kidney as reported by Farooqui *et al*. (2011) in goat foetuses. At this stage the mesonephric kidney had regressed completely. In the mid gestation period, the testes were still intra-abdominal location. The shape of testis in general was egg shaped (Fig. 2c).

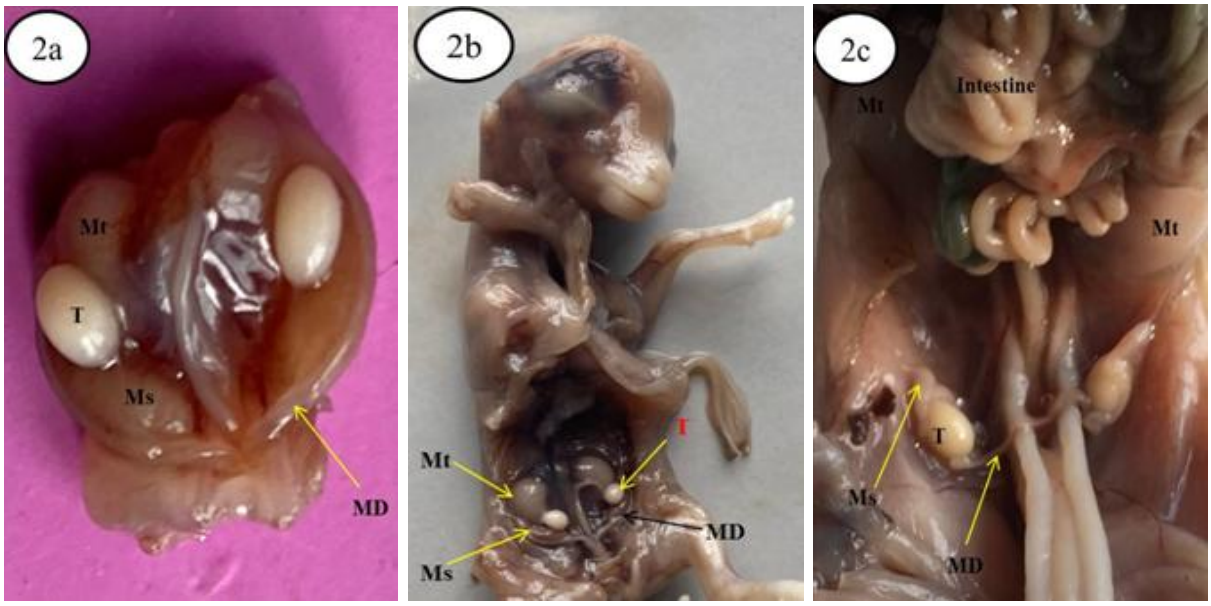

Fig. 2a (CRL- 9.80 cm), 2b (CRL- 11.57 cm), 2c (CRL- 16 cm); Ventral view showing metanephros (Mt) mesopnehros (Ms), testis (T) & mesonephric duct (MD)

Late gestation: Grossly at 114.24 (CRL-37.4) days, the testes were completely extra abdominal and descended into the scrotal sac (Fig.3a). Externally the testes were enclosed by a parietal layer of peritoneum. The mesonephric duct was modified into epididymis, and the spermatic cord along with the pampiniform plexus was visible for the first time at the dorsal extremity of testis. The epididymis at this stage was clearly divided into head, body and tail, which were attached to proximal extremity, caudal border and distal extremity of testis, respectively (Fig. 3b). The shape of testis at this stage was again oval shaped. At 118.86 days (CRL- 39.6), there were no significant changes when compared with previous age group. The testicular artery and vein were grossly visible for the first time. It is however worth to mention that the testis had not reached to the base of the scrotum. These findings are in agreement with the reports of Hejazi *et al*. (2011) in sheep foetuses of the comparable age. Therefore it can be concluded that the testicular descent had initiated but was not completed, at least around 120 days of gestation period. This is contrast to the findings of Basavaiah *et al*. (2021), who reported that the testicular descent was completed by 94 days.

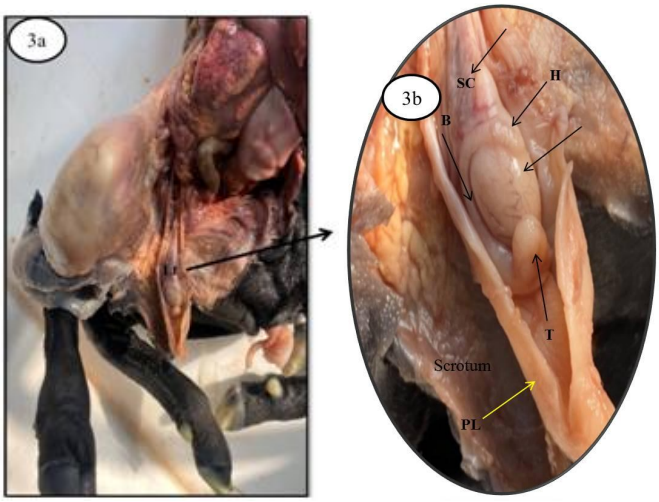

Fig. 3a & 3b (CRL- 37.4 cm) showing left testis (Lt), parietal layer (PL) of vaginal tunic, head (H), body (B), tail (T) of epidydimis & Spermatic cord (SC)

Post-natal development: The testes, which were oval shaped at this stage, remained well outside the abdomen in the scrotal sac. At one month of age, the structures of spermatic cord were well differentiable. Except in size, the structures of spermatic cord did not show any significant differences among different age groups. The testicular artery and vein emerged from the abdominal aorta and vena cava, respectively (Fig.4). The cremaster muscle was present around the entire area of spermatic cords, except at its caudal aspect (Fig.5a, 5b, 5c, 5d). It was closely associated with the vas deferens towards the caudo-medial aspect (Fig. 5d). The muscle was inserted at the parietal layer of tunica vaginalis, which was itself attached to testis at its caudal border (Fig.6a, 6b, 6c), in agreement with the reports of Perez *et al*. (2013) in pampas deer. The cremaster muscle appeared as single bundle of fibers, in contradiction with the reports of Perez *et al*. (2013). According to these researchers, the cremaster muscle in pampas deer was divided into three bundles of fibers. With the advancement of age, the shape of testicular artery and pampiniform plexus changed from cylindrical to conical shape, the base of which was attached to the proximal extremity (Fig.6d). This kind of transition in shape of spermatic cord is to cope up with the increased size of testis so that there is more effective cooling of arterial blood. This kind of arrangement has also been reported in the testes of Plateau-type Tibetan sheep and low –altitude small - tailed Han sheep (Liu *et al*., 2022), where it was concluded that such a morphological arrangement is beneficial for temperature regulation and maintenance of spermatogenesis in the epididymis. The shape of the testis was observed to be oval at all age groups, and except in size, no significant changes were observed (Fig.7a). The testis in general presented two surfaces, two borders and two extremities. The caudal border was attached to epididymis, whereas, the anterior border was free (Fig.7b). The proximal extremity was associated with the structures of spermatic cord, and the distal extremity was associated with the tail of epididymis (Fig.6d). The medial surface was flat and the lateral surface was convex. Upon dissection, the sagittal section of testis showed a well-developed mediastinum testis in both the pubertal and post-pubertal groups, whereas, it could not be confirmed in pre-pubertal sheep (Fig. 7b).

The average weights of left and right testis in grams, recorded at pre-pubertal, pubertal and post-pubertal ages were (8.58 ± 8.46^{a} and 8.47 ± 8.73^{a}), (63.34 ± 7.57^{b} and 63.85 ± 7.81^{b}) and (119.08 ± 7.97^{c} and 118.44 ± 8.23^{c}), respectively (Table 2). Thus there were no significant differences between the weight of left and right testis at different age groups. However, with the advancement of age, the testicular weights were differing significantly between these groups. Further, while moving from pre-pubertal to pubertal stage, there has been a 7.5 fold increase in the weight of testis. Similarly, from the period of pre-pubertal to post-pubertal stage, the weight of testis has increased to nearly double. In the present study, the average testicular weight of testis (right or left) at pre-pubertal stage (4-9 months) was around 8.5 grams. This value was much lower when compared with the Najdi lambs, whose average weight of testis was 392.00 ± 2.00 grams at 9 months of age (Al-Kawmani *et al*., 2014). Similarly, the average testicular weight of Awassi and Arrabi sheep at 8 months of age was recorded as

(49.60 ± 0.20 and 47.60 ± 0.17) grams, respectively (Al-Sadoon *et al*., 2019). Thus the reports of the current study and the literature cited clearly indicate that the rate of growth in the testes of Gurez sheep was less when compared with the other sheep breeds of the same age group. There was no difference in the pattern of development in the development of testis in Gurez sheep, although the timing events were slightly delayed when compared with other sheep breeds.

Table.2: Grouping of post-natal sheep testis based upon pattern of dentition

Group	Age (Range) months	Weight of testis		*P* value
		Left testis 0.000	Right testis 0.000	
Pre pubertal	4-9 months (**7.19±1.04**)	8.58±8.46[a]	8.47±8.73[a]	0.975
Pubertal	9 - 13 months (**10.40**± 0.93)	63.34±7.57[b]	63.85±7.81[b]	0.938
Post-pubertal	15-30 (**18.78±0.98**)	119.08±7.97[c]	118.44±8.23[c]	0.972

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