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Evaluation of goat ovaries and follicles by histological study

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ABSTRACT

The present study evaluated slaughterhouse goat ovaries and follicles to know the effect of corpus luteum on the number and quality of ovarian follicles. For this study, 18 goat ovaries were collected and categorized as ovaries with corpus luteum (CL+) and without corpus luteum (CL−) group. Both groups were evaluated by morphometric measurements and histological observation. Among 18 ovaries, 12 ovaries contained CL (66.67%) and 6 ovaries were without CL (33.33%). Based on the morphometric measurements, the length and weight of the ovaries with CL were numerically higher ($p>0.05$) than those of the ovaries without CL. But the diameter of the ovaries with CL was significantly higher ($p<0.05$) than those of the ovaries without CL. In comparison to the CL− group, the CL+ group had a numerically higher ($p>0.05$) number of follicles with a diameter of 2-6 mm and > 6 mm. Histological analysis revealed that the total number of follicles (primary to antral) in CL− group (11.10±2.47) was numerically higher ($p>0.05$) than that of the CL+ group (10.07±2.47). Presence of primary (4.44±1.59), secondary (1.45±0.83) and antral (6.27±1.31) follicles were found higher ($p>0.05$) in ovaries without CL than that of with CL (4.05±1.61, 0.92±0.21, 5.08±1.21 respectively). On the other hand, the number of degenerated primary (1.29±0.56), secondary (0.52±0.14) and antral (3.38±0.66) follicles were found comparatively higher ($p>0.05$) in the ovaries with CL than that of the ovaries without CL (1.14±0.69, 0.37±0.19, 2.40±0.40 respectively). Since, there was no statistical difference found among the number of total and different types of follicles between ovaries with or without corpus luteum; hence, it may be summarized that both type of ovaries may be used for the retrieval of oocytes for further reproductive biotechnological study.

Introduction

Goats are one of the utmost productive animals (Bhardwaj *et al.*, 2018); having short generation intervals and possibly high reproductive rates. These production efficiencies make goats the most effective substitute for large farm animal production in Bangladesh. For the goat industry, the reproduction rate is the crucial element focused on economic importance (Islam *et al.,* 2018); hence, reproduction is an essential part that must be taken under consideration (Sharma, 2000).

Schlafer and Foster (2016) denoted the ovaries as the critical part of the female reproductive system that produces ova and female sex hormones. The ovarian cortex encompasses a large number of follicles at different phases of development, among which only a few go through ovulation and the rest higher portions become degenerated (Bailey *et al.*, 1958). After ovulation, the corpus luteum is formed in the ovary, which maintains the pregnancy through the secretion of progesterone hormone (Hafez, 1993). Miranda-Moura *et al.,* (2010) defined corpus luteum as a temporary endocrine gland maintaining the estrous cycle and pregnancy.

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The ovarian follicular development may be negatively affected by the presence of corpus luteum (Ananthan *et al.,* 2021) and the regression of the CL initiates the next estrous cycle (Wen *et al.,* 2020).

Although some morphological and biometric studies have been conducted on the goat ovary and follicles (Jaji *et al.*, 2012; Ramsingh *et al.*, 2013; Aliyu *et al.*, 2016; Haque *et al.*, 2016), but histological study related to goat reproductive organs is still inadequate. Bearing it in mind present study was carried out to deliver a scientific interpretation through the histological exploration of goat ovary, which might be a potential source for getting good quality oocytes that might potentiate both the qualitative and quantitative *in vitro* embryo production in practice. Keeping this view in mind, present study was designed to know the effect of CL on the number and quality of ovarian follicles and to identify the different types of normal and degenerated follicles depending on the cellular integrity of the granulosa cells.

Materials and Methods

The research was carried out in the Reproductive Biotechnology Laboratory at the Department of Animal Breeding and Genetics at Bangladesh Agricultural University in Mymensingh, Bangladesh, from January 2018 to December 2018.

Collection and transportation of the reproductive organs

The reproductive tracts of 9 adult female goats were randomly collected immediately after slaughtering animals from the abattoirs of Mymensingh City Corporation in Bangladesh. The health and reproductive background of the goats were unknown. The age of slaughtered goats was determined by dentition technique (Wilson and Durkin, 1984), which varied between 9 to 12 months. The collected reproductive tracts were kept into Thermo flask (containing 0.9% normal saline solution) at 25°C and brought to the Reproductive Biotechnology Laboratory.

Ovary processing and morphometric analysis

Collected ovaries were detached from the reproductive tract and trimmed to remove the surrounding tissues and overlying bursa. After that, the ovaries were graded as ovaries with corpus luteum (CL+ group) and ovaries without corpus luteum (CL– group) based on the existence of the corpus luteum. Among the 18 ovaries, 12 ovaries contained CL and 6 ovaries had no CL. The length and diameter of the ovaries were measured using digital slide calipers, and the weight was measured by a digital balance (Precisa, XB-220A, Switzerland). The number of visible follicles on the surface of the ovary was counted by naked eyes and the follicles were classified as small follicles (having the diameter 2-6 mm) and large follicles (having the diameter >6 mm) as described by Talukder *et al.* (2011).

Histological Analysis

The ovaries were cut into 2-4 pieces and all the pieces were fixed in the Bouin's solution (picric acid, 37-40% formalin and glacial acetic acid) for 8-10 hours. Ovaries were dehydrated in 70% alcohol for 12 hours, 80% alcohol for 1 hour, 90% alcohol for 1 hour and 100% alcohol for 1 hour for three times. After that, xylene was used to clean the dehydrated ovary samples. For the first hour, the ovary samples were submerged in a 50/50 combination of xylene and alcohol. After that, ovarian tissue samples were soaked in pure xylene (100 percent) for an hour. The cleared samples were moved from the clearing agent to a mixture of 50 percent paraffin and 50 percent xylene, then kept into incubator (60°C) for 1 hour. The ovarian tissues were placed in the empty mould after it was filled with melted paraffin wax. Thus, the ovarian tissues turned into paraffin blocks that were then allowed for hardening for 24 hours at room temperature. The blocks were cut into 6 µm thick sections by using a sliding microtome machine (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Serial sectioning was maintained and every 9^{th} or 10^{th} section was taken for slide preparation to avoid the repetition of same follicles. From each ovary, 16 sections were taken for examination.

Ovarian sections were air-dried before being deparaffinized with xylene, dehydrated in alcohol, and hematoxylin and eosin stained. Finally, using DPS mounting reagent, the dyed sections were securely mounted with a coverslip. Every section of both kinds of ovaries was observed by light microscope (10×10X and 10×40X) and follicles of different stages were determined. The follicles were classified into four classes: a) Primordial follicle having flattened granulosa cells monolayer surrounding the oocyte; b) Primary follicles having single layer of cuboidal cell; c) Secondary follicles having two or more layer of granulosa cells but no antrum and d) Antral follicles having an antral cavity with granulosa cells manifold layers.

This characterization was done according to Alam *et al.* (2014). In both types of ovaries, the total number of primary, secondary, and antral follicles was counted. Follicular degeneration was also noticed and degenerated follicles numbers in every section was counted. Degenerated follicles may have various criteria (pycnotic nuclei, large vacuoles, condensed cytoplasm, swollen or loss of granulosa cells) but in this study the degenerated follicles were identified by observing the granulosa cells integrity of the surrounding the oocyte. The follicles having no homogenous distribution of granulosa cells at the granulosa cell layer were marked as degenerated follicles. Using an Olympus light microscope (Olympus CX41; Olympus Industrial America Inc., Orangeburg, NY, USA), we counted the follicles and examined the granulosa cell layer.

Statistical analysis

Data analysis was performed using the GLM method in SAS software (USA) using this model: $Y_{ijk}= \mu + S_i + T_j + e_{ijk}$ where, Y_{ijk} is the observation/dependent variable, μ is the population mean, S*i* is the influence of the ovary with corpus luteum (CL+), T_j is the ovary's effect without corpus luteum (CL−) and e_{ijk} is the random error. Ovary with and without CL served as independent variables, whereas ovarian dimensions (including weight, length, and diameter) and follicles characteristics (including size and type) served as dependent variables. Results were shown as the mean±SEM, and differences at the $p<0.05$ level were considered significant.

Results

Quantitative evaluation of ovaries

Among the 18 goat ovaries, 12 ovaries contained corpus luteum (66.67%), whereas 6 ovaries had no corpus luteum (33.33%).

Morphometric evaluation concerning corpus luteum in ovaries

Morphometric measurements of ovaries with corpus luteum (CL+) or without corpus luteum (CL−) are presented in Table 1. The length and weight of the CL+ ovaries were numerically higher than that of the CL− ovaries but no significant differences ($p>0.05$) were found. Ovaries with CL were larger ($p<0.05$) in diameter than those without it (1.20±0.06 cm *vs.* 0.99±0.08 cm). Based on the diameter of the follicles, the visible follicles of the ovarian surface were categorized into two groups like 2-6 mm and >6 mm diameter follicles (Table 1). The follicles number having 2-6 mm diameter (21.00±1.84) and > 6 mm diameter (0.92±0.22) in CL+ ovaries was numerically higher ($p>0.05$) than CL− ovaries (17.50±2.51 and 0.50±0.22, respectively).

Table 1. Impact of corpus luteum on the morphometric measurements and visible follicles numbers based on their size (Mean ± SEM) in goat ovaries

Ovarian Types	Morphometric parameters			Number of visible follicles	
	Length (cm)	Diameter (cm)	Weight (g)	2-6 mm	> 6 mm
Ovaries with CL (n=12)	1.58±0.09	1.20[a]±0.06	1.03±0.16	21.00±1.84	0.92±0.22
Ovaries without CL (n=6)	1.47±0.10	0.99[b]±0.08	0.63±0.13	17.50±2.51	0.50±0.22
Level of sig.	NS	*	NS	NS	NS

*n= Number of ovaries; CL= Corpus luteum; NS= Non-significant; *= Significant at p < 0.05*

Table 2. Variation of ovarian follicles (Mean ± SEM) in response to corpus luteum's presence and absence

Ovary (18)	Number of follicles per ovary			
	Primary	Secondary	Antral	Total (Primary to Antral)
Ovaries with CL (n=12)	4.05±1.61	0.92±0.21	5.08±1.21	10.07±2.47
Ovaries without CL (n=6)	4.44±1.59	1.45±0.83	6.27±1.31	11.10±3.39
Level of sig.	NS	NS	NS	NS

CL=Corpus luteum, NS= Non significant

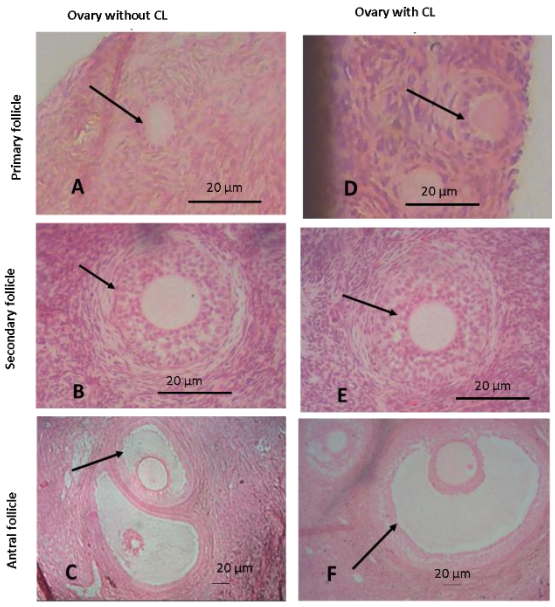

Fig. 1. Sections of ovarian histology demonstrating different kinds of follicles in CL+ ovary and CL– ovary. *A) Primary follicle (at 10 ×40X); B) Secondary follicle (at 10 ×40X) and C) Antral follicle in CL– ovary (at 10 ×10X); D) Primary follicle (at 10 ×40X); E) Secondary follicle (at 10 ×40X) and F) Antral follicle in CL+ ovary (at 10 ×10X). Directional arrows denote follicles, and the 20 µm scale bars provide a sense of proportion.*

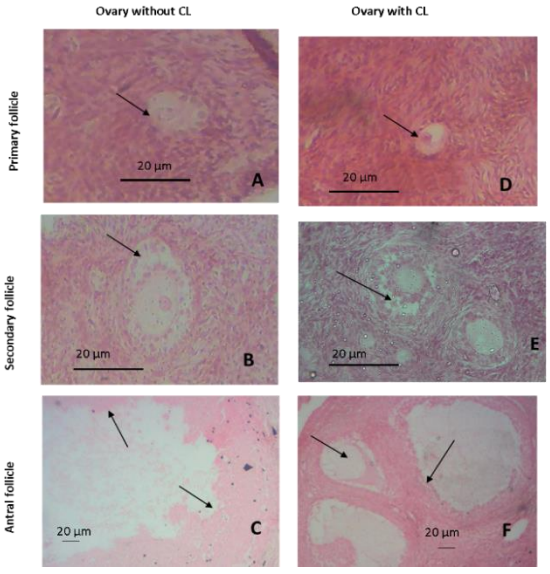

Fig. 2. Histological section of CL+ and CL– ovaries showing the degeneration of follicles. *A) Degeneration of primary follicle (at 10 ×40X); B) Degenerated secondary follicle (at 10 ×40X) and C) Degenerated antral follicle in CL– ovary (at 10 ×10X); D) Degeneration of primary follicle (at 10 ×40X); E) Degeneration of secondary follicle (at 10 ×40X) and F) Degenerated antral follicle (at 10 ×10X) in the CL+ ovary. Directional arrows denote follicles, and the 20 µm scale bars provide a sense of proportion.*

Histological evaluation of ovaries

Diverse kinds of follicles in CL+ and CL– ovaries were determined histologically and the findings are mentioned in Table 2. Histological analysis revealed that the number of total follicles in CL– ovaries was higher (p>0.05) than CL+ ovaries. In addition, the number of primary, secondary, and antral follicles was observed to be greater (p>0.05) in ovaries without CL compared to ovaries with CL. Figure 1 depicts the follicular structure of an ovary. The number of diverse sorts of degenerated follicles for CL+ ovary and CL– ovary was presented in Table 3. The number of degenerated primary (1.29±0.56), secondary (0.52±0.14) and antral (3.38±0.66) follicles were found numerically higher (p>0.05) in CL+ ovaries compared to the CL– ovaries (1.14±0.69, 0.37±0.19, 2.40±0.40, respectively). Different types of degenerated follicles for the CL+ and CL– groups are illustrated in Figure 2.

Table 3. Effect of the presence of the corpus luteum on histologically counted degenerated follicles per ovary (Mean ± SEM)

Ovary (18)	**Degenerated follicles per ovary**		
	Primary	**Secondary**	**Antral**
Ovaries with CL (12)	1.29±0.56	0.52±0.14	3.38±0.66
Ovaries without CL (6)	1.14±0.69	0.37±0.19	2.40±0.40
Level of sig.	NS	NS	NS

CL=Corpus luteum, NS= Non-significant

DISCUSSION

Among the collected ovaries, 66.67% ovaries contained CL, and 33.33% ovaries had no CL. The greater percentage of corpus luteum presence on the ovary revealed that the goats were in cyclic condition. Although the goats were cyclic, the reasons for slaughtering them might be their lower reproductive or productive performance.

Morphometric measurements of ovary and follicles

The present study revealed that numerically higher (p>0.05) numbers of small (follicles having 2-6 mm diameter) and large (follicles having >6 mm diameter) follicles were found in the ovaries with CL than CL– ovaries which is not similar to the results of Mahzabin *et al.* (2020). Mahzabin *et al.* conducted a research on cattle ovary and found that significantly

greater number of follicles having diameter 2-6 mm in the CL (–) ovary (15.90±1.73) compared to the CL (+) ovary (9.83±2.23). In the current study the difference was found non-significant and the reason behind this may be the smaller sample size and lower number of CL absent (CL–) ovaries.

Consistent with the findings of Alsafy and El-Shahat (2011) for sheep, Asad et al. (2016) for goats, and Hoque et al. (2016) for both sheep and goats, the mean diameter of the CL+ ovaries was substantially bigger ($p<0.05$) than CL– ovaries. The mean weight of the CL+ ovaries was also numerically higher ($p>0.05$) compared to the CL– ovaries. This result partly concurs with the study of Islam et al. (2007), Asad et al. (2016), Bhajoni et al. (2018), and Mahzabin et al. (2020) on goat and cattle ovaries, they reported significantly higher weight in CL+ ovaries. The mean length of CL+ ovaries was also found numerically higher ($p>0.05$) than CL– ovaries, which negates the finding of Asad *et al.* (2016). Jablonka-Shariff *et al.* (1993) characterized CL as an extracellular material in the ovary possessing a certain level of its development, maintenance and regression which might cause variations in weight, diameter and length of the ovary having CL.

Histological study

The total number of primary, secondary, and antral follicles was numerically greater ($p>0.05$) in CL– ovaries compared to CL+ ovaries, as determined by histological analysis. Again, the numbers of degenerated primary, secondary and antral follicles were found numerically lower ($p>0.05$) in CL– ovaries compared to the CL+ ovaries. These findings are not similar to Mahzabin *et al.* (2020) who conducted a histological study on cattle ovaries and found that CL– ovaries contained significantly higher number of total follicles and significantly lower degenerated follicles than ovaries with CL.

The corpus luteum slows down the development of follicles (Hafez, 1993). Webb *et al.* (1999) explained that the existence of CL in a cyclic female's ovary produced a higher level of progesterone hormone that directs negative feedback to the anterior pituitary gland for the constraint of gonadotropin secretion, and finally, follicular degeneration takes place. As ovaries without CL contain higher number of growing follicles and lower numbers of degenerated follicles, indicates good-quality oocytes might be possible to harvest from these ovaries.

CONCLUSIONS

There was no statistically significant difference between the two types of ovaries however; ovaries without CL had a greater total follicle count and a lower number of degenerated follicles. So, it can be determined that both types of ovaries are good for the retrieval of oocytes for further reproductive biotechnological study. However, immunohistochemistry study is required to evaluate the ovaries more accurately.

Author's contribution:

Conception and design of study: MAMY Khandoker; **Methodology:** SJ Shathi, R Mahzabin, MA Jahan, A Khatun; **Supervision:** MAMY Khandoker and S Debnath; **Data analysis:** MR Islam; **Writing—original and draft preparation:** SJ Shathi; **Writing—review and editing:** MAMY Khandoker.

Conflicts of Interest statement: There are no competing interests, as stated by the authors.

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Access to data: In response to a fair request, the data can be obtained from the first author at the following email address: sarmin.jahan505@gmail.com.

Ethical approval: This research complies with the ethical standard required for the research concerning the handling of biological material. The approval number: Ref: /BAURES/ESRC/AH/25.

***Consent for publication*:** All authors have read and agreed to the published version of the manuscript.

REFERENCES

Alam MH, Kabir ME, Sarker MB, Saha BK, Khan RI, Moniruzzaman M (2014). Development and survivability of ovarian follicles of goat in different feeding systems. *Bangladesh Journal of Animal Science,* 43: 175-179. https://doi.org/10.3329/bjas.v43i3.21644

Aliyu A, Peter ID, Zakariah M, Maina VA, Malgwi KD, Bitrus AA (2016). Biometry of the ovary in Balami, Yankassa sheep and Sahel goat. Nigerian Veterinary Journal, 37: 223-229.

Alsafy MA, El-Shahat KH (2011). Morphological evaluation of ovary in relation to recovery, quality of oocytes and steroid production in sheep. Journal of Applied Biological Sciences, 5: 27-31.

Ananthan PAS, Khandoker MAMY, Akter T (2021). Qualitative Evaluation of Slaughterhouse Bovine Ovaries by Histological Study. Journal of Bangladesh Agricultural University.
https://doi.org/10.5455/JBAU.54052

Asad L, Rahman ANMI, Hossain MM, Akter M (2016). Ovarian category, follicles and oocytes analysis of Goat ovaries in view of *in vitro* production of embryos. International journal of Animal Research, 1: 27-34.

Bailey FR (1958). Bailey's Textbook of Histology. pp. 656-657.

Bhajoni M, Bhuyan D, Biswas RK, Dutta DJ (2018). Morphometric study of ovary and rate of recovery of oocyte from medium size follicle by aspiration technique in cattle. International Journal of Chemical Studies, 6: 499-503.

Bhardwaj JK, Kumar V, Saraf P, Kumari P, Mittal M (2018). Current status and changing national scenario of goat population: A review. Agricultural Reviews, 39: 91-103.
https://doi.org/10.18805/ag.-1752

Hafez ESE (1993). Folliculogenesis, egg maturation and ovulation. In: Reproduction in Farm Animals. 5th Ed, Lea and Febiger Press. Philadelphia. pp. 114-142.

Haque Z, Haque A, Quasem MA (2016). Morphologic and morphometric analysis of the ovary of black bengal goat (*capra hircus*). International Journal of Morphology, 34: 13-16.
https://doi.org/10.4067/S0717-95022016000100002

Hoque SM, Islam MM, Selim ASM (2016). Interspecies differences on ovarian parameters between Black Bengal goat and indigenous Bengal sheep in view of *in vitro* maturation. Advancements in Life Sciences, 6: 54-60.

Islam MA, Hossain MN, Chokraborti SS, Rahman S, Tasnim A, Al Zabir A (2018). Socio-economic profile of goat rearing farmers and their management practices in Sylhet, Bangladesh. Journal of Agriculture and Ecology Research International, 15: 1-10.
https://doi.org/10.9734/JAERI/2018/43818

Islam MR, Khandoker MAMY, Afroz S, Rahman MGM, Khan RI (2007). Qualitative and quantitative analysis of goat ovaries, follicles and oocytes in view of *in vitro* production of embryos. Journal of Zhejiang University: Science B, 8: 465-469.
https://doi.org/10.1631/jzus.2007.B0465
PMid: 17610325
PMCid: PMC1906591

Jablonka-Shariff A, Grazul-Bilska AT, Redmer DA, Reynolds LP (1993). Growth and cellular proliferation of ovine corpora lutea throughout the estrous cycle. Endocrinology, 133: 1871-1879.
https://doi.org/10.1210/endo.133.4.8404629
PMid: 8404629

Jaji AZ, Buduwara RA, Akanmu AL, Zachariah M, Luka J, Gambo B (2012). Pregnancy related biometric changes in the ovaries and uterus of the Sahelian goat. Sokoto Journal of Veterinary Sciences, 10: 17-21.
https://doi.org/10.4314/sokjvs.v10i1.4

Mahzabin R, Khandoker MAMY, Husain SS, Islam MR, Shathi SJ, Habib MR, Vargas-Bello-Perez E (2020). Evaluation of cattle ovaries and follicles by histological analysis for potential *in vitro* production of embryos in tropical conditions. Tropical and Subtropical Agroecosystem, 23: 1-9.

Miranda-Moura MTM, Fonseca VU, Silva NB, Freitas ML, Almeida OB, Rocha HAO, Papa PC, Moura CEB (2010). Morphological features and vascularization study of caprine cyclic corpus luteum. Pesquisa Veterinária Brasileira 30:351-357.
https://doi.org/10.1590/S0100-736X2010000400012

Ramsingh L, Sadasivarao K, Muralimohan K (2013). Ovarian biometrics and oocyte grading percentage of yield in local goats of Andhra Pradesh. IOSR Journal of Pharmacy and Biological Sciences, 3: 47-49.

https://doi.org/10.9790/3013-31304749

Schlafer DH, Foster RA (2016). Female genital system. Jubb, Kennedy & Palmer's Pathology of Domestic Animals: Volume 3, pp.358.
https://doi.org/10.1016/B978-0-7020-5319-1.00015-3
PMCid: PMC7158333

Sharma RK (2000). Follicular atresia in goat: A review. Indian Journal of Animal Science, 70: 1035-1046.

Talukder MNS, Iqbal A, Khandoker MAMY, Alam MZ (2011). Collection grading and evaluation of cumulus-oocyte-complexes for *in vitro* maturation in sheep. *Bangladesh Veterinarian,* 28: 31-38.
https://doi.org/10.3329/bvet.v28i1.8811

Webb R, Campbell B, Garverick H, Gong J, Gutierrez C, Armstrong D (1999). Molecular mechanisms regulating follicular recruitment and selection. Journal of Reproduction and Fertility, 54: 33-48.

Wen X, Liu L, Li S, Lin P, Chen H, Zhou D, Tang K, Wang A, Jin Y (2020). Prostaglandin F2α induces goat corpus luteum regression via endoplasmic reticulum stress and autophagy. Frontiers in Physiology, 11: 868.
https://doi.org/10.3389/fphys.2020.00868
PMid: 33013430
PMCid: PMC7516216

Wilson RT, Durkin JW (1984). Age at permanent incisor eruption in indigenous goats and sheep in semi-arid Africa. Livestock Production Science, 11: 451-455.
https://doi.org/10.1016/0301-6226(84)90056-3