Original Article

# Histological and Histochemical Developmental Study of the Duodenal Glands in Rabbit and Mice During Different Ages

**Ali Thoulfikar A. Imeer[1]*, Hassan Thoulfikar A. Alamir[2], Thoulfikar A. Albassam[3], Abdul Amir H. Kadhum[4]**

[1] Faculty of Medicine, University of Al-Ameed, Karbala, Iraq.
https://orcid.org/0000-0003-0842-1072
Email: albassamali76@gmail.com
[2] Faculty of pharmacy , University of Al_Ameed.
Email: dr.hassanalbassam80@gmail.com
[3] Faculty of Medicine, University of Al-Ameed, Karbala, Iraq.
Email: amir8@ukmadu.my
[4] Faculty of Medicine, University of Al-Ameed, Karbala, Iraq.
Email: amir1719@gmail.com

## Abstract

**Objective:** The objective of this study is to investigate the development, growth, and maturation of the Brunner's glands, as well as their distribution in the duodenum of rabbits and mice. **Methods:** A total of 20 animals from each species were selected and categorized into four groups, with each group consisting of 5 animals. The categorization was based on the age of the animals in days, specifically One, Seven, Fifteen, and Forty. Samples were collected from various parts of the duodenum, and these samples were subsequently fixed using formalin and processed using histological techniques. The sections were stained using various techniques, including hematoxylin and eosin, Alcian blue PH 2.5, Periodic acid Schiff, a combination of Alcian blue and Periodic acid Schiff, and Masson's trichrome stains. In all studied species, the epithelium of the villi exhibited a substantial presence of vacuolated columnar cells at one day of age. The villi themselves were observed to be short and asymmetrical, with noticeable intervillus space. Additionally, the bases of the villi were characterized by clusters of immature cells, while the lamina propria lacked crypts. Furthermore, it was observed that the muscularis mucosa was not continuous. Furthermore, it was seen that the submucosa did not have a Brunner's gland. However, in mice at seven days of age and rabbits at ten days of age, the mucosa exhibited slender cylindrical villi and the muscularis mucosa had a poorly developed circular layer. During the histogenesis process, both crypts and Brunner's gland were observed to initiate their development. **Results:** Over a span of fifteen days, the presence of rabbits and mice resulted in a reduction in cell vacuolation. Additionally, the villi exhibited a mature morphology, while the crypts displayed evident growth. The muscularis mucosa acquired a circular layer, accompanied by an increase in the quantity of Brunner's gland within the submucosa. **Conclusion:** At the age of forty days, across various species, the mucosa exhibited mature villi with a leaf-like morphology. The crypts displayed a higher level of development, while the muscularis mucosa exhibited a well-developed circular muscular layer. In rabbits, the duodenal glands extended in close proximity to the jejunum, whereas these glands were absent in the latter segments of the mice duodenum. The glands in mice throughout their early developmental stages had serous characteristics, but the glands in rabbits displayed mucous characteristics. As rabbits age, the Brunner's glands in their digestive system exhibit a mixture of acini, whereas in mice, the majority of acini are mucous in nature. The aging process significantly influences the morphology of the villi, as well as the quantity of crypts and Brunner's glands. Furthermore, this study provides extensive morphometrical analyses that illustrate the alterations in the duodenal wall associated with aging.

**Keywords:** Development, Brunner's Gland, Duodenum, Rabbit, Mice

## Introduction

In the human body, the Brunner's glands are distributed throughout the submucosa of the duodenum. The initial moments of the incident were characterized by their overwhelming presence. However, as the duodenum transitions into the jejunum, there is a notable decrease in size observed in other segments of the organ.[1] Stem cells exhibit a relatively low rate of division in mouse and rat models, occurring around once per 24 hours. The utilization of adult stem cells for cell regeneration has been observed,

Access this article online

Quick Response Code:

Website: www.jnsbm.org

DOI: https://doi.org/10.4103/jnsbm.JNSBM_14_2_21

**Address for Correspondence:** Faculty of Medicine, University of Al-Ameed, Karbala, Iraq
Email: albassamali76@gmail.com

**Submitted:** 24th October, 2023 **Received:** 25th October, 2023
**Accepted:** 04th November, 2023 **Published:** 10th December, 2023



**How to cite this article:** Imeer A T A, Alamir H T A, Albassam T A, Kadhum A A H. Histological and Histochemical Developmental Study of the Duodenal Glands in Rabbit and Mice During Different Ages. J Nat Sc Biol Med 2023;14:246-252

although recent research has indicated that this process is significantly slower in humans, with a reported eight-fold decrease in efficiency.[1-3] During the developmental stages from birth to adulthood, the duodenal glands undergo a gradual process of growth and differentiation. The glands undergo continuous growth, resulting in the formation of a well-defined and condensed glandular cluster.[4,5] At birth, the submucosa of the duodenum in Mole-rats generates coiled tubules.[6] The gut is a vital organ that serves crucial functions in the host's immune response, elimination of waste materials, and absorption of nutrients.[7] The intestinal tract has evolved a mechanism to enhance its absorptive surface area through a morphogenetic process that generates finger-like structures called villi, enabling it to meet the daily needs.[8,9] The villi present in the adult gut harbor specific cell types that play a crucial role in the host's absorption of nutrients from food and in protecting against both commensal and pathogenic bacteria.[10,11] The utilization of laboratory animals is prevalent in modern biomedical research, serving as subjects for investigation in various disciplines such as surgery, biology, medicine, physiology, toxicology, and pharmacology. Additionally, they are employed as experimental models in immunological, reproductive, and numerous other studies pertaining to different diseases.[12,13] According to the study conducted by Dyce *et al*.[14], it has been proposed that many signaling pathways play a crucial role in effectively regulating the differentiation process of the gut mucosa. The formation of immature cells is commonly observed when the epithelial lining of the colon undergoes frequent and rapid renewal processes.[15,16] The proliferation of these cells occurs rapidly as they rise along the axis that extends between the crypt and the villus, thereby forming a region of transit amplification. Enterocytes, enteroendocrine cells, goblet cells, and Paneth cells are representative examples of the differentiated epithelial cells that arise from this particular location.[17,18]

## Materials and Method

This study was conducted utilizing a sample size of twenty healthy rabbits (Oryctolagus cuniculus), who are known to be herbivorous, as well as mice. Mus musculus, often known as the house mouse, is classified as an omnivorous rodent. This categorization is applicable throughout the time period spanning from February to May in the year 2023. The parental individuals of each species were confined in enclosures to facilitate controlled breeding and reproduction. These enclosures were subjected to artificial lighting conditions, alternating between periods of darkness and light for 8 to 16 hours. Additionally, a feeding system was implemented to ensure proper nourishment for the offspring of each species. The offspring were categorized into four groups based on their age in days: newborns, those at the age of seven days during the suckling period, those at the age of fifteen days during the preweaning stage, and those at the age of forty days after weaning. The diet provided to the offspring differed depending on the species: rabbits were fed a solid diet with a greenish component, while other species were fed a mixture of seeds, grains, and animal-derived feedstuffs. The animals were subjected to euthanasia using an excessive dosage of ketamine, with the exception of those that were one day old. The ventral abdominal wall of each animal was dissected, followed by the separation of the duodenum by sectioning the pylorus just before the duodenum to jejunum junction. Samples were collected from various regions of the duodenum and subsequently fixed in formalin. Histological techniques were employed to process the samples, including staining with hematoxylin and eosin (H&E) for general histological analysis, as well as periodic acid Schiff (PAS) for the visualization of specific histological components. The staining technique involving the use of Alcian blue at pH 2.5 is commonly employed to detect the presence of carbohydrates and mucopolysaccharides. Specifically, this method allows for the visualization of acid mucopolysaccharides when Alcian blue is combined with them. The utilization of Periodic Acid Schiff (PAS) staining technique, along with Masson's trichrome staining, enables the visualization of neutral mucin and the identification of collagen and smooth muscles.[19] The objective of this study was to measure the thickness of the tunicae and quantify the number of Alveoli or acini present in the Brunner's glands within a permiroscopic area at a magnification of X 200. The mean (x-) ± standard error (SE) was computed for each of the measurements taken from fifteen slides of the duodenum (n=20).

## Results

The duodenal wall of each studies animals and ages was composed of mucosa, submucosa, muscularis, serosa (Figure. 1), mucosa; consists of columnar epithelium, loose connective tissue of lamina propria and thin smooth muscle layer of muscularis mucosa, crypts of Lieberkuhn line with columnar epithelium, the dense connective tissue of submucosa with glands, two layers of smooth muscles of muscularis externa and thin simple squamous layer of serosa (Figure. 1-12). At one-day age in all studies species, the villi's epithelium contained more number of the vacuolated absorptive cells, the villi were short, asymmetrical with intervillus space, and the villi's bases contain clusters of an immature cells, lacked crypt in the lamina propria, and the muscularis mucosa was not continuous. Additionally, the submucosa lacked a Brunner's gland (Figure. 1,2), the thickness of each of mucosa, submucosa, muscularis, serosa in duodenum at age one day, in rabbit (186.2±3.1; 84.2±1.4; 114.2±0.1; 13.1±0.1µm) respectively, while in mice was (148.6±2.1; 68.6±0.2; 98.6±0.1; 11.2±0.2µm) respectively (Table 1).

Whereas on age of 7 day in mice and 10 day in rabbit, the mucosa had slender cylindrical villi and muscularis mucosa was poorly developed of circular smooth layer, the crypts of Lieberkuhn were start in the histogenesis (Figure. 4,5,6), A few number of acini of the Brunner's glands were in submucosa of the duodenum (Figure. 5), the thickness of

each of mucosa, submucosa, muscularis, serosa and number of alveoli of Brunner's gland in duodenum in this age, in rabbit (211.2±2.5; 91.2±0.3; 121.2±0.2; 17.2±0.3; 15±2µm) respectively, while in mice was (178.6±3.2; 74.6±1.6; 92.6±1.7; 15.1±0.5; 17±1µm) respectively (Table 1).

In 15 day of rabbit and mice, the cell vacuolation was decreased, the villi assumed their mature morphology, and the crypts were clearly growing and muscularis mucosa was developed circular smooth layer with increase in the number of the Brunner's gland acini an in the duodenal submuosa (Figure. 7,8), the thickness of each of mucosa, submucosa, muscularis, serosa and number of alveoli of Brunner's glands in duodenum in fifteen day age, in rabbit (261.4±3.5; 106.4±2.1; 196.4±2.3; 18.4±0.2; 23±1µm) respectively, while in mice was (215.9±4.1; 93.2±3.2; 171.2±3.4; 19.3±0.3; 27±3µm) respectively (Table 1).

In 40 day age in all studies species; the mucosa was composed of mature villi, were leaf shape, absent of the cell vacuolation in the duodenal epithelium, the crypts were very well established, and their depth and number had also grown, muscularis mucosa was very developed circular muscular layer and the duodenal glands were found at length of the rabbit duodenum, while absent in last parts of mice duodenum (Figure. 9), these glands were mucous type in mice, while serous and mucous type in rabbit (Figure. 9,10), the thickness of each of mucosa, submucosa, muscularis, serosa and number of alveoli of Brunner's gland in duodenum in forty day age, in rabbit (371.2±2.6; 167.2±1.4; 217.2±1.2; 22.3±0.1; 44±2µm) respectively, while in mice was (310.2±3.7; 113.1±2.1; 193.1±1.9; 21.4±0.3; 52±1µm) respectively (Table 1). Within the submucosa, in preweaned ages, the duodenal glands were mucous type in mice (Figure. 8), while serous type in rabbit (Figure. 7), the glands were made up of acini that were tightly packed and in mice were take positive reaction with only Periodic acid Schiff,[11] while in rabbit take positive reaction with Alcian blue (Figure. 7) and reactions to PAS (Figure. 4), with progress age, the Brunner's glands as mixed acini and had neutral mucin, according to combined PAS-AB reaction (Figure. 8). and weak reactions to PAS (Figure. 11), the progress in age has a pronounced effect on the shape the villi, and on the number each of the crypts and Brunner's glands; Additionally, the numerous morphometrical analyses demonstrated how the duodenal wall changed with age in this study.

**Table (1): Measurement of thickness of the wall layers and number of acini of Brunner's gland in duodenum of rabbit and mice µm (X- ± S.E)**

| Tunica Part | Mucosa | Submucosa | Muscularis Externa | Serosa | Number of acini |
|---|---|---|---|---|---|
| In 1 day age Rabbit | 186.2±3.1 | 84.2±1.4 | 114.2±0.1 | 13.1±0.1 | - |
| mice | 148.6±2.1 | 68.6±0.2 | 98.6±0.1 | 11.2±0.2 | - |
| In 7 day age Rabbit | 211.2±2.5 | 91.2±0.3 | 121.2±0.2 | 17.2±0.3 | 15±2 |
| mice | 178.6±3.2 | 74.6±1.6 | 92.6±1.7 | 15.1± 0.5 | 17±1 |
| In15 day age Rabbit | 261.4±3.5 | 106.4±2.1 | 196.4±2.3 | 18.4±0.2 | 23±1 |
| mice | 215.9±4.1 | 93.2±3.2 | 171.2±3.4 | 19.3±0.3 | 27±3 |
| In 40 day age Rabbit | 371.2±2.6 | 167.2±1.4 | 217.2±1.2 | 22.3±0.1 | 44±2 |
| mice | 310.2±3.7 | 113.1±2.1 | 193.1±1.9 | 21.4±0.3 | 52±1 |

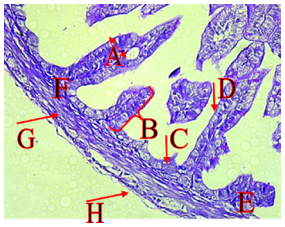


Figure 1. Cross section of rabbit duodenum at 1 day age; mucosa (A), villi (B), intervillus space (C), epithelium (D), lamina propria (E), submucosa (F), muscularis (G), Serosa (H), H&E 40X.

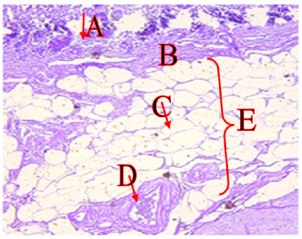


Figure 2. Longitudinal section of mouse duodenum at 1 day age; crypt (A), muscularis mucosa (B), adipose tissue (C), blood vessel (D), submucosa(E), PAS 100X.

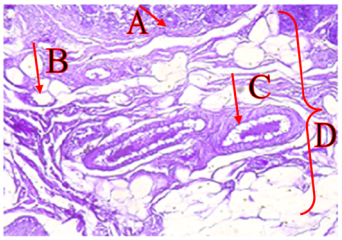


Figure 3. Cross section of rabbit duodenum at 1 day age; crypt (A), adipose tissue (B), blood vessel (C), submucosa(D), PAS 200X.

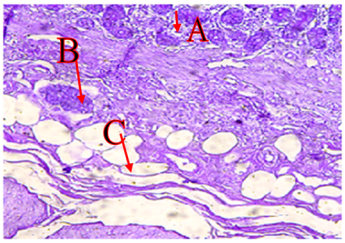


Figure 4. Cross section of rabbit duodenum at 7 day age: crypt (A), Brunner's glands (B), adipose tissue (C), PAS-AB 100X.

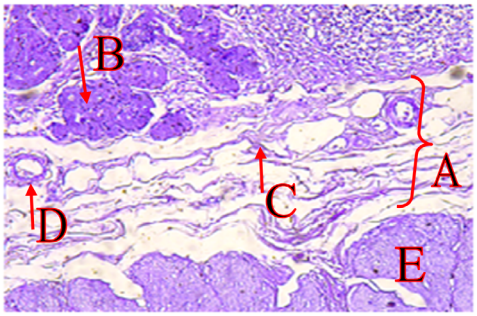


Figure .(5): Cross section of mice duodenum at 7 day age, Submucosa (A), Brunner glands (B), adipose tissue (C), blood vessel (D), muscularius (E), AB 100X.

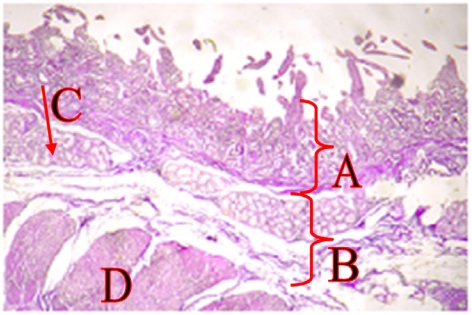


Figure 6. Cross section of the mice duodenum at 7 day age: mucosa (A), Submucosa (B), Brunner glands (C), muscularius (D), Masson trihrome 40X.

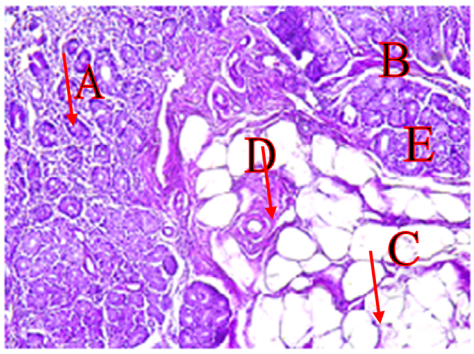


Figure 7. Cross section of rabbit duodenum at 15 day age; crypt (A), muscularis mucosa (B), adipose tissue (C), blood vessels (D), Brunner glands (E), AB 200X.

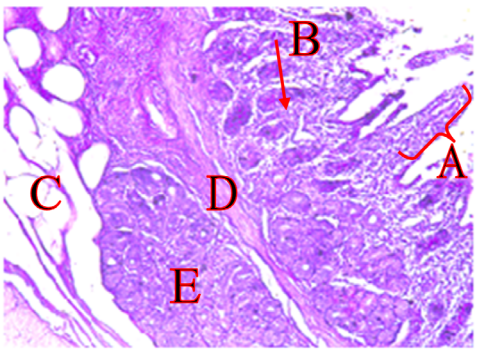


Figure 8. Cross section of mice duodenum at 15 day age; villi (A), crypt (B), adipose tissue (C), muscularis mucosa (D), Brunner glands (E), PAS-AB 200X.

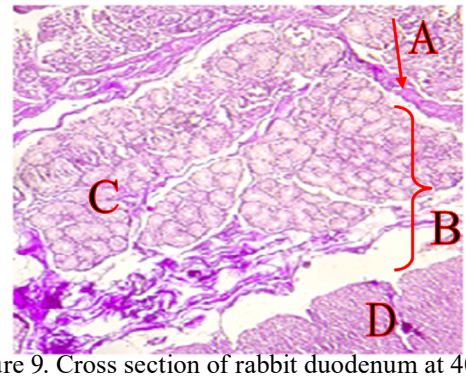


Figure 9. Cross section of rabbit duodenum at 40 day age; muscularis mucosa (A), submucosa (B), Brunner glands (C), muscularis (D), masson trihrome 200X.

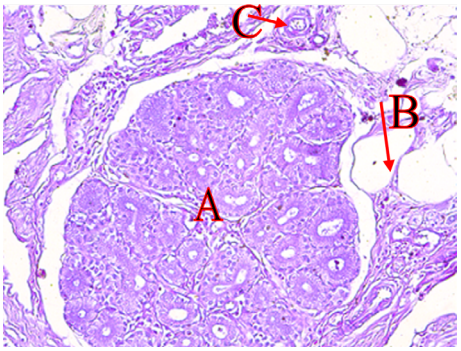


Figure 10. Cross section of mice duodenum at 40 day age; acini of Brunner glands (A), adipose tissue (B), blood vessels (C), H@E 400X.

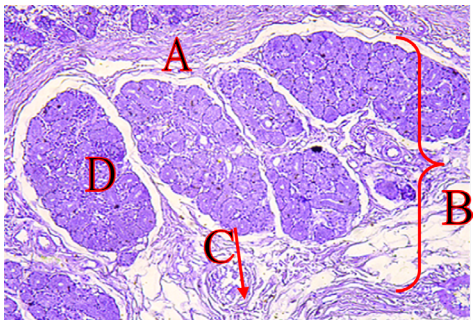


Figure 11. Cross section of mice duodenum at 40 day age; muscularis mucosa (A), submucosa (B), connective tissue (C), Brunner glands (D), PAS 200X.

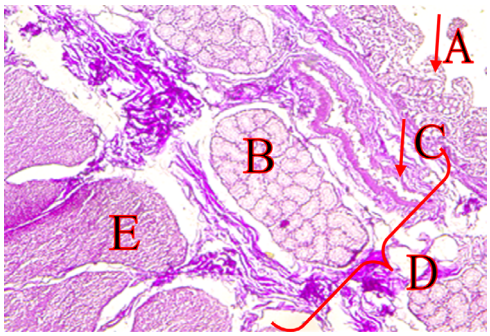


Figure 12. Cross section of mice duodenum at 40 day age; epithelium (A), Brunner glands (B), blood vessels (C), submucosa (D), muscularis (E), masson trihrome 100X.

## Discussion

The presence of duodenal glands is not observed immediately after birth, but rather within the initial ten days in rabbits and the first week in mice. In accord with the results reported by previous studies.[17,20] The aforementioned discovery was consistent with the most recent observations in pups exceeding one day of age.[8] Conversely, it was shown that rats at the age of one day possessed Brunner's glands.[21] The gastrointestinal glands in mice exhibited a serous-type composition during the early stages, whereas the duodenal glands in rabbits displayed a mucous-type composition. As individuals age, there is a development of mixed acini and neutral mucin in Brunner's glands. The association between the structural changes seen and the protective role of Brunner's glands in safeguarding the mucosa against the harmful effects of gastric juice has been established.[22] The secretion of mucus, its alkaline nature, and the potential ability of its bicarbonate concentration to serve as a buffer against the acidic conditions in the digestive tract. Consequently, a recent study has revealed that the majority of acini in mice aged ten and fifteen days have a serous composition, but acini in older animals around 40 days of age predominantly display a mucous nature. The duodenal glands in rabbits are found in close proximity to the jejunum, whereas they are absent in the final parts of the duodenum in mice, which is consistent with previous findings.[23] The distribution of Brunner glands is more extensive in herbivores compared to omnivores, as evidenced by previous research.[15] The glands in question secrete substances that serve to preserve and lubricate the lining of the duodenum. Additionally, these glands generate urogastrone, which acts as an inhibitor of gastric acid secretion and serves as a demonstrator of immunoglobulin. Lysozyme is present within the cellular composition of secretory units, indicating a perpetual secretion of bactericidal enzyme by these glands.[14] The increased focus on changes in mucosal architecture and mucin's histochemical composition is due to their connection with the development of various inflammatory and intestinal diseases, as well as their role in regulating intestinal functions.[1] Previous research has identified various other constituents, including peptides, proteinase inhibitors, bactericidal agents, and surface active lipids, which are formed by mucin glycoproteins in the duodenal glands together with a little amount of bicarbonate.[4] The incidence of various types in this study was found to be similar to that documented in Angora rabbits[17] as well as in rabbits and rats.[24]

The adult gastrointestinal tract is a highly intricate organ that fulfills numerous essential functions. Following the process of gastric breakdown and digestion, the resultant food substance proceeds to engage with the small intestine. Intestinal enterocytes has the capability to uptake proteins, carbohydrates, lipids, and nutrients through a complex and interconnected vascular network.[25] The intestinal tract serves vital functions in both immune response and nutrient absorption. The luminal contents of the intestine come into contact with the epithelium layer, which serves as a protective barrier against external factors and helps defend the body against local diseases and pathogens. Epithelial tight junctions play a crucial role in maintaining the integrity of the barrier by selectively regulating the movement of substances between adjacent epithelial cells.[14,26] The epithelium furthermore produces mucus, a dense protective layer that envelops the gastrointestinal system. This mucus serves the dual purpose of impeding the entry of pathogens, thus reducing the risk of infection, and providing nourishment to the resident commensal bacteria. Specialized intestinal epithelial cells play a substantial role in the immune response by producing peptides that possess antibacterial and antifungal properties. Furthermore, the immune system is activated through the establishment of commensal bacterial colonization during the process of birth.[27] The shape of the villi exhibited variability, with the height of the villi showing variation. The presence of very tall villi potentially contributes to the creation of a large surface area, which may indicate that absorption is most pronounced in older animals. This observation aligns with findings reported in a study conducted on Angora rabbits.[17] At the 24-hour mark following birth, the presence of vacuolated enterocytes can be observed in the villi. This phenomenon has been historically associated with the absorption of macromolecules. Similar discoveries were also reported by studies examining the crypts of Lieberkuhn, which are characterized by a lining of columnar epithelium.[14] The crypts are actively producing and releasing digestive juices in order to facilitate the processes of digestion and absorption of various substances.[3] The formation of the crypt in mice typically occurs around postnatal day 14. The differentiation of Paneth cells in the gastrointestinal tract coincides with the establishment of crypts. These specialized cells are responsible for releasing defensive proteins that possess antibacterial properties, so providing protection against pathogenic infections.[17] According to recent studies, it has been discovered that significant alterations in the metabolic processes of the intestinal epithelium play a crucial role in the maturation of the intestinal epithelium.[28]

## Conclusion

The current study contributes novel insights and provides documentation regarding the structural disparities in the development of duodenal glands between rabbits and mice. It is observed that the advancement in age significantly influences the morphology of the villi, as well as the quantity of crypts and Brunner's glands. Furthermore, a multitude of morphometric analyses have elucidated the alterations occurring in the duodenal wall as a consequence of aging.

## References

1. Salva MN, Gupta C, Pandey AK, Kumar N, Kotian SR, Kalthur SG. Histogenesis and Histomorphometric study of Human Fetal Small Intestine. Ethiop J Health Sci. 2019; 29(6): 689-96. doi: https://doi.org/10.4314/ejhs.v29i6.5.

2. Holladay SD, Smialowicz RJ. Development of the murine and human immune system: differential effects of immunotoxicants depend on time of exposure. Environ Health Perspect. 2000; 108(Suppl 3): 463-73. doi: https://doi.org/10.1289/ehp.00108s3463.
3. Kliwinski C, Cooper PR, Perkinson R, et al. Contribution of FcRn binding to intestinal uptake of IgG in suckling rat pups and human FcRn-transgenic mice. Am J Physiol Gastrointest Liver Physiol. 2013; 304(3): G262-70. doi: https://doi.org/10.1152/ajpgi.00340.2012.
4. Davies RR, Davies JA. Rabbit gastrointestinal physiology. Vet Clin North Am Exot Anim Pract. 2003; 6(1): 139-53. doi: https://doi.org/10.1016/s1094-9194(02)00024-5.
5. Chin AM, Hill DR, Aurora M, Spence JR. Morphogenesis and maturation of the embryonic and postnatal intestine. Semin Cell Dev Biol. 2017; 66: 81-93. doi: https://doi.org/10.1016/j.semcdb.2017.01.011.
6. Kotze SH, van der Merwe EL, O'Riain M J. The topography and gross anatomy of the gastrointestinal tract of the Cape dune mole-rat (Bathyergus suillus). Anat Histol Embryol. 2006; 35(4): 259-64. doi: https://doi.org/10.1111/j.1439-0264.2005.00676.x.
7. Karim AJ, Hassan AH, Kadhim KH, Kadhim KK. The immunity function of rodlet cells in the intestine of Binni fish (Mesopotamichthys sharpeyi). J Adv Vet Anim Res. 2022; 9(2): 282-89. doi: https://doi.org/10.5455/javar.2022.i594.
8. Elnagy TMM, Osman DI. Anatomical study on the postnatal development of the gastrointestinal tract in rabbits. University of Khartoum Journal for Veterinary Medicine and Animal Production. 2013; 1(2): 174-83. Available from: https://typeset.io/papers/anatomical-study-on-the-postnatal-development-of-the-cn8wyz0uiy.
9. Salama WA, Refaie AM, Amin HF, Abdel-Mawla LF. Use of untreated and autoclave-treated wheat germ meal in growing Rabbit diets. World's Veterinary Journal. 2019; 9(3): 192-200. doi: https://doi.org/10.36380/scil.2019.wv25.
10. Perrin MR, Curtis BA. Comparative morphology of the digestive system of 19 species of Southern African myomorph rodents in relation to diet and evolution. African Zoology. 1980; 15(1): 22-33. Available from: https://www.ajol.info/index.php/az/article/view/152108.
11. Alves A, Pinheiro V, Mourão JL, Pires I, Oliveira J, Gama A. Measurement of rabbit's intestinal villus: Preliminary comparison of two methods. In: Proceedings of the 8th World Rabbit Congress. 2004:422-26. Available from: http://world-rabbit-science.com/WRSA-Proceedings/Congress-2004-Puebla/Papers/Pathology/P-Alves.pdf.
12. Holmes ZA, Wei SF, Harris DJ, Cheeke PR, Patton NM. Proximate Composition and Sensory Characteristics of Meat from Rabbits Fed Three Levels of Alfalfa Meal. J Anim Sci. 1984; 58(1): 62-67. doi: https://doi.org/10.2527/jas1984.58162x.
13. Brewer NR. Biology of the rabbit. J Am Assoc Lab Anim Sci. 2006; 45(1): 8-24. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5129783/pdf/jaalas2006000008.pdf.
14. Dyce KM, Sack WO, Wensing CJG. Textbook of Veterinary Anatomy. 4th ed. Philadelphia: W. B. Saunders Company; 1987.
15. Dellmann HD, Eurell JA. Textbook of Veterinary Histology. 5th ed. Awoters Kluwer company Philadelphi; 1998.
16. Besoluk K, Eken E, Sur E. A morphological and morphometrical study on the sacculus rotundus and ileum of the Angora rabbit. Veterinarni Medicina. 2006; 51(2): 60-65. Available from: https://vetmed.agriculturejournals.cz/pdfs/vet/2006/02/04.pdf.
17. Ergun E, Ergun L, Ozen A, Kurum A, Bayraktaroglu AG. Histomorphology of the Brunner's Glands in the Angora Rabbit. Journal of Animal and Veterinary Advances. 2010; 9(5): 887-91. doi: https://doi.org/10.3923/javaa.2010.887.891.
18. Sababi M, Nilsson E, Holm L. Mucus and alkali secretion in the rat duodenum: effects of indomethacin, N omega-nitro-L-arginine, and luminal acid. Gastroenterology. 1995; 109(5): 1526-34. doi: https://doi.org/10.1016/0016-5085(95)90640-1.
19. Suvarna KS, Layton C, Bancroft JD. Bancroft's Theory and Practice of Histological Techniques. 8th ed. Elsevier; 2018. Available from: https://shop.elsevier.com/books/bancrofts-theory-and-practice-of-histological-techniques/suvarna/978-0-7020-6864-5.
20. Andleeb RR, Bhardwaj RL, Sharma KB. Histochemical studies on the small intestine of Gaddi goat. Indian Journal of Animal Physiology. 2009; 2: 75-78.
21. Ainsworth MA, Koss MA, Hogan DL, Isenberg JI. Higher proximal duodenal mucosal bicarbonate secretion is independent of Brunner's glands in rats and rabbits. Gastroenterology. 1995; 109(4): 1160-6. doi: https://doi.org/10.1016/0016-5085(95)90574-x.
22. Al-Shamary ER, Jarad AS, Taher IA, Al-Saffar FJ, Naji WA. Some histo-morphometric and histochemical comparsion aspect of the duodenum in Collard Dove (Frivaldszky), Ruddy Shelduck (Pallas) and Owl (Otus Scors brucei) in south Iraq. J Entomol Zool Stud. 2017; 5(6): 923-28. Available from: https://www.entomoljournal.com/archives/2017/vol5issue6/PartM/5-5-405-600.pdf.
23. Mohammad HJ, Ali AK, Al-Ali ZAJR. Histomorphologal and Histochemical Structure in the Duodenum of Sheep (Ovis Aries) and Rabbit (Oryctolagus Cuniculus); A Comparative Study. Online Journal of Animal and Feed Research. 2020; 10(6): 251-57. doi: https://doi.org/10.51227/ojafr.2020.34.
24. Jawad I, Kadhim KH, Kadhim DM, Sadiq DH. A comparative histomorphological and histochemical study of the goblet cells and brunner's glands in the duodenum of Rabbits and Rats. Research Journal of Pharmacy and Technology. 2019; 12(5): 2421-24. doi: http://doi.org/10.5958/0974-360X.2019.00406.2.
25. Kadhim KH. Light microscopic study on the absorptive cells and goblet cells in the intestine of adult common carp Cyprinus carpio: 1Khalid Hadi Kadhim, 2Abdulkarim J Karim and 2Khalid Kamil Kadhim. The Iraqi Journal of Veterinary Medicine. 2019; 43(1): 148-55. doi: https://doi.org/10.30539/iraqijvm.v43i1.485.

26. Mohammadpour AA. Morphological and histochemical study of Guinea pig duodenal submucosal glands. Bulgarian Journal of Veterinary Medicine. 2011; 14(4): 201-08. Available from: http://tru.uni-sz.bg/bjvm/BJVM-December%202011%20p.201-208.pdf.
27. Alboghobeish N, Ghamber AZ. Histological study of ileum and caecum with special reference to their lymphoid tissue of Le-Lapin Albinos rabbit. Indian J Anim Sci. 1996; 66(7): 666-69. Available from: https://www.researchgate.net/publication/293613905.
28. Kadhim AAAKH. Histological and histochemical study on the structural development of the small intestine in cape hare Lepus capensis. European Journal of Molecular & Clinical Medicine (EJMCM). 2023; 10(03): 860-69. Available from: https://ejmcm.com/uploads/paper/f1e70f4753a3f24b101c7f1c5ae37eb5.pdf.