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Original Research Article

Variation of Alkaline Phosphatase Activity in Fallopian Tube: A Semiquantitative Histochemical Assessment in Clinical Perspective

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Abstract:

Introduction: Body proteins perform a number of functions, of which the most remarkable and specialized function is their ability to act as biological catalysts. Catalytic proteins called enzymes, stimulate the rate of several reactions in the body without themselves getting consumed in the process. Enzymes are highly selective as biocatalysts. A catalyst increases the rate of reaction without altering its equilibrium position and without changing itself

The alkaline phosphatases (ALP) are a group of enzymes that hydrolyses the organic phosphates at alkaline Ph. They are present in most tissues but are in particularly high concentration in intestinal epithelial cells, kidney tubules, bones (osteoblasts), liver and placenta. In adult plasma ALP is derived mainly from bone and liver in approximately equal proportions. Increased osteoblastic activity increases the level of plasma ALP, it may be physiological as seen in children. The total activity is about 2.5 times more than the normal upper limit in adults. In adults it is 40-125 IU/L. during pregnancy, plasma ALP level rises due to contribution of placental isoenzymes.

The fallopian tube is the vital organ of female reproductive system, which acts as a conduit to give passage to ova from ovary to uterus. The fallopian tube undergoes histochemical variations with oestrus cycle.

Aim: To study the phasial and segmental variation of ALP activity in rabbit's fallopian tube and to correlate the anatomy, physiology and Biochemistry of fallopian tube which may be useful to understand the different anomalies/ pathologies or problems related to female reproductive system.

Materials and Methods: The cross-sectional study was done on 50 Rabbit's fallopian tube at SNMC Agra from June 1976 – June 1979. The fallopian tubes of both sides were taken by the section and cut into segments from medial to lateral end viz intramural, isthmus, ampulla and infundibulum. The phases of oestrus cycle i.e. Pro-oestrus, oestrus, meta-oestrus and dioestrus were decided by cytological observation of a papaniculus stained vaginal smear. ALP activity was observed in various phases of sexual cycle and of different segments of fallopian tube by the Gomori's calcium cobalt method (after Gomori, Pears 1946). One way analysis of variance (ANOVA) was applied for the analysis of data.

Results: The ALP activity was higher in pro-oestrus (4.33±0.25), oestrus (4.08±0.25) and low in meta-oestrus (3.25±0.25) and dioestrus (2.08±0.25) of oestrus cycle. The activity was seen higher in infundibulum (4.25±0.31) ampulla (3.92±0.31) and low in Isthmus (3.00±0.31) Intramural (2.58±0.31). The maximum difference 1.7 was noticed between infundibulum and intramural segments ($p<0.0001$). a maximum difference (2.25) in pro-oestrus was noticed between pro-oestrus and dioestrus ($p<0.0001$).

Conclusion: The oestrogenic phase of the sexual cycle has higher enzymatic activity whereas in luteal phase it was low. The infundibulum and ampulla show higher activity. It suggests these segments are more functionally active as compared to intramural and isthmus segments of fallopian tube.

Keywords: Alkaline phosphatases, Ampulla, Di-oestrus, Enzyme, Fallopian tube, Infundibulum, Intensity, Meta-oestrus, Oestrus Phase, Oestrous cycle, Proestrus, Segments.

Introduction

Enzymes are biocatalysts that are essential for biochemical reactions to proceed in the human body. Biochemical activity of enzymes is dependent on the structural confirmation of the enzyme proteins. Alkaline phosphatase is a nonspecific enzyme which hydrolyses aliphatic, aromatic or heterocyclic compounds at Ph between 9 and 10. It is localised in cell membrane and is associated with transport mechanism in liver, kidney and intestinal mucosa. The ALP is present in most tissues of the body but are in particularly in high concentration in intestinal epithelial cells, kidney tubules, bones, liver and placenta. In adults, plasma ALP is derived mainly from bones and liver in approximately equal proportions.

Normal serum ALP value 40-125 U/L. the physiological increase ALP is seen in children due to increased osteoblastic activity and during pregnancy due to contribution of placental iso-enzymes. Pathological increase in ALP is seen in hepatic disorders, moderate increase 2-3 times such as infective hepatitis, Alcoholic hepatitis or hepatocellular carcinoma.

It is very high ALP (10-12 times of upper limit). It may be noticed in extrahepatic obstruction (obstructive jaundice) caused by gall stone or by pressure on bile duct by carcinoma of head of pancreas. Intrahepatic cholestasis may be due to virus (infective hepatitis) or by drugs (chlorpromazine), ALP is produced by epithelial cells of biliary canaliculi and obstruction of bile with consequent irritation of epithelial cells leads to secretion of ALP into serum. Drastically high levels of ALP (10-25 times upper limit) are seen in bone diseases where the osteoblastic activity is enhanced such as Paget's disease (osteitis deformance), Rickets, osteomalacia, osteoblastoma, metastatic carcinoma of bone and hyperparathyroidism, bone diseases etc. Hence it is an important bio marker [1, 2].

ALPs are post-translationally modified, resulting in the production of several different iso-enzymes, which differs in abundance in different tissues eg elevated ALP with Gamma glutamyle transferase (GGT) is suggestive of non- hepatic origin eg bone disease or pregnancy [3]. The bone specific ALP (SALP), a marker of osteoblastic activity is raised in Paget's disease of bone, metastatic disease of bone, osteomalacia and osteoporosis. However, it is important to exclude hepatobiliary diseases where ALP level is also high especially in patient with cholestasis. The bone specific ALP (SALP) fraction has better productive value in evaluating bone disease [4]. Fallopian tube epithelium and its secretions are important for the survival and transportation of gamete and provide favourable condition for sperm capacitation and fertilization which are essential for productivity. As far as the authors are aware, work on histochemical aspect of fallopian tube is less considered and previous work have not comprehensively considered various segments of fallopian tube and various phases of sexual cycle. The present study was conducted to find out segmental and phasial variations of ALP activity in fallopian tube. The obtained histochemical data may be of value in understanding the physiology and pathological aspects of fallopian tube.

Materials and Methods

A cross sectional observational study was done on 50 female rabbits at SN medical college, Agra during June 1976 to June 1979. The healthy mature female rabbits were chosen for this study.

Inclusion criteria: Physically active female rabbits in reproductive age group without pregnancy

Exclusion criteria: Unhealthy and pregnant female rabbits with visible congenital malformations, altered physical and feeding behaviour.

Study procedure

The phase of Oestrus cycle was decided by cytological appearance of Papaniculous stained vaginal smear of experimental animal. These animals were treated with ether to anaesthetise and abdomen was opened by giving mid vertical incision in the anterior abdominal wall. The fallopian tubes were obtained by dissection and cut into four anatomical segments from medial to lateral ends i.e. intramural, isthmus, ampulla and infundibulum in each phase of oestrus cycle viz, pro-oestrus, oestrus, meta-oestrus and dioestrus. The cut segments were fixed in cold acetone (-4°C) for 24 hours.

Tissues were then routinely processed and embedded in paraffin blocks, transverse microsection of 6 micron were cut and stained with modified Gomori's calcium cobalt stain method (after Gomori 1946 was used). The slides were studied under light microscope, ALP activity appears as black precipitate. The staining was controlled by staining the microsections of kidney by same procedure simultaneously. The specificity was confirmed by taking the boiled microsections for the staining. The stained slides were given to three independent different observers to grade the intensity of ALP activity in various phases and in different anatomical sections.

The grading criteria was fixed as ± irregular and or in traces + week, ++ mild, +++ moderate, ++++ intensive and +++++ very intensive for ALP activity. The results of observations were not known to each other.

The mean of the grading given by three observers were taken into consideration for evaluation of ALP activity, depicted in table 1.

Table1: Semiquantitative assessment of alkaline phosphatase activity

Phase of sexual cycle	Segments of fallopian tube			
	Infundibulum	Ampulla	Isthmus	Intramural
Proestrus	+++++	++++	+++	+++
Oestrus	+++++	++++	+++	+++
Metaestrus	++++	++++	+++	+++
Diestrus	+++	++	++	++

Chemistry of phosphatase activity

ALP+Beta Glycerophosphate→ Alkaline phosphate→ (Calcium Chloride)→Calcium Phospahate → (Cobalt Nitrate) → Cobalt phosphate → (Yellow ammonium sulphide) → Cobalt sulphide (black precipitate).

The sections from different segments of fallopian tube of rabbit in various phases of sexual cycle were stained by modified Gomori's calcium cobalt stain. The black precipitate showed the presence of ALP activity. It was seen more towards the free luminal surface of lining epithelial cell of fallopian tube where the activity of ciliary cells is known to be maximum. Lindenbaum ES etal (1982) stated that ALP reaction product was located along the apical and lateral plasma membranes of the secretory cells only, regardless of stages of the cycle. In secretory cells of lining epithelium, the activity was seen negligible.

The enzyme was found exclusively in the cytoplasm of the epithelial cells. The cytoplasm was stained black or brownish black in colour and varied in different phases of sexual cycle in various segments of fallopian tube [5].

Statistical analysis: Mean grading of stained slides was converted into quantitative data. The grade for intensity of ALP activity by three independent observers was considered equivalent to +1, ++2, +++3, ++++4, +++++5. The rounding of mean values of grading was taken into consideration. One way ANOVA was applied for the analysis of data, using 16 version of JMP software.

Statistical Analysis of Anatomical Variation:

Table 2: Mean values of ALP Activity in various segments.

Segments of fallopian tube	Mean ± SEM	P value	
Infundibulum	4.25 ± 0.31	0.0011	Statistically significant
Ampulla	3.92 ± 0.31		
Isthmus	3.00 ± 0.31		
Intramural	2.58 ± 0.31		

When comparing the means of different segments of fallopian tubes for ALP activity with that of intramural segment, we obtained the following results.

Table 3: Comparison of means of different segments of Fallopian tube for ALP activity. ANOVA was used

Segments of fallopian tube	Segments of fallopian tube	Difference	P value	Results
Infundibulum	Intramural	1.666667	0.0001	Statistically significant
Ampulla	Intramural	1.333333	0.0001	Statistically significant
Isthmus	Intramural	0.416667	0.0775	Not significant

Statistical Analysis of Physiological Variation:

Table 4: Mean values of ALP Activity in various phases.

Phases of sexual cycle	Mean ± SEM	P value	
Proestrus	4.33333 ± 0.257	0.0001	Statistically significant
Oestrus	4.08333 ± 0.257		
Metaestrus	3.25000 ± 0.257		
Diestrus	2.08333 ± 0.257		

When comparing the means of different phases of sexual cycle for ALP activity with that of Diestrus phase, following results were obtained.

Table 5: Comparison of means of different phases of sexual cycle for ALP activity, ANOVA was used.

Phases of sexual cycle	Phases of sexual cycle	Difference	P value	Results
Proestrus	Diestrus	2.250000	0.0001	Statistically significant
Oestrus	Diestrus	2.000000	0.0001	Statistically significant
Metaestrus	Diestrus	1.166667	0.0024	Statistically significant

Results

The sections stained with Modified Gomori's Calcium Cobalt stain revealed ALP activity as black precipitate. The ALP activity was located more towards the free luminal surface of the lining epithelial cells of the Fallopian tube, where the activity of the ciliary cells is known to be maximum. The secretary cells of the lining epithelium, the activity was negligible. The enzymatic activity was found exclusively in the cytoplasm of epithelial cells. The cytoplasm was stained black or brownish black in colour and varied in reaction in different phases of sexual cycle in various anatomical segments of fallopian tube. The ALP activity was seen more towards the free luminal surface of the lining epithelial cells where the ciliary apparatus is known to be developed and located. The ALP activity was negligible in secretary cells of the lining epithelium. Weeth and Hermann (1952) and Friedriesson (1957) observed ALP activity exclusively on ciliary tufts in tubal cells in cattle [6, 7]. Bjorkmann and Friedricsson (1960) supported this observation and they remarked that this activity is associated with microvilli present on the free surface of ciliary cells [8]. Ono K (1974) also found with light microscope study that the ALP activity was on the free luminal surface of fallopian tube epithelium and confirmed it by electron microscope [9]. Our findings suggest that the enzymes are related with cellular functional activity. Hence their occurrence in ciliary cells in tubal epithelium is accounted by the increased workload required for ciliary motion during conduction of ova through the tube.

Lindeubaum, Ella S et. al. (1982) however observed in ultrastructural localization of ALP activity that ALP product was located along the apical and lateral plasma membrane of secretary cells only, regardless of the stages of sexual cycle [5]. The ciliated cells were almost devoid of any reaction product at all stages of the cycle. M Krajnicakova (2004) studied the activity of Alkaline and Acid phosphatase and non-specific esterase in the endometrium and oviduct of post-partum does, observed the ALP activity in apical parts of the goat's oviductal epithelium, which was increased during the observed intervals of observation period [10]. Santhi Lakshmi M et. al. (2018) stated that the ALP activity was strong in the basement membrane and apical part of lining epithelium of all the regions of the oviduct, submucosal connective tissue and tunica muscularis region of infundibulum, uterus, vagina. Strong reaction for ALP was observed in lamina propria and muscular layer of isthmus, while moderate reaction was noticed in submucosa and muscularis region of magnum [11].

Abe H et. al. (1993) reported that the luminal surface of epithelial cells in various regions of the oviduct of goats at follicular and luteal phases of oestrus cycle presented marked cyclic changes in scanning electronic microscopy. Marked cyclic changes were observed on the surface of epithelium in fimbria, ampulla and ampullary isthmus junction of oviduct in the follicular phase was extensively ciliated and most of the cilia extended above the apical processes of the non-ciliated cells. In luteal phase, many ciliated cells were hidden by bulbous processes of the non-ciliated cells in the isthmus. In the isthmus and at the utero-tubal junction, the apical surfaces of the non-ciliated cells were flat or gently rounded at both phases of the oestrus cycle [12]. The result demonstrate that regional variation are associated with the cyclic changes in the epithelial cells of the goat oviduct. Dhruv Lowe et. al. (2023) stated that ALP are a group of iso enzymes located on the outer layer of the cell membrane. They catalyse the hydrolysis of organic phosphate esters present in extra cellular space. ALP is present in decreasing concentration in the placenta, ileal mucosa, kidney, bone and liver. The majority of ALP in serum (more than 80%) is released from the liver and bone and in small amounts from the intestine. Even though ALP are present in many tissues throughout the body, their precise physiological function remains largely unknown [13].

Anatomical variation of ALP activity

The ALP activity in our study was found as intense to very intense in ampullary and infundibulum segments of fallopian tube respectively. It is moderate to mild in isthmus and intramural segments. The ALP activity in infundibulum and ampulla suggesting that the segments are more physiologically active during sexual cycle. The lesser ALP activity in isthmus and intramural segments seems to be physiologically less active segments of fallopian tube.

Kumar V and Srivastava A 1995 reported that secretory and ciliated cells are crowded in infundibulum. It results in increased mechanical activity needed for transportation of ova towards the uterine end. Therefore, ALP activity is maximum in infundibulum [14].

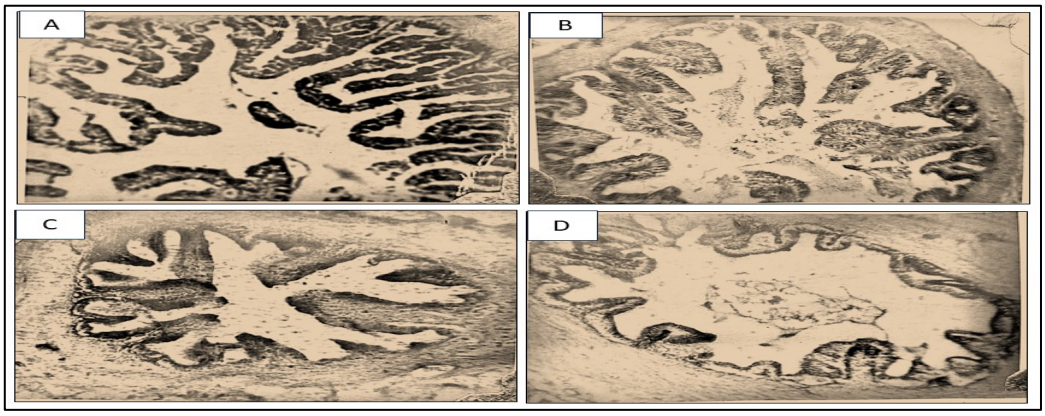

Figure 1: Photomicrograph: Modified Lead Nitrate Staining (X100). A) Infundibulum, B) Ampulla, C) Isthmus, D) Intramural.

Physiological variation of ALP activity

The results showed maximum ALP activity in tubular epithelium in proestrus and oestrus phases and minimum in di-oestrus through meta-oestrus phase of sexual cycle. Catallan and Calaprie (1963) also observed high ALP activity during proestrus and oestrus and low in meta-oestrus and diestrus phases of sexual cycle [15]. McDanial et. al. (1968) studied ALP activity in relation to the hormones and found it to be increased by estrogen and decreased by progesterone treatment [16].

It has also been suggested by Fredricsson B and Holm S 1974 and Vajpayee et al 1977 that oviductal epithelial cells show regional differences in their responses to steroid hormone [17, 18]. The increased activity in proestrus and oestrus phases in our study may be related to the hyper-estrogenic status of the animal. The minimal amount of ALP in diestrus phase of sexual cycle may be due to lower estrogen level. Odor D Louise et. al. (1983) stated that most of the epithelial cells in early follicular phase are non-ciliated i.e. 89.9% (fimbria), 80.4% (ampulla), 65.0% (isthmus) where as an extensive deciliation in fimbria and ampulla during second half of cycle leads to a significant increase of non-ciliated cells in these regions during late luteal phase i.e. 92.2% (fimbria), 81.2% (ampulla) and there is no appreciable increase in isthmus i.e. 62.2%. since this segment does not participate to any significant degree in cycle shedding and renewal of cilia occurs in other segments [19]. Odor et. al. (1988) further worked on light and electron microscopic observation on cervical epithelium of rabbit and reported the percentage of non-ciliated secretary cells that are 49.6% in ovulatory, 43.6% in oestrus and 23.7% in long term ovariectomized Rabbit and ciliated cells are 50.2% in ovulatory, 56.2% in oestrus and 76.3% in long term ovariectomized animals. The value for the ovulatory and oestrus rabbits are significantly different at $p < 0.05$ level from those of ovariectomized animal. These statements endorsed our findings that ALP activity was maximum in follicular phase and minimum in luteal phases [20].

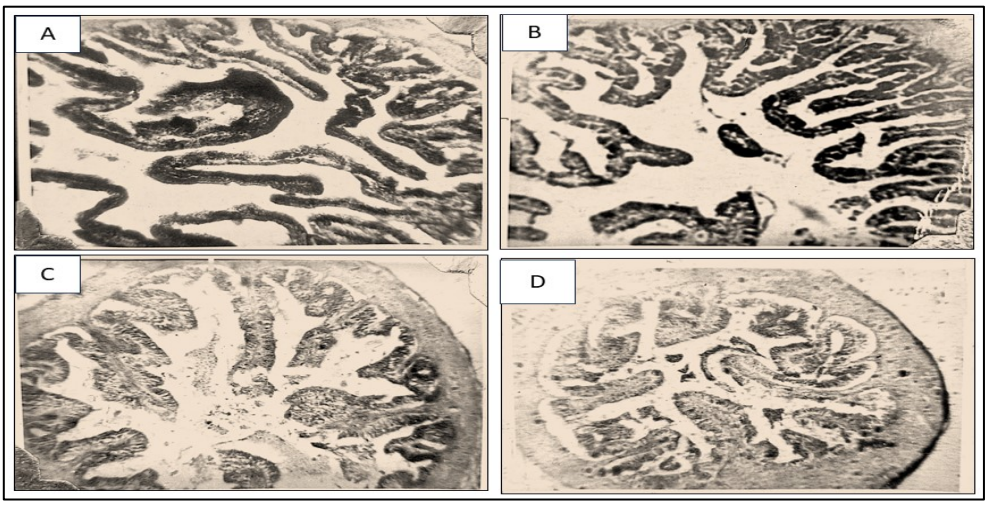

Figure 7: Photomicrograph: Modified Lead Nitrate Staining (X100). A) Pro oestrus, B) Oestrus, C) Meta Oestrus, D) Diestrus

Abe H et. al. (1999) studied the cyclic and segmental variations in proportion and cell height of ciliated and secretary cells in goat oviduct epithelium and found that ciliated cells in fimbria and ampullary epithelium were more abundant than those in the isthmic region in follicular phase [21].

Barbara S and Richard L Reeder (1996) reported that during the course of oestrus cycle, there were changes in relative numbers of secretory and ciliated cells in the ampullae. Secretary cells increased in numbers at oestrus, became dominant cell type at metestrus, and became rare at diesrtus-1, a day on which ciliated cells prevailed.

On the remaining days of the cycle, both cell types were evident with more secretory cells at diestrus-2 and ciliated cells seen at proestrus phase of oestrus cycle [22]. Abe H and Hoshi H (2007) in ultrastructural cyto-morphometric study in Chinese Meishan Pig observed marked regional variations in the epithelial cells of the oviduct that are associated with cyclic changes.

During follicular phase, ciliated cells were predominant in fimbrial epithelium and the height of ciliated cells decreased subsequently in fimbria and ampulla at the luteal phase which is responsible for the reduction in the number of cilia on the surface of oviductal epithelium in luteal phase whereas the secretary activity of epithelial cells is highest in ampulla and isthmus at follicular phase of oestrus cycle. From oestrus to meta-oestrus of oestrus cycle, fertilization occurs in the ampulla and the embryo migrate in the isthmus towards uterus [23].

Kumar V and Srivastava A (2018) in histological study of fallopian tube in various phases of oestrus cycle observed that the epithelial surface area was maximum in oestrus phase, the secretory cells were most active in metaestrus and diestrus phases. Ciliary cells were prominent in proestrus and oestrus phases of sexual cycle.

The study explains the basis of increased activity of enzymes in proestrus and estrus phases of sexual cycle [24]. Stastna D et. al. (2019) reported the significant changes between ciliary and secretary cells during oestrus cycle in various parts of fallopian tube. Ciliary cells dominated through the cycle in infundibulum and ampulla, whereas secretary cells in isthmus. Their changes and differentiations are the manifestations of hormonal changes that direct the oestrus cycle [25].

The biological significance of regional differences in the secretary cells of fallopian tube needs further investigations to improve our understanding of the reproductive process. Chang-You YU et. al. (2011) studied the relationship of ALP and the reproductive activity in dairy goats, observed the serum ALP value in virgin (13 cases) 320.69$\pm$176.67, nonpregnant (7 cases) 213.57$\pm$52.87, pregnant (7 cases) 414.71$\pm$452.57 and lactating (15) 249.07$\pm$411.70 which shows the maximum serum ALP u/l in pregnancy.

The study has definitely found a way for researchers to investigate a possible role of ALP activity of genital origin in different reproductive stages [26].

Kumar V et. al. (2017) studied acid and alkaline phosphatase activity in uterus of goat in different stage of pregnancy, observed that acid and alkaline phosphatases increased with the advancement of pregnancy.

Endometrial glands showed mild to intense activity of ALP and ACP activity during initial stages of pregnancy. The activity of ALP increased with the advancement of pregnancy. However, ACP activity was moderate to intense during mid stage and moderate during advance stage of pregnancy [27]. Kumar V et. al. (2023) reported that ACP activity is higher in estrogenic phase and low in luteal phase of sexual cycle.

The ALP activity follows the same pattern except that the intensity of ALP reaction is much higher as compared to ACP in their study [28]. Our histochemical study thus attracts further investigations which may help in solving the reproductive problems such as miscarriages, infertility and other Pathologies.

Conclusion

The ALP activity was found concentrated in ciliary cells, its presence might be related with increased mechanical ciliary activity, caused by ciliary cells. It was more in estrogenic phase suggesting their relationship with estrogen level of the animal. It was found more in infundibulum and this might be related to mechanical activity needed for transportation of ova towards the uterine end.

The study is useful in understanding of various problems related to female reproductive system. It also attracts for further investigation as regards to have much more detailed physiological, clinical and biochemical aspects of fertility, miscarriages and other pathologies.

These investigations can also find out the way to have certain more information regarding chronic diseases such as carcinoma of female reproductive system for its early detection.

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References

1. Vasudevan DM. Text book of biochemistry for medical students. 10 ed. New Delhi: Jaypee brothers medical publishers (p) ltd; 2023.
2. Puri D. Text book of Medical Biochemistry. 4 ed. New Delhi: Elsevier; 2020.
3. Penman ID, Ralston SH, Strachan MWJ, Hobson R, editors. Davidson's Principles and Practice of Medicine. Gurgaon, Haryana (India) ELSEVIER; 2023.
4. Kamath S. API Textbook of Medicine. 11 ed: CBS Publishers & Distributors; 2019.
5. Lindenbaum ES, Beach D, Peretz BA. Ultrastructural Localization of Alkaline and Acid Phosphatases in the Human Fallopian Tube Epithelium During the Menstrual Cycle The Anatomical Record. 1982;203:67-72.
6. Weeth HJ, Herman H. A histological and histochemical study of the bovine oviducts, uterus and placenta. Biology. 1952;501:54.
7. Fredricsson B. histochemical studies on the epithelium in the human fallopian tube and comarison between different animal species. Ark Zool (stockh) Ser. 1957;11(11):110-1.
8. Bjorkman N, Fredricsson B. The ultrastructural organization and the alkaline phosphatase activity of the epithelial surface of the bovine fallopian tube. Zeitschrift fiir Zellforschung. 1960;51:589--96.
9. Ono K. Fine structural localization of alkaline phosphatase activity of the female genital canal surface during the estrous cycle and after castration in rats. Cells Tissues Organs. 1974;89(4):577–90.
10. Krajnicáková M, Lenhardt L, Valocký I, Cigánková V, Kostecký M, Maracek I. Activity of alkaline and acidic phosphatase and non-specific esterase in the endometrium and oviduct of postpartum does. Small Ruminant Research 2004;53:47–51.
11. Lakshmi MS, Naik KR, Kumar DP. Histochemical studies on the oviduct of emu bird (Dromaius novaehollandiae). The Pharma Innovation Journal. 2018;7(1):576-9.
12. Abe H, Onodera M, Sugawara S. Scanning electron microscopy of goat oviductal epithelial cells at the follicular and luteal phases of the oestrus cycle. Journal of Anatomy. 1993;183(2):415-21.
13. Lowe D ST, Zubair M, John S. Alkaline Phosphatase. 2022 Nov 4. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan–. PMID: 29083622.
14. Kumar V, Srivastava A. Histological differences in various segments of Rabbit's fallopian tube. j Anat sci. 1995;14(2):22-8.
15. Catallan G, Calaprie A. Alkaline phosphatase and PAS positive substance in the fallopian tube of baffalos during the estrus cycle and pregnancy. Acta Med Vet Napolli. 1963;8:261-73.
16. McDaniel JW, Scalzi H, D L Black. Influence of Ovarian Hormones on Histology and Histochemistry of the Bovine Oviduct. Journal of Dairy Science. 1968;51(5):754-61.
17. Fredricsson B, Holms S. Dissociated response of the epithelium of the Rabbit oviduct to estrogen. Biology of Reproduction. 1974;11:40-9.
18. Vajpayee BK, Shipstone AC, Gupta DN, Karkun JN. Differential response of the ampullary and isthmic cells to ovariectomy and estrogen: an ultrastructural study. Endokrinologie. 1977;69:11-20.
19. Odor DL, Gaddum-Rosse P, Rumery RE. Secretory Cells of the Oviduct of the Pig-Tailed Monkey, Macaca nernestrina, During the Menstrual Cycle and After Estrogen Treatment The American Journal Of Anatomy. 1983;166(2):149–72.
20. Odor DL, Blandau RJ. Light and Electron Microscopic Observation on the Cervical Epithelium of the Rabbit 1. The American Journal Of Anatomy. 1988;181:289-319.
21. Abe H, Onodera M, Sugawara S, Satoh T, Hoshi H. Ultrastructural features of goat oviducatl secretory cells at follicular and luteal phases of the oestrus cycle. J Anat. 1999;195:515-21.
22. Shirley b, Reeder RL. Cyclic Changes in the ampulla of the rat oviduct. the journal of Experimental Zoology. 1996;276:164-73.
23. Abe H, Hoshi H. Regional and Cyclic variations in the ultrastructural features of secretory cells in the oviductal epithelium of the chinese meishan pig. reprod dom Anim. 2007;42:292-8.
24. Kumar V, Srivastava A. Histological variations in fallopian tube during various phases of oestrus cycle. J Anat Science. 2018;26(1):44-8.
25. Šťastná D, Šťastný P, Miluchová M, Gábor M. The morphological changes of oviductal mucose in oestral cycle of sows. Acta fytotechn zootechn. 2019;22(2):34–41.
26. Yu C-Y, Chou S-J, Wu J-T, Lin K-Y, Chan K-W, Su Y-C, et al. The Relationship of Alkaline Phosphatase and the Reproductive Activity in Dairy Goats. taiwan vet. 2011;37(3):152-8.
27. Kumar V, Singh SP, Farooqui MM, Gangwar C, Kumar P, Prakash A. Acid and Alkaline Phosphatase Activity in Uterus of Goat in Different Stage of Pregnancy. International Journal of Livestock Research. 2017;7(7):92-7.

28. Kumar V, Mohan G, Airan N, Srivastava A, Dwivedi AK. Variation of Acid Phosphatase Activity in the Fallopian Tube of Rabbits: A Semiquantitative Histochemical Assessment. Journal of Clinical and Diagnostic Research. 2023;17(4):1-4.