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Histochemical study of the uterus during various phases of estrus cycle in buffalo (*Bubalus bubalis*)

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Abstract

Uterus of total sixty non-descript buffaloes were studied during proestrus, estrus, metestrus, diestrus, anestrus and early pregnancy. Each group was consisted of ten samples. Histochemical reactions of Periodic Acid Schiff, PAS Alcian blue, Best Carmine, alkaline phosphatase, Acid phosphatase activity was demonstrated as per the standard protocol. Staining reactions of correlative changes in the carbohydrate and enzymes in the epithelium, lamina propria, uterine glands, myometrium and perimetrium of uterine horns were observed. The greatest secretory activity of uterine glands was observed during the diestrus. The reactions for presence of glycogen in the lamina propria, uterine glands and myometrium showed maximum reaction for glycogen material in the early pregnancy. The PAS-positive material in the uterine epithelium, lamina propria and uterine glands showed intense reactions during proestrus and estrus. It is hoped that this study would be helpful for better interpretation of changes caused in diseased conditions of the uterus in buffalo.

Keywords: Buffalo, uterus, estrus, pregnancy, histochemistry

Introduction

The uterus forms an important part of the reproductive tract. The four phases of estrus cycle namely, proestrus, estrus, metestrus and diestrus are the manifestations of the cyclic changes in the uterus due to the influence of hormones of the pituitary acting on ovary. The uterine structure and functions vary according to the reproductive status (Deshpande, 1994) [5]. Endometrium and endometrial fluid plays major role in reproduction. The contraction of the uterus helps transport of spermatozoa from the site of deposition to the site of fertilization. The developing blastocysts before implantation gets nourishment from endometrial secretions. The uterus undergoes a considerable change in size, structure and position to accommodate and help proper growth of the fetus during pregnancy. (Bandyopadhyay *et al.*, 2007) [2]. Initial preparations of uterus take place during the estrus cycle, in which it undergoes various important histological and histochemical changes. Much information on histology and histochemistry of uterus during different phases of sexual cycle is available in laboratory animals and other domestic animals but literature on this aspect in buffalo is scanty (Singh, 1983) [14]. Considering the enormous potential of the uterus in reproduction being an organ of female reproductive tract and the importance, scope and significance of the uterus in the field of biomedical research, there is a great need of advanced study on histochemical reports of uterus during different phases of estrus cycle in buffalo.

Materials and Methods

The present study was carried out on 60 buffaloes. The samples were classified into six groups according to different phases of the estrus cycle *viz.*, proestrus, estrus, metestrus, diestrus, anestrus and early pregnancy. Each group was consisted of ten samples. Samples were cleaned under running tap water for removing tissue debris, fascia and blood clots. Histochemical reactions were observed for study of correlative changes in the carbohydrate and enzymes in the uterus. Following staining techniques were carried out for demonstration of histochemicals and histoenzymes in uterus.

1. Periodic Acid Schiff reaction (pH 2.5) stain for mucopolysaccharide (Culling, 1969) [4].
2. PAS Alcian blue (pH 2.5) stain for neutral mucopolysaccharide (Culling, 1969) [4].

3. Best Carmine method for glycogen (Luna, 1968) ^[10].
4. Gomori's modified alkaline phosphatase cobalt method was used for alkaline phosphatase activity at pH 8.5 - 9.5 (Singh and Sulochana, 1996 - 97) ^[15].
5. Gomori's modified lead nitrate method was used for Acid phosphatase activity at pH 5 (Singh and Sulochana, 1996 - 97) ^[15].

Results and Discussion

1. PAS for mucopolysaccharide

The distribution of the PAS-positive material in the epithelial cells of the uterus demonstrated variation in its quantity, depending upon the estrogen level as influenced by the condition of the ovary.

The intensity of the PAS reaction at the apical border of lining epithelium was mild in diestrus and anestrus (Fig. 1). A moderate reaction was observed during metestrus and early pregnancy (Fig. 2). During the proestrus and estrus, an intense reaction was observed at the apical border of the lining epithelium. This might be due to the maximum concentration of carbohydrate complexes present in surface epithelium. The cytoplasm remained mild to moderate and homogeneously reactive for PAS. It decreased as the cycle proceeded towards the anestrus phase. Similar observations have been reported by Likar and Likar (1964) ^[9], Larson *et al.* (1965) ^[8], Marinov and Lovell (1968) ^[11], Kalkar (1971) ^[7] in buffalo, Wordinger *et al.* (1971) ^[20] in cows, Dwivedi and Singh (1972) ^[6], Sundaravandanan and Venkatswami (1973) ^[18], Agarwal *et al.* (1978) ^[1] and Sinha (1980) ^[16] in buffaloes. The increased concentration of the carbohydrate in the proestrus and estrus has a significant role as a source of energy to the conceptus. This was in contrast with the findings of Bhattacharya and Saigal (1984) ^[3] in goat where, they observed the histochemical changes in the uterus during the reproductive cycle. They reported that the PAS-positive material was confined to the luminal border of the glandular epithelia of goat endometrium with no appreciable cyclic variations. Relatively stronger PAS reaction was reported at similar sites during metestrus and early diestrus.

The intensity of the PAS reaction in lamina propria was increased from proestrus to estrus. It was intense in estrus, and then it diminished in metestrus, diestrus and anestrus, where it was mild. In early pregnancy the intensity of reaction was of moderate type. The intensity of the reaction was maximum in superficial propria as compared to the deeper zone. Similar observations were reported by Wordinger *et al.* (1970) ^[19] in cattle and Sinha (1980) ^[16] in buffalo. The tunica intima of the blood vessels in the uterine wall exhibited moderate to intense PAS-positive reaction.

In proestrus and estrus the PAS reaction in uterine glands was strong but limited to the apical border of the glandular epithelium. The PAS activity was higher in glands of spongiosa than that of glands of basalis. In metestrus, diestrus and early pregnancy the intensity of the reaction was moderate, whereas in anestrus, mild intensity was observed. These observations are in accordance with Dwivedi and Singh (1972) ^[6], Sundaravandanan and Venkatswami (1973) ^[18] and Agarwal *et al.* (1978) ^[1] in buffaloes and Moss *et al.* (1954) ^[12], Likar and Likar (1964) ^[9], Larson *et al.* (1965) ^[8] and Marinov and Lovell (1968) ^[11] in cows.

The myometrium was mildly reactive for PAS in anestrus and throughout all the phases of estrus cycle. The reaction was remarkable in the connective tissue in between the

muscle fibers of the myometrium. Similar findings were reported by Singh (1983) ^[14] in buffalo and Bhattacharya and Saigal (1984) ^[3] in goat. In early pregnancy moderate intensity of PAS reaction was noticed. The reactions in the myometrium remained unchanged with the changes in the estrus cycle. The reactions indicated that the myometrium of buffalo was relatively passive. Possibly the blood vessels play more role in maintaining uterine tonicity and turgidity in different phases of estrus cycle.

The perimetrium was generally very negative for most of the histochemical staining. It did not show any specific cyclic changes except more vascularization and lymphatic dilatation in the proestrus and estrus phase.

2. PAS-AB for neutral mucopolysaccharide

The intensity of the PAS-AB reaction in epithelium was observed maximum in estrus and mild in metestrus, diestrus, anestrus and early pregnancy. The reaction was moderate in proestrus (Fig. 3). The PAS-AB reaction in Lamina propria of the uterus was moderate in estrus, whereas, in proestrus, metestrus, diestrus, anestrus and early pregnancy the reaction was mild.

Moderate PAS-AB reaction in the apical border of the glandular epithelium was noticed in the estrus. In proestrus, metestrus, diestrus and early pregnancy the intensity of the reaction was of mild type. In anestrus, the uterine glands exhibited almost negative reaction for PAS-AB. In all the groups the PAS-AB reaction was of mild type in myometrium. The perimetrium was generally very negative for most of the histochemical staining. It also did not show any specific cyclic changes except more vascularization and lymphatic dilatation in the proestrus and estrus phase.

3. Best Carmine method for glycogen.

In all the six groups under this study showed mild intensity for presence of glycogen in lining epithelium of the uterus. Mild intensity for presence of glycogen in lamina propria was observed in metestrus, diestrus and anestrus. In proestrus and estrus the reaction was moderate. Maximum reaction was observed in the early pregnancy, where, intensity of the reaction was maximum in superficial propria as compared to the deeper zone (Fig. 8). The uterine glands in proestrus, metestrus, diestrus and anestrus showed mild reaction for presence of glycogen. In estrus, the glands showed moderate reaction (Fig. 7). The intensity of reaction was noted maximum in early pregnancy (Fig. 8).

The myometrium was mildly reactive for Best Carmine in anestrus. Whereas, in proestrus, estrus, metestrus and diestrus moderate reaction was noticed. The intensity of reaction for glycogen was observed maximum in the early pregnancy. The perimetrium was negative for the PAS-AB reactions in all the phases of the estrus cycle, except in early pregnancy where, mild reaction was observed.

4. Alkaline phosphatase

In proestrus, estrus and anestrus mild ALK reaction was observed in epithelium of the uterus (Fig. 9). In the metestrus and early pregnancy the reaction was moderate in epithelium. It was intense in the basement membrane of the epithelium was in the diestrus (Fig. 10). This increase in alkaline phosphatase enzyme concentration along the supranuclear zone of the surface epithelium during diestrus might indicate that the cells were more active in this phase. The alkaline phosphatase activity is, concerned with

membrane transport; hence the luminal border is very active in the secretory phase which showed a high concentration of this enzyme. These observations are in agreement with the observations of Moss *et al.* (1954)^[12], Skjerven (1956) and Larson *et al.* (1965)^[8] in cattle and Dwivedi and Singh (1972)^[6] in buffalo and Ramchandraiah *et al.* (1980)^[13] in ewe.

In all the groups the alkaline phosphatase enzyme concentration of the lamina propria was generally negative. A mild alkaline phosphatase activity was observed along the endothelium of the blood vessels, particularly in the superficial propria. These observations are in accordance with Singh (1983)^[14].

The alkaline phosphatase activity in the uterine glands was negative in anestrus and mild in proestrus and estrus. In metestrus moderate alkaline phosphatase reactivity was observed along the apical border of the superficial glandular epithelium which intensified in the diestrus and early pregnancy (Fig. 11). The reaction was stronger in glands present in spongiosa than that of the glands of basalis. It is believed that the superficial glands have more secretory activity while basal glands have more synthesizing activity.

The present study confirmed the observations of Moss *et al.* (1954)^[12] and Larson *et al.* (1965)^[8] in cow.

The myometrium showed negative reactions for the alkaline phosphatase enzymes during all the phases of the estrus cycle. The perimetrium of the uterus did not show any ALK activity throughout all the groups studied in present investigation.

5. Acid phosphatase

Mild reaction for acid phosphatase was noticed in estrus, metestrus and early pregnancy (Fig. 12). Whereas, in proestrus and diestrus the reaction was nearly negative. Lamina propria, uterine glands, myometrium and perimetrium was negative for the acid phosphatase enzymes in all the groups studied. The acid phosphatases are the degradative enzymes, mostly contained in lysosomes. Therefore, it appears that the nearly negative to mild lytic activity which continued throughout the estrus cycle and in early pregnancy. These observations are in agreement with Larson *et al.*, (1970)^[8] in cow and Singh (1983)^[14] in Buffalo.

Table 1: Histochemical and histoenzymic activity of various structures of uterus in different phases of estrus cycle in buffalo (*Bubalus bubalis*)

Group	Structure	PAS	PAS-AB	Best Carmine	ALK	ACP
Proestrus	Epithelium	+++	++	+	+	±
	Lamina Propria	++	+	++	±	-
	Glands	+++	+	+	+	-
	myometrium	+	+	+	-	-
	Perimetrium	-	-	-	-	-
Estrus	Epithelium	+++	+++	+	+	+
	Lamina Propria	++	++	++	±	-
	Glands	+++	++	++	+	-
	myometrium	+	+	++	-	-
	Perimetrium	-	-	-	-	-
Metestrus	Epithelium	++	++	+	++	+
	Lamina Propria	+	+	+	±	-
	Glands	++	+	+	++	-
	myometrium	+	+	++	-	-
	Perimetrium	-	-	-	-	-
Diestrus	Epithelium	+	+	+	+++	±
	Lamina Propria	±	+	+	±	-
	Glands	++	+	+	+++	-
	myometrium	+	+	++	+	-
	Perimetrium	-	-	-	-	-
Anestrus	Epithelium	+	+	+	+	-
	Lamina Propria	±	+	+	±	-
	Glands	+	±	+	±	-
	myometrium	+	+	+	-	-
	Perimetrium	-	-	-	-	-
Early Pregnancy	Epithelium	++	+	+	++	+
	Lamina Propria	++	+	+++	±	-
	Glands	++	+	+++	+++	-
	myometrium	++	+	+++	±	-
	Perimetrium	-	-	+	-	-

+++ : Intense, ++ : Moderate, + : Weak, ± : Very Weak and - : Negative

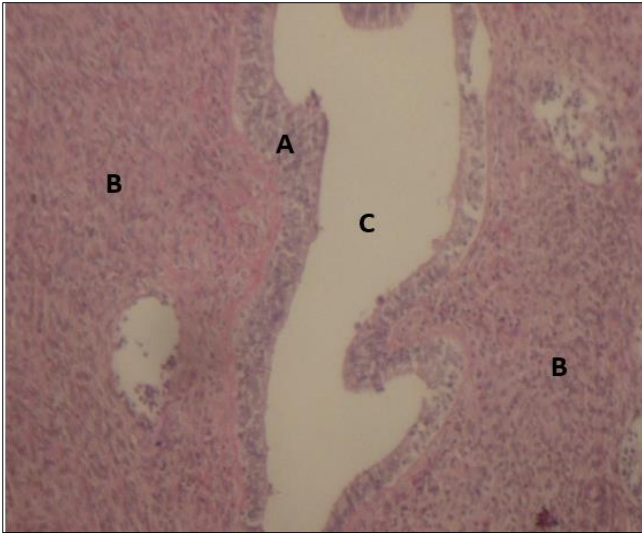


Fig 1: Microphotograph of uterus during anestrus showing mild intensity A. at the apical border of epithelium, B. caruncle and C. lumen (PAS-100X)

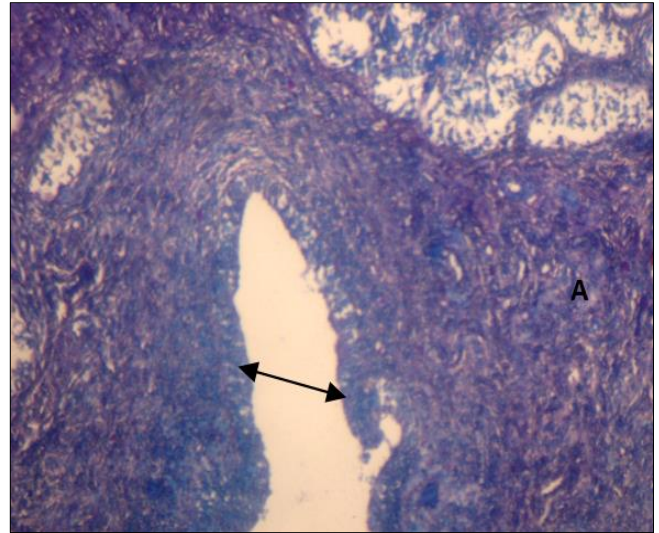


Fig 4: Microphotograph of uterus during estrus showing intense reaction (arrow) in apical border of epithelium and A. moderate in lamina propria (PAS-AB-100X)

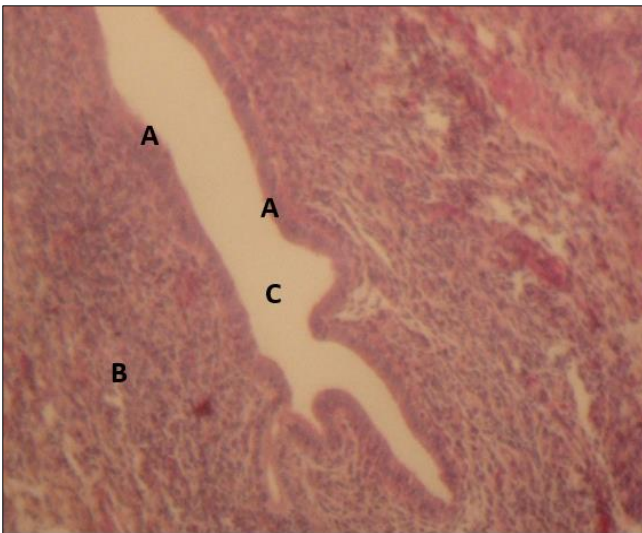


Fig 2: Microphotograph of uterus during early pregnancy showing moderate intensity A. at the apical border of epithelium, B. lamina propria and C. lumen (PAS-100X)

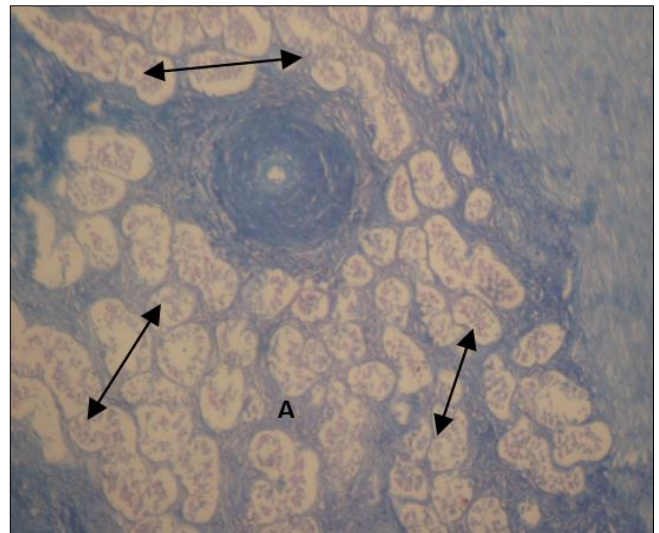


Fig 5: Microphotograph of uterus during diestrus showing mild reaction (arrow) in glandular epithelium and A. lamina propria (PAS-AB-100X)

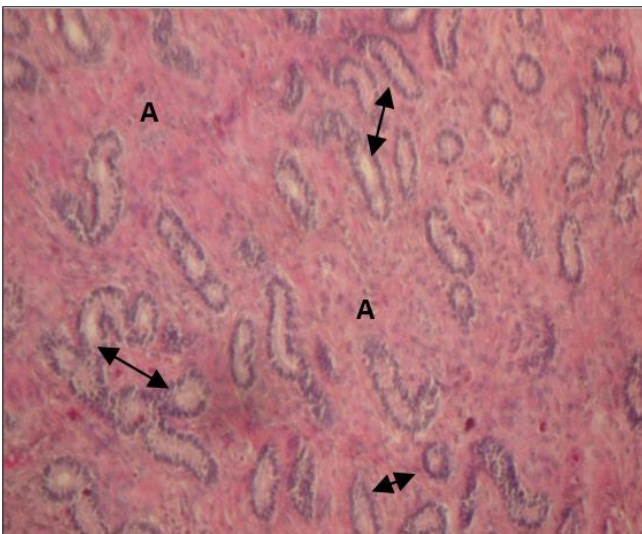


Fig 3: Microphotograph of uterus during estrus showing intense reaction at the apical border of glandular epithelium (arrow) and A. lamina propria (PAS-100X)



Fig 6: Microphotograph of uterus during early pregnancy showing mild reaction (arrow) in glandular epithelium and A. lamina propria (PAS-AB-100X)

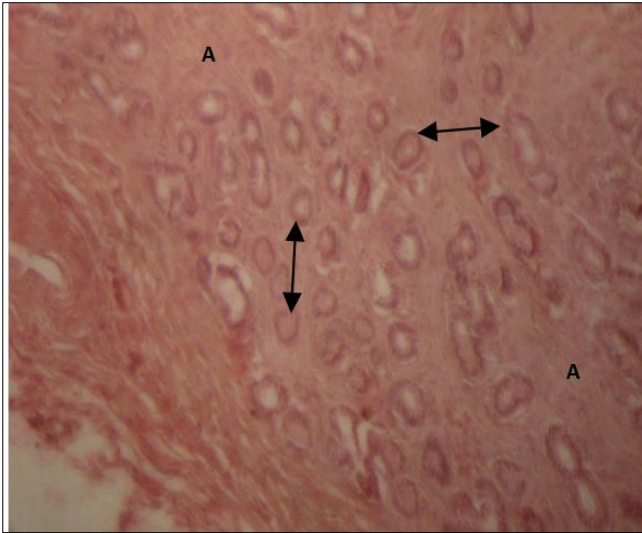


Fig 7: Microphotograph of uterus during estrus showing moderate reaction in glandular epithelium (arrow) and A. lamina propria (Best Carmine-100X)

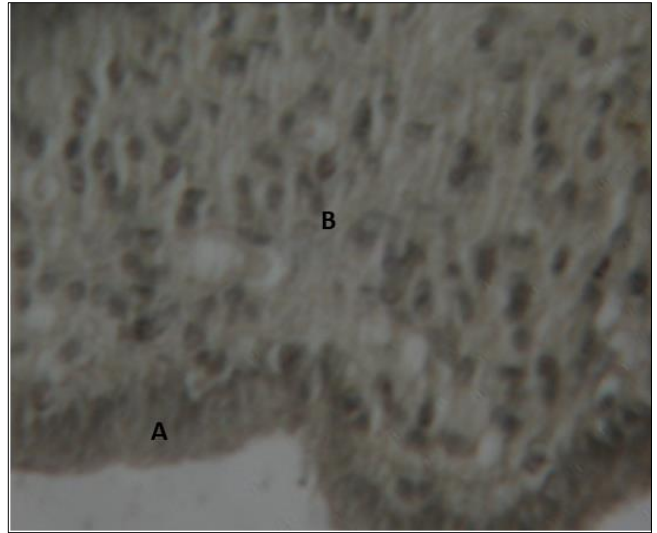


Fig 10: Microphotograph of uterus during diestrus showing intense reaction in A. surface epithelium and B. lamina propria (ALK-400X)

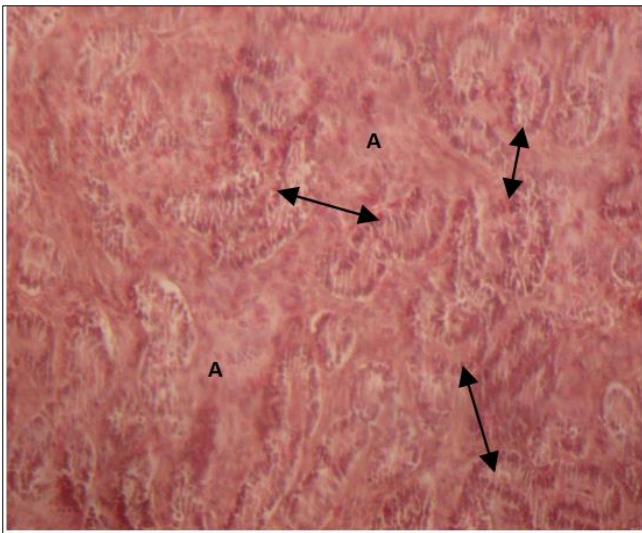


Fig 8: Microphotograph of uterus during early pregnancy showing intense reaction in glandular epithelium (arrow) and A. lamina propria (Best Carmine-100X)

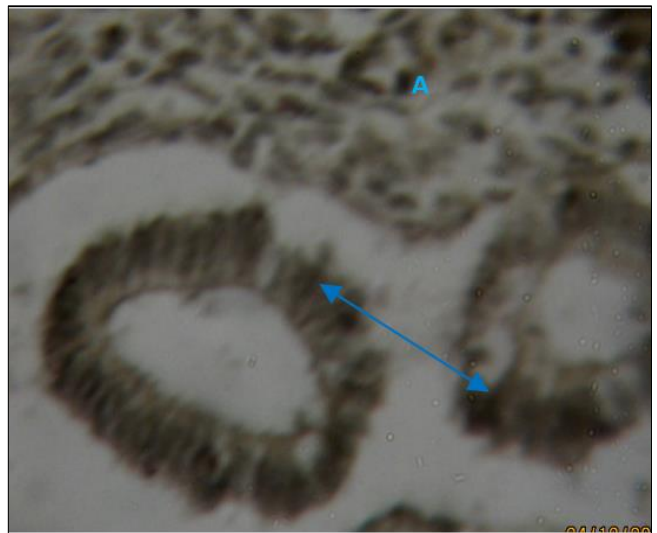


Fig 11: Microphotograph of uterus during early pregnancy showing Intense reaction (arrow) in glandular epithelium and A. lamina propria (ALK-400X)

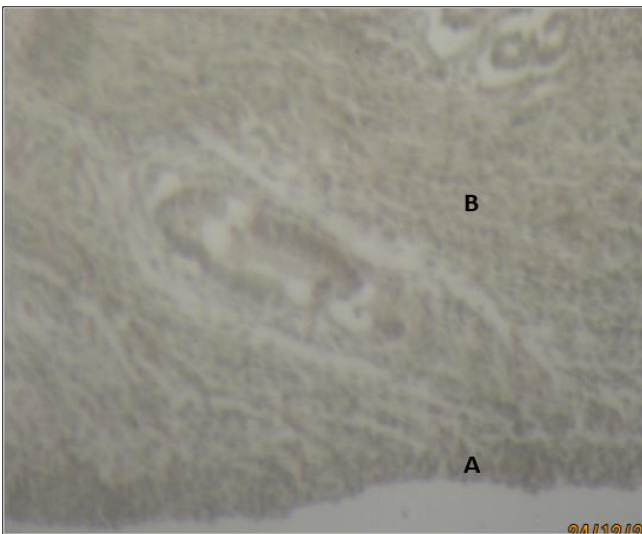


Fig 9: Microphotograph of uterus during anestrus showing mild reaction in A. surface epithelium and B. lamina propria (ALK-400X)

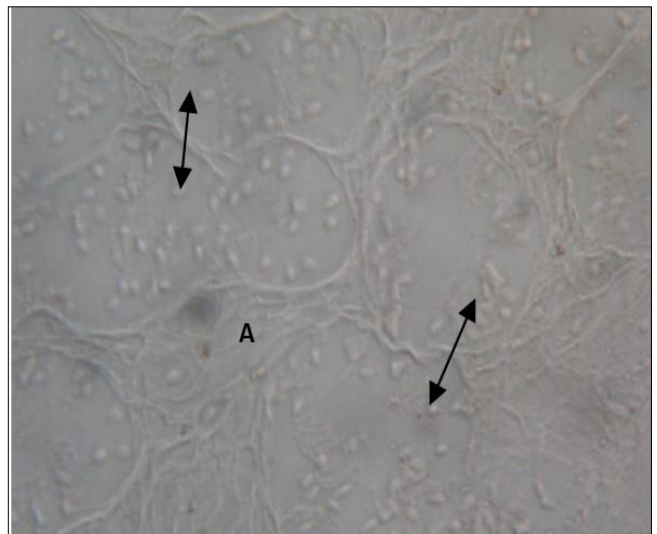


Fig 12: Microphotograph of the uterus during estrus showing mild reaction (arrow) in glandular epithelium and A. lamina propria (ACP-400X)

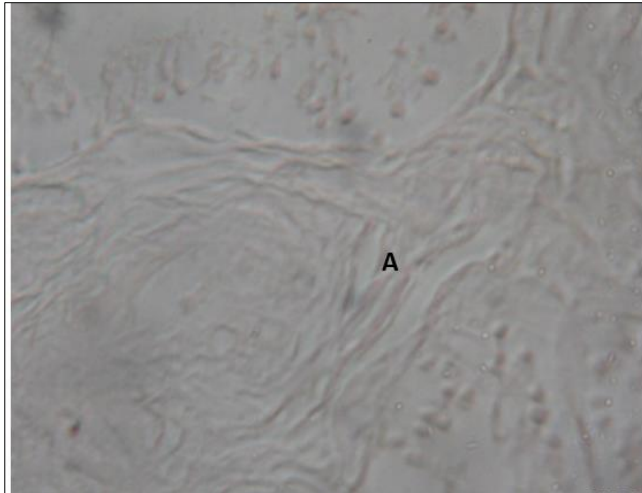


Fig 13: Microphotograph of the uterus during diestrus showing negative reaction in glandular epithelium and A. lamina propria (ACP-400X)

Conclusion

The PAS-positive material in the uterine epithelium, lamina propria and uterine glands showed intense reactions during proestrus and estrus. The increased concentration of the carbohydrate may have a significant role as a source of energy to the conceptus. The reactions for presence of glycogen in the lamina propria, uterine glands and myometrium showed maximum reaction for glycogen material in the early pregnancy. This increase in the ALK phosphatase enzyme concentration along the supranuclear zone of the surface epithelium during diestrus indicated that the cells were more active in this phase. The secretory cells with secretory material were observed relatively more in number in the metestrus and diestrus. The greatest secretory activity of uterine glands was observed during the diestrus.

Ethical approval

Not applicable.

Data statement

The authors confirm that the data supporting the findings of this study are available within the article.

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Declaration of Interest

All authors declare that no commercial or financial relationships exist that could, in any way, lead to a potential conflict of interest.

Authors' contribution

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