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Histochemical study of the goat placentome

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Abstract
The histochemical study was conducted on 18 gravid goat uteri divided into three groups according to their gestational ages as Gr I (0-50 days), Gr II (51-100 days) and Gr III (101 till term). The Perodic acid Schiff 's (PAS), acid mucopolysaccharides (AMPs), alkaline phosphatase (ALK) and acid phosphatase (ACP), Sudan Black B and Feulgen reaction were studied. In 1st group, cryptal epithelium, intercryptal column core, trophoblast epithelium were mild postive with AMP and moderately positive for PAS. In the Caruncles; Uni and multinucleate giant cells showed mild positive with PAS, ALK and Feulgen's reaction. Cryptal epithelium and binucleate giant cells exhibited moderate activity with PAS, AMP and Feulgen's reaction and mild activity with ACP in 2nd and 3rd group. In the villous epithelium; uninucleate cells showed moderate activity and binucleate showed intense activity with PAS and mild activity with AMP in 2nd group. Uni and multinucleate giant cells during 3rd group showed mild activity with PAS, AMP and moderate activity with ALK and Sudan Black- B. In the arcade epithelium; Uninucleate giant cells showed mild activity and binucleate giant cells showed moderate activity in 2nd and 3rd group with PAS. Binucleate giant cells in 2nd group, uninucleate giant cells during 3rd group showed mild activity with AMP, ALK, ACP, and Sudan black B and Feulgen's reaction.

Keywords: Goat, histo chemical study, placentome, pregnancy

Introduction
During pregnancy, the functional layer of the endometrium undergoes specific molecular, enzymatic, morphological and structural changes, which is under control of ovarian hormonal changes [1, 2]. At the time of implantation in mammals, the cell surface of luminal epithelium of endometrium undergoes changes, named "plasma membrane transformation". The epithelium of mammalian endometrial is the first site of contact between trophoectoderm and maternal tissue during the period of attachment and implantation of embryo [2].
In mammals, glycogen, carbohydrates and some enzymes are very important for early phases of implantation and embryonic nutrition in the early stages of pregnancy and the majority of these elements are produced from uterine glandular cells [3]. The activities of Alkaline (ALP) and acid phosphatase (ACP) enzymes play a important role in nourishment and implantation of the blastocyst [4]. ALP and ACP enzymes are closely involved in the metabolism of monosaccharide' s. ALP plays very important role in early phases of implantation [3]. Especially in luteal phase, the acid phosphatase (ACP) activities are responsible for hydrolysis of organic phosphoesterase [5]. Acid mucopolysaccharides (AMPs), which constitute an important component of the connective tissue ground substance present in the female genital tract. AMPs in the mammalian uterus are of great importance since the alteration in the quantity and quality of the ground substance may prove to be important in the pathogenesis of uterine infections and in other disorders. In the female genital tract the cytoplasmic carbohydrate and lipid accumulations differ depending on sexual hormones (estrogen and progesterone) alterations [6].

Objective: In the view of above observations the objective of the current study was to perform a histochemical study of the neutral mucopolysaccharides, acid mucopolysaccharides, alkaline and acid phosphatase, distribution of lipase and DNA in the plancentome of the goat during different stage of pregnancy.

Materials and Method
The present study was conducted on the 18 gravid uteri of goats collected from the slaughter house. Immediately, after collection foeti were cleaned. Each foeti was measured for its crown

rump length (CRL) in centimeters with the help of nylon tape as per the technique described by [7] and weighed in grams with the help of electronic weighing balance machine. The approximate age of foeti above 30 days was estimated by using the formula derived by [8] in goat. Below 30 days approximate age of foeti was estimated by its crown-rump length. The uteri were divided into three groups according to their gestational ages as Gr I (0-50 days), Gr II (51-100 days) and Gr III (101 till term). Tissue were collected and fixed in 10% neural buffered formalin. Paraffin blocks were prepared by acetone benzene schedule by using low melting point paraffin of 46-48 ^{0}c and 15 µm thick sections were obtained on the glass slides with the help of rotary microtome. The sections were stained for demonstration of Polysaccharides (PAS) (Periodic Acid Schiffs [9] Acid mucopolysaccharides (AMPS) (Muller's Colloidal (hydrous) ferric oxide, [9], Gomori's method for study of alkaline and acid phosphatase [9], Lipids (Sudan Black B method [10] DNA (Feulgen's reaction) [11].

Results and Discussion

1. Early pregnancy

Periodic Acid Schiff's reaction (PAS)

The cryptal epithelium, intercryptal column core, trophoblast epithelium was moderately positive for PAS similar observations were made by [11, 13] in bovine plancentome.

Caruncle, Villous epithelium and Arcade epithelium showed no activity with PAS in initial stage of pregnancy.

Acid Mucopolysaccharide

Epithelial cells exhibited mild activity with acid mucopolysaccharide **(**AMP). Superficial connective tissue zone showed mild and deeper connective tissue zone was showed moderate activity with AMP. Stromal tissue showed no reaction for AMP (Table 1). Similar observations were made by [13] in bovine placentome.

Caruncle, Villous epithelium and Arcade epithelium showed no activity with Acid Mucopolysaccharide in initial stage of pregnancy. Similar observations were made by [13] in bovine placentome.

Alkaline phosphatase (Gomori's method)

Superficial connective tissue zone showed intense activity, deeper connective tissue zone showed moderate to intense activity with alkaline phosphatase. Stromal tissue showed no reaction with alkaline phosphatase (table 1). Increased alkaline phosphatase activity in the caruncular area during early pregnancy was reported in ewe by [19, 15] Boshier mentioned that alkaline phosphatase activity in this species was present in the apical cell membrane of the opposing trophoblast and uterine epithelium. This enzyme has been associated with carbohydrate metabolism and production of fibrinoid [20] reported the presence of alkaline phosphatase in the placenta of all form animals [12, 13] in bovine placenta observed that during early and mid pregnancies caruncular epithelium and trophoblastic epithelium show negative to small activity respectively but during late pregnancy moderate activity for alkaline phosphatase was observed. Whereas [21] reported that the cryptal epithelium in bovine placenta during early pregnancy contain abudunt alkaline phosphatase between 25 to 47 days of pregnancy. PAS similar observations were made by [11, 13] in bovine plancentome.

Caruncle, Villous epithelium and Arcade epithelium showed no activity with Alkaline phosphatase in initial stage of pregnancy. similar observations were made by [11, 13] in bovine plancentome.

Acid phosphatase

No reaction for acid phosphatase observed in the epithelial cell, superficial, deeper connective tissue and stromal tissue. PAS similar observations were made by [11, 13] in bovine plancentome.

Caruncle, Villous epithelium and Arcade epithelium showed no activity with acid phosphatase in initial stage of pregnancy. PAS similar observations were made by [11, 13] in bovine plancentome.

Sudan black B (Fat)

No reaction for Sudan Black-B observed in the epithelial cell, superficial, deeper connective tissue and stromal tissue with Sudan Black-B similar observations made by [14] in Goat and [12] in the bovine placentome (table.1).Caruncle, Villous epithelium and Arcade epithelium showed no activity with Sudan black B (Fat) in initial stage of pregnancy.

2. Mid pregnancy

Periodic Acid Schiff's reaction (PAS)

PAS positive reaction were observed (table-1) similar observations were made by [11, 13] in bovine plancentome.

Caruncle

Cryptal epithelium and binucleate cells exhibited moderate PAS positive reaction; uninucleate and multinucleate cells showed mild reaction with PAS as observed by [14] in goat and [13] in bovine placentome.

Villous epithelium

Uninucleate cells showed moderate activity and binucleate showed intense activity with PAS. The syncytial mass of inter crypt columns was mild PAS positive while cytoplasm of binucleate giant cells were strongly PAS positive similar observation was made by [15] in sheep.

Arcade epithelium

Uninucleate giant cells showed mild activity with PAS. Binucleate giant cells showed moderate activity with PAS. (Table-1) Similar observations were made by [15] in sheep and [14] in Goat. Whereas [16] reported that giant cells were negative for glycogen staining in bovine [17] had also reported that BNC-specific proteins accumulated as periodic acid Schiff (PAS) positive secretory granules in the giant cells to be released at the basal membrane of the uterine epithelium that seemed to be their primary functions.

[18] in buffalo reported that these giant cells showed strong PAS-positive activity indicating high content of neutral mucopolysaccharides [15] in **s**heep suggested that the strong/ moderate PAS reaction in the giant cell in main core of chorionic villi is due to the presence of mostly glycogen. The strong PAS reaction in the giant cells indicates that these cells can synthesized and store glycoprotein.

Acid Mucopolysaccharide

Epithelial cells exhibited mild activity with acid mucopolysaccharide **(**AMP). Superficial connective tissue zone showed mild activity and deeper connective tissue zone showed moderate activity with AMP. (table - 1). Similar observations were made by [13] in bovine placentome.

Caruncle
Uninucleate and multinucleate giant cells during showed mild activity with AMP. Binucleate cells showed moderate activity with AMP. Similar report made by [12] in bovine and [14] in Goat placentome.

Villous epithelium
Uninucleate, binucleate and multinucleate giant cells showed mild activity with AMP as reported by [14] in goat. [13] Observed moderate activity for acid mucopolysaccharide in the cryptal depth, trophoblastic epithelium and intercryptal coloumn of bovine placentome.

Arcade epithelium
Binucleate giant cells showed mild activity with AMP similar observations were made by [15] in sheep.

Alkaline phosphatase (Gomori's method)
Superficial connective tissue zone showed intense activity, deeper connective tissue zone showed moderate to intense activity with alkaline phosphatase. Stromal tissue showed no reaction with alkaline phosphatase (table 1, fig.1). Increased alkaline phosphatase activity in the caruncular area was reported in ewe by [19, 15] Boshier mentioned that positive alkaline phosphatase activity in the uterine epithelium.

Caruncle
Uninucleate cells showed mild reaction with alkaline phosphatase (table-1) similar observations were made by [12] in bovine placenta and [14] in the goat.

Villous epithelium
Uninucleate and multinucleate giant cells showed mild activity with alkaline phosphatase. (table-1) as observed by [12] in bovine placentome.

Arcade epithelium
Uninucleate and binucleate giant cells showed moderate activity with alkaline phosphatase. (Table-1 and fig. 63) similar observations were made by [14] in Goat.

Acid phosphatase
No reaction for acid phosphatase observed in the epithelial cell, superficial, deeper connective tissue and stromal tissue similar observations were made by [12] in Bovine placenta.

Caruncle
Uninucleate and multinucleate giant cells showed no reaction with acid phosphatase. Binucleate giant showed mild activity with acid phosphatase (table-1) similar observations were made by [14] in Goat and [12] in Bovine placenta. Whereas [13] observed moderate activity of acid phosphatase was observed in the caruncular epithelium during mid pregnancy.

Villous epithelium
Cuboidal cells and multinucleate giant cells showed no activity with acid phosphatase. Columnar cells and uninucleate giant cells showed moderate activity with acid phosphatase (table-1). Whereas, [13, 15] observed similar observations during mid pregnancy. [15] Further mentioned that this enzyme was responsible for the release of lysosomal hydrolysin [22]. in ewe, observed that alkaline phosphatase activity in the maternal caruncles was high and did not change during pregnancy.

Arcade epithelium
Uninucleate and binucleate giant cells showed mild activity with acid phosphatase. Similar observations were made by [12] in bovine placentome.
Reaction of PAS positive substances, acid mucopolysaccharides and alkaline phosphatase in the arcade zone epithelium varied from negative to moderate and generally resembled the description given under villus epithelium. Such extreme variation may be suggestive of the physiological status of a particular cell as mentioned by [23] or it may be attributed to the complexity and multifarious activities assigned to a single cell which resulted in its diverse appearance.

Sudan black B (Fat)
No reaction for Sudan Black-B observed in the epithelial cell, superficial, deeper connective tissue and stromal tissue with Sudan Black-B similar observations made by [14] in Goat and [12] in the bovine placentome (table.1, fig 2).

Caruncle
Uninucleate, binucleate and multinucleate giant cells showed no activity with Sudan Black-B (table. 1). [13] observed caruncular epithelium showed moderate staining reaction for Sudan Black B where as caruncular core showed no reaction for the fat.

Villous epithelium
Uninucleate and binucleate giant cells showed mild activity with Sudan Black-B. (table-1) [24] also observed lipid droplet in cryptal epithelium. Whereas [12] reported increased staining for the fat in all parts of placentome with advancement of pregnancy. [25] Observed that the cryptal epithelium and trophoblastic epithelium were rich and poor respectively in fatty acid material.

Arcade epithelium
Cuboidal cells and uninucleate giant cells showed no activity with Sudon Black-B. Columnar and binucleate cells showed mild activity with Sudon Black-B (table-1). Similar observation was made by [14] in goat.

Feulgen's reaction (DNA)
Deeper connective tissue zone during early pregnancy showed mild activity with Feulgen's reaction. Similar observations were made by [14] in Goat (table.1, fig. 3).

Caruncle
Uninucleate and multinucleate giant cells showed mild activity with Feulgen's reaction. Binucleate giant cells showed moderate activity with Feulgen's reaction. (table-1) as reported by [12] in bovine placenta.

Villous epithelium
Uninucleate giant cells showed mild activity with Feulgen's reaction. Binucleate and multinucleate giant cells showed moderate activity with Feulgen's reaction as observed by [14] in Goat and [12] in bovine placenta (table-1).

Arcade epithelium
Cuboidal and columnar cells and uninucleate giant cells showed mild activity with Feulgen's reaction similar observations were made by [14] in Goat (table-1)
In conclusions, there were remarkable changes in activity of

various enzymes/ histochemical substances in the placentome during different stage of pregnancy and these enzymatic activities vary according to the different physiological state of the uterus.

3. Late pregnancy

Periodic Acid Schiff's reaction (PAS)

PAS positive reaction were observed (table1, fig. 4) similar observations were made by [11, 13] in bovine plancentome.

Caruncle

Cryptal epithelium and binucleate cells exhibited moderate PAS positive reaction; uninucleate and multinucleate cells showed mild reaction with PAS as observed by [14] in goat and [13] in bovine placentome.

Villous epithelium

Uninucleate, binucleate and multinucleate giant cells showed mild activity with PAS. Similar observation was made by [15] in sheep.

Arcade epithelium

Uninucleate giant cells during showed mild activity and binucleate giant cells showed moderate activity with PAS. (Table-1) Similar observations were made by [15] in sheep and [14] in Goat. Whereas [16] reported that giant cells were negative for glycogen staining in bovine [17] had also reported that BNC-specific proteins accumulated as periodic acid Schiff (PAS) positive secretory granules in the giant cells to be released at the basal membrane of the uterine epithelium that seemed to be their primary functions.

[18] in buffalo reported that these giant cells showed strong PAS-positive activity indicating high content of neutral mucopolysaccharides [15] in **s**heep suggested that the strong/ moderate PAS reaction in the giant cell in main core of chorionic villi is due to the presence of mostly glycogen. The strong PAS reaction in the giant cells indicates that these cells can synthesized and store glycoprotein.

Acid Mucopolysaccharide

Epithelial cells exhibited mild activity with acid mucopolysaccharide **(**AMP). Stromal tissue showed no reaction for AMP (table 1, fig. 5). Similar observations were made by [13] in bovine placentome.

Caruncle

Uninucleate giant cells showed mild activity and binucleate cells showed moderate activity with AMP. Uninucleate giant cells showed no activity with AMP similar report made by [12] in bovine and [14] in Goat placentome.

Villous epithelium

Uninucleate and multinucleate giant cells showed mild activity with AMP. Binucleate giant cells showed no activity with AMP as reported by [14] in goat. [13] Observed moderate activity for acid mucopolysaccharide in the cryptal depth, trophoblastic epithelium and intercryptal coloumn of bovine placentome.

Arcade epithelium

Binucleate giant cells during mid pregnancy; uninucleate giant cells showed mild activity with AMP. Cuboidal and columnar cells showed no activity with AMP. Binucleate giant cells showed moderate activity with AMP similar observations were made by [15] in sheep.

Alkaline phosphatase (Gomori's method)

Superficial and deeper connective tissue zone showed moderate activity with alkaline phosphatase. Stromal tissue showed no reaction with alkaline phosphatase (table 1, fig.3). Increased alkaline phosphatase activity in the caruncular area during was reported in ewe by [19. 12, 13] in bovine placenta observed that moderate activity for alkaline phosphatase was observed.

Caruncle

Uninucleate, binucleate and multinucleate giant cells showed no activity with alkaline phosphatase (table-1) similar observations were made by [12] in bovine placenta and [14] in the goat.

Villous epithelium

Uninucleate and binucleate giant cells showed mild activity with alkaline phosphatase. Multinucleate giant cells showed moderate activity with alkaline phosphatase (table-1) as observed by [12] in bovine placentome.

Arcade epithelium

Uninucleate and binucleate giant cells showed mild activity with alkaline phosphatase. (table-1) similar observations were made by [14] in Goat.

Acid phosphatase

No reaction for acid phosphatase observed in the epithelial cell, superficial, deeper connective tissue and stromal tissue.

Caruncle

Uninucleate, binucleate and multinucleate giant cells showed mild activity with acid phosphatase (table-1) similar observations were made by [14] in Goat and [12] in Bovine placenta. Whereas [13] observed strong activity in the caruncular epithelium.

Villous epithelium

Cuboidal and columnar cells and uninucleate giant cells showed no activity with acid phosphatase. Binucleate and multinucleate giant cells showed moderate activity with acid phosphatase (table-1). Whereas [21, 15 13] observed strong activity of acid phosphatase in the cryptal epithelium can be attributed to be degenerative changes observed in epithelial cells indicating lysosomal activity. Further mentioned that this enzyme was responsible for the release of lysosomal hydrolysin [22] in ewe, observed that alkaline phosphatase activity in the maternal caruncles was high and did not change during pregnancy.

Arcade epithelium

Uninucleate and binucleate giant cells showed mild activity with acid phosphatase. Similar observations were made by [12] in bovine placentome.

Sudan black B (Fat)

No reaction for Sudan Black-B observed in the epithelial cell, superficial, deeper connective tissue and stromal tissue with Sudan Black-B similar observations made by [14] in Goat and [12] in the bovine placentome (table.1).

Caruncle

Uninucleate binucleate and multinucleate cells giant cells showed no activity with Sudan Black-B (table. 1) [13] observed

caruncular epithelium showed moderate staining reaction for Sudan Black B where as caruncular core showed no reaction for the fat.

Villous epithelium

Multinucleate giant cells showed mild activity and Uninucleate giant cells showed moderate activity with Sudan Black-B (table-1) [24] also observed lipid droplet in cryptal epithelium. Whereas [12] reported increased staining for the fat in all parts of placentome with advancement of pregnancy [25] observed that the cryptal epithelium and trophoblastic epithelium were rich and poor respectively in fatty acid material.

Arcade epithelium

Columnar cells and uninucleate giant cells showed no activity with Sudon Black-B. Cuboidal cells and binucleate giant cells showed mild activity with Sudon Black-B (table-1). Similar observation was made by [14] in goat.

Feulgen's reaction (DNA)

Deeper connective tissue zone during early pregnancy showed mild activity with Feulgen's reaction. Similar observations were made by [14] in Goat (table.1,).

Caruncle

Uninucleate, binucleate and multinucleate giant cells showed mild activity with Feulgen's reaction. (table-1) as reported by [12] in bovine placenta.

Villous epithelium

Uninucleate giant cells showed mild activity with Feulgen's reaction. Binucleate and multinucleate giant cells showed moderate activity with Feulgen's reaction as observed by [14] in Goat and [12] in bovine placenta (table-1).

Arcade epithelium

Uninucleate giant cells during late pregnancy showed mild activity with Feulgen's reaction similar observations were made by [14] in Goat (table-1)

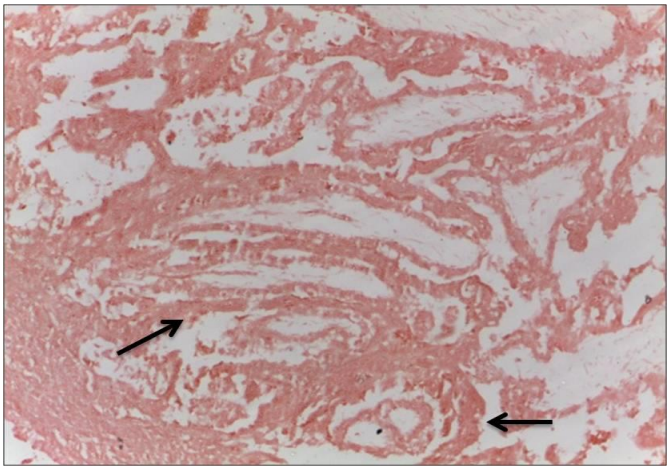

Fig 1: Photomicrograph showing Alkaline phosphatase reaction in goat placentome at 65days of pregnancy. (Arrow) shows mild activity. (Alkaline phosphatase X100)

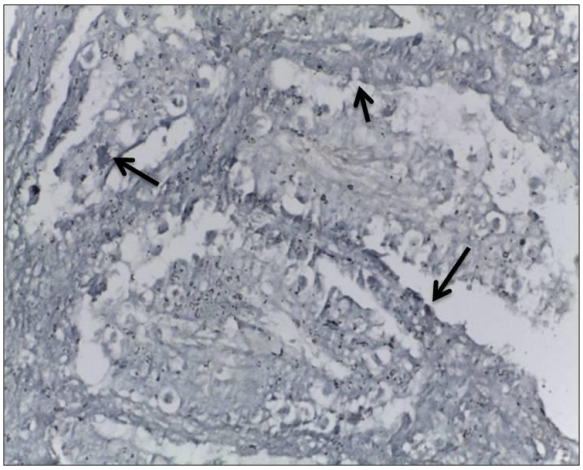

Fig 2: Photomicrographshowing Sudan Black-B reaction in goat placentome at 65 days of pregnancy. (Arrow) shows mild activity. [Sudan Black-B X200]

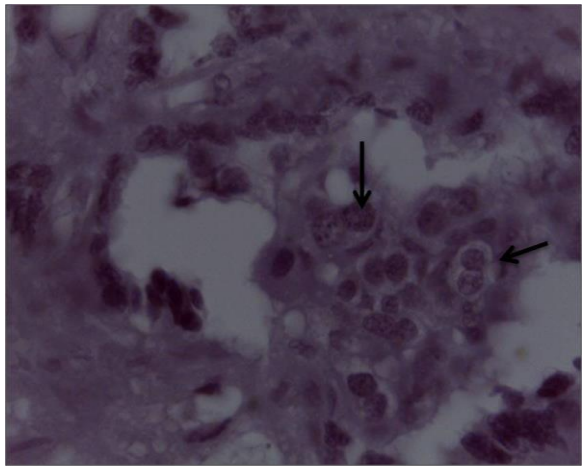

Fig 3: Photomicrograph showing Feulgen's reaction in nuclei of goat placentome at 65 days of pregnancy. (Arrow) shows moderate activity. [Feulgen's reaction X400]

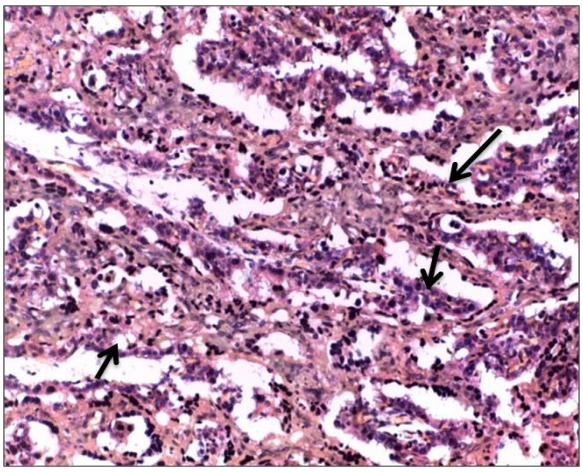

Fig 4: Photomicrograph showing periodic acid Schiff's reaction in goat placentome from the mid region of gravid horn at 120 days of pregnancy. (Arrow) shows mild activity with PAS. [Periodic Acid Schiff's stainX200]

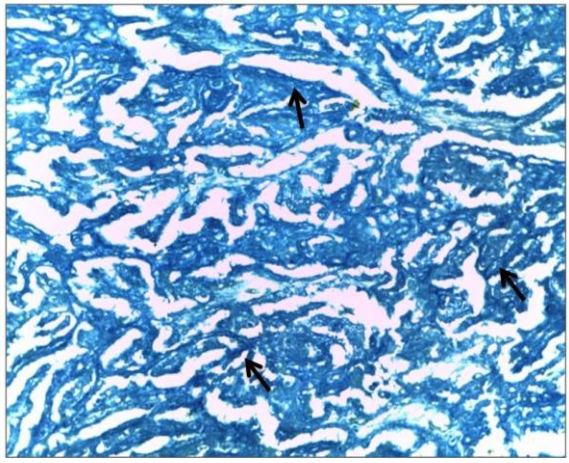

Fig 5: Photomicrograph showing Acid mucopolysaccharides reaction in goat placentome at 120 days of pregnancy. (Arrow) shows mild activity. [Muller's colloidal (hydrous) ferric oxide stainX100]

Table 1: showing Some Histochemical variation of in the placentome of gravid horn during different stage pregnancy in uterus of goat

Group			PAS		AMP		Alkaline phosphatase		Acid phosphatase		Sudan Black B		Feulgen reaction (DNA)	
			IInd	IIIrd	IInd	IIIrd	IInd	IIIrd	IInd	IIIrd	IInd	IIIrd	IInd	IIIrd
Caruncle		UN	+	±	+	±	+	-	+	-	-	-	+	±
		BN	++	++	++	++	++	-	++	-	-	±	++	+
		MN	+	+	+	-	++	-	++	-	-	-	+	±
Cotyledon	Villus epithelium	Cub	-	±	+	-	-	-	-	-	± to +	++	±	-
		Col	+	+	+	±	+	-	+	-	+	+	±	-
		UN	++	+	+	+	+	+	+	+	± to +	++	+	+
		BN	+++	+	+	-	+	+	++	+	± to +	-	++	++
		MN	+	±	+	±	++	++	+	++	-	± to +	++	++
	Arcade epithelium	Cub	-	+	±	-	+	-	±	-	-	± to +	±	-
		Col	±	+	±	-	±	-	+	-	± to +	-	±	-
		UN	+	+	-	±	+	±	++	±	-	-	+	±
		BN	++	++	+	++	++	+	++	+	± to +	+	++	+

UN: Uninucleate cells, BN: Binucleate cells, MN:Multinucleate cells, Cub: Cuboidal cells, Col: Columnar cells
(± to +) very mild, (+) mild, (++) moderate, (+++) intanse, (-) no reaction

Conclusions

There were remarkable changes in activity of various enzymes/ histochemical substances in the placentome during different stage of pregnancy and these enzymatic activities vary according to the different physiological state of the uterus. The enzymatic activity helps in diagnosing the normal pregnancy status in goats.

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