Better expression profile of CD8 and CD4 gene in uterus of pregnant ewe in comparison to non-pregnant - a novel report

Md Mofijul Islam, Aruna Pal*, Partha Das, Samiddha Banerjee

West Bengal University of Animal and Fishery Sciences, 37, K.B.Sarani, Kolkata-37.

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Abstract:

CD8 and CD4 T cells play a central role in the immune response to viruses and intracellular pathogens as well as functions for the maintenance of both the mother and fetus. The present study was conducted to explore the differential gene expression profile for CD8 and CD4 present in the uterus with reference to the gravid and non-gravid Garole sheep and confirmation through immuno histochemical studies. Better CD8 and CD4 gene expression was observed in the mid uterus of pregnant ewes compared to that of non-pregnant ewes, where CD8 expression was better to that of CD4. Gene expression profiling of CD8 and CD4 are reported here for the first time in sheep. CD8 and CD4 expression may be regarded as the useful factor for maintenance of pregnancy. The current observations demonstrate that during pregnancy in ewe the immune system may respond to changes in the maternal environment to maintain the size and function of the CD8 and CD4 T-cell compartment. CD8 and CD4 expression may be employed as marker for pregnancy detection in sheep, which remains always a challenge for sheep breeders.

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Key Words: Garole sheep; Reproductive immunology; CD8/Cytotoxic T cells; CD4, Immunohistochemistry; Gene expression

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*Corresponding Author: Dr. Aruna Pal, Assistant Professor, West Bengal University of Animal and Fishery Sciences, 37, K.B. Sarani, Kolkata-37, West Bengal, Pin: 700037. E mail: arunachatterjee@gmail.com

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Introduction:

Abortion or preterm pregnancy is one of the major problem in both human and livestock sector. Lymphocytes play key roles in the defense of the body. T-lymphocytes regulate acquired immunity and are responsible for cell mediated immune response. CD8+ T-cell is a T-lymphocyte also known as killer cell or cytotoxic T cell and they act as class-I MHC receptor molecules. It is very important for immune defense against intracellular pathogens, including viruses and bacteria. CD8+ T cells serve as important mediator for fetal tolerance and antiviral immunity. Cytotoxic T cells act through T-cell receptors (TCRs)

38 recognizing a specific antigen. Antigens within the cell are attached to class I
39 MHC molecules and in the surface of the cell, they are recognized by the T cell and
40 destroyed.¹

41 CD8 T cells play a central role in the immune response to viruses and intracellular
42 pathogens, so the maintenance of both the number and function of these cells are critical to
43 protect both the mother and fetus. The proportion of maternal CD8 T cells in the spleen and
44 the uterine draining lymph nodes are gradually increased at mid-gestation, which correlates
45 with enhanced CD8 T-cell proliferation and an increased relative expression of both pro-
46 survival and pro-apoptotic molecules.²

47 CD4 and CD8 T cells are surface molecules play a role in T cell recognition³ and they
48 are activated by binding to the MHC-II and MHC-I ligands respectively on an antigen
49 presenting cell (APC)⁴. CD4 cells are also known as T helper cells because they produce
50 various cytokines required for the activation of leukocytes and send signals to other types of
51 immune cells, including CD8 killer cells, which then destroy the infectious particle. CD4 T
52 cells secrete IFN- γ that maximizing bactericidal activity of phagocytes such as macrophages
53 and digest intracellular bacteria⁵ and protozoa⁶. Sometime these cells suppress immune
54 responses. If CD4 cells become depleted, for example in untreated HIV infection, the body is
55 left vulnerable to a wide range of infections^{7, 8}. Understanding exactly how T helper cells
56 respond to immune challenges is currently of major interest in immunology, because such
57 knowledge may be very useful in the treatment of disease and in increasing the effectiveness
58 of vaccination.

59 Garole sheep (*Ovis aries*) are distributed in the Sundarban region of West Bengal in
60 India. It is known for its prolificacy and adaptation to the saline marshy land. It is believed
61 that these sheep contributed to the prolificacy gene (Boorola fecundity) in Merino sheep of
62 Australia. Flocks are stationary and average flock size ranges from three to five. Twin and
63 triplet births are common. Average adult weights for male and female Garole was observed
64 to be 10.3 ± 0.31 kg 9.72 ± 0.28 kg, respectively⁹. They are reared for mutton production¹⁰.
65 Since Garole sheep possess the unique characteristic for reproduction, Garole sheep was
66 choosen as model organism for the current study. Earlier we had studied the female
67 reproduction of Ghungroo pig using mitogenetics approach¹¹.

68 Successful pregnancy requires the maternal immune system to tolerate the semi-allogenic
69 fetus. A failure in immune tolerance may result in abnormal pregnancies, such as recurrent
70 spontaneous abortion and it happens due to several etiological factors. Out of this, immune
71 deficiency causes several infections in the female reproductive system which results in
72 misconception or abortion. The most notable infectious diseases of the reproductive tract of
73 sheep are those that infect the placenta and cause abortion. These infections outreach when
74 the natural immune barrier broken down¹².

75 Considering the above facts, the current study was conducted to explore the differential
76 mRNA expression profile and immunohistochemical studies for the identification of immune
77 cells (Lymphocytes) as CD8 and CD4 expression in both the gravid and non-gravid Garole
78 sheep.

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80 **Results:**

81 **Gene expression profiling with real time PCR:**

82 We analysed gene expression of CD8 and CD4 mRNA, normalized for 18S rRNA
83 expression and was quantified by using SYBR green PCR system from samples (Tip and
84 middle horn of the uterus) of gravid and non-gravid ewes.

85 Out of the two different parts of the uterus, the middle horn of the uterus revealed
86 better expression of both CD8 (Fig 1A) and CD4 (Fig 1B) in comparison to the tip of Horn
87 of the uterus.

88 An increased expression of both CD8 (Fig 2A) and CD4 (Fig 2B) mRNA in gravid
89 uterus (pregnant) as compared to the non-gravid (non-pregnant) uterus was reported. The
90 current observations demonstrate that during pregnancy in ewe the immune system responds
91 to changes in the maternal environment to maintain the size and function of the CD8 T-cell
92 compartment.

93 It has also been observed that CD8 expression was higher in comparison to CD4
94 expression in both the cases of middle and tip of the horn of the uterus (Fig 3A). The similar
95 phenomenon of better expression profile of CD8 compared to that of CD4 was also observed
96 in case of both pregnant and non-pregnant uterus of ewe (Fig 3B)

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98 **Immunohistochemistry:**

99 The distribution of lymphocytes were identified by routine H & E staining of the tissues with
100 a characteristics of a dark staining round nucleus that occupied virtually the entire cells.
101 Lymphocytes were found in all the tissue samples under investigation. Lymphocytes were not
102 equally distributed in the different parts of uterus and they were especially abundant in the
103 basal zone of endometrium. Irrespective of the stage of the physiological status of the uterus,
104 lymphocytes were found to infiltrate the caruncular and inter caruncular epithelium
105 Lymphocyte population was also identified in the sub epithelial area, the stroma.
106 Lymphocytic aggregation was however found in none of the tissue irrespective of
107 physiological condition. The epithelium of the endometrial glands and their ducts were
108 frequently infiltrated by lymphocytes. However, the lymphocyte population was
109 comparatively more at the middle horn of the uterus as compared to the tip and body of the
110 uterus in both gravid and non-gravid ewes.

111 Immunohistochemical study evidenced the presence of cells displaying
112 immunoreactivity for CD8+ antibody, in the luminal, glandular epithelial layers and stroma
113 of the uterus of both gravid and non-gravid ewes.

114 In gravid uterus of ewe, the CD8+ lymphocyte sub population were more at the
115 middle of the horn (Fig 4A) compared to the tip of the horn (Fig 4B). The occurrence of
116 CD8+ cells were found within the luminal and glandular epithelium of the caruncular and
117 inter caruncular areas. The CD8 population was observed to be better in the stroma compared

to that of the epithelium. In some areas within the caruncular stroma, positive cells appeared in the form of clusters. Similarly, CD4 cells were observed to be better expressed in middle of the horn (Fig 5A) in comparison to that of tip of the horn (Fig 5B).

In gravid uterus (Fig 6A) the population of CD8+ cell was observed to be better in comparison to that of non-gravid uterus (Fig 6B). Pregnancy was associated with an increase in the thickening of the epithelial lining of the endometrial glands and lumen but the distribution of lymphocyte was unaffected by pregnancy status. However the number of CD8+ cells was more in stromal region compared to luminal epithelium. CD4+ cells were also observed to be distributed mostly in the stroma and sub-epithelial region of both the caruncular and inter caruncular area of uterus like CD8 cells. CD4 expression was also observed to be better in the uterus of pregnant ewe (Fig 7A) compared to that of non-pregnant ewe (Fig 7B).

Comparison of CD8 and CD4 has revealed some interesting findings. In gravid uterus, the expression of CD8 was observed to be higher compared to that of CD4 gene expression.

Discussion:

CD8 (cluster of differentiation 8) is a transmembrane glycoprotein that serves as a co-receptor for the T cell receptor (TCR). **CD8** binds to a specific class I MHC protein (major histocompatibility complex) molecules. Endometrial mucosa contains a set of immune cells, which apart from providing host-pathogen immunity, have immense role in implantation and maintenance of pregnancy. The mucosal surface of the uterus (endometrium) contains both innate and adaptive immune cells. The implanted foetus has different genetic makeup from its mother and simultaneously differ in immunological makeup to that of the mother. Endometrial mucosal cells of the mother tolerate the semi allogenic foetus without loss of host immunity¹³. The peculiarity of endometrial CD8-T cells has memory CD8-T cells. Memory CD8 T-cells were less in the patients with recurrent miscarriage^{14,15}.

Abortion or tissue rejection is one of the major problems in vogue. Tissue rejection is generally mediated by T lymphocytes. In the current study, it was observed that CD8 was genetically expressed more in pregnant uterus compared to non-pregnant. It was also observed that CD8 was highly expressed in middle of the horn compared to that of tip of the horn which clearly indicated that CD8 expression is essential for maintenance of pregnancy. Similar results were also observed by other researchers. The greatest number of T-lymphocytes in the sheep endometrium was observed in epithelial layer of the glandular and luminal epithelium or in stroma immediately adjacent to the epithelium^{16, 17}. The near absence of stromal T-lymphocyte may indicate that blood was not a major source of endometrial T-lymphocytes. In fact many endometrial inter epithelial lymphocytes developed extra thymically rather than being derived from a circulating pool of lymphocytes¹⁸.

In sheep, cytotoxic (CD8+) T cells were reported to be located primarily adjacent to the epithelial layer of the uterus^{19, 20} and similar observation in sow were reported²¹. The presence of cells displaying immunoreactivity for both CD 4+ and CD 8+ antibodies in the epithelial layer and stroma of the cervix and uterine horn of female goats were reported²². However, only an occasional CD8+ cell was observed to be present in the caruncular and

intercaruncular stroma and the loose connective tissue in the vicinity of the endometrial glands in sheep¹⁷. It was identified that in most sections, CD4+ and CD8+ cells were rarely observed or absent¹⁶, although for a few ewes positively staining cells were readily apparent. This was not in accordance with our present findings.

In non-gravid uterus the CD8+ lymphocyte sub population was found to be more at the middle of the horn. However, the CD8+ cells increased markedly during the mid-to-late luteal and follicular phases, respectively, of the cycle²⁰.

In gravid uterus the population of CD8+ cell was found to be comparatively greater. There was a tendency for higher number of CD8+ cells in luminal epithelium in pregnant ewes²³ and there was no significant effect of uterine type on number of CD8+ cells in either luminal or glandular epithelium. The role of immune competent cells was documented²⁴ who observed that acute inflammation in the pig endometrium in response to fertile mating which included marked changes in the tissue and immune cell components of the endometrium. There appeared to be suppression and activation of various immune cell components in the uteri of pregnant pigs. This phenomenon was presumably in response to foetal or trophoblast antigens, suggesting that the local immune system was involved in the uterine response to pregnancy. The population was very less at the palcentomal region of the endometrium and also in the inter placental endometrium^{24,25}.

Leucocytes were a significant population in the human endometrium (EM), accounting for 10–20% of all endometrial cells. Of the leucocyte population, ~50% were T cells and at least two-thirds of these were CD8⁺ T cells. These CD8⁺ cells were found in three distinct locations within the EM: as scattered stromal cells; as intra epithelial lymphocytes; and in lymphoid aggregates (LA). LA develop in the stratum basalis of the human EM during the proliferative phase of the menstrual cycle, but were absent in the EM of post menopausal women, suggesting that their development was hormonally influenced²⁶.

In sheep very few lymphocytes carrying classical B and T cell markers (CD5, surface immunoglobulin) were detected in the uterine epithelial cell suspensions and all intra epithelial lymphocytes (IEL) expressed the CD8 surface marker although with varying intensities²¹, which was in support of the present findings. It was revealed that MHC-I and -II⁺ cells were expressed by individual cells in organ layers, while CD8+ T cells and CD68+ macrophages were dominant in epithelium and muscle layer of bovine²⁷.

Natural killer (NK) cells were observed to be essential for establishment of human and rodent pregnancies. The function of these and other cytotoxic T cells (CTL) was controlled by stimulatory and inhibitory signalling²⁸. An increased proportion of NKp46+ and CD8+ cells were observed in the uterus of pregnant heifers, which supports our observation.

During human pregnancy, the tolerance of the semi-allogeneic fetus presents a significant challenge to the maternal immune system. In a woman, who had previous pregnancy, T cells were detected with specificity for foetal epitopes. Although it was previously thought that for maintaining maternal tolerance of the foetus, such foetal specific cell were generally deleted during pregnancy. Fetal- specific CD8+ T lymphocytes were

observed in half of all pregnancies and expression increased from the first trimester upto the postnatal period. Fetal-specific cells lead to an effector memory phenotype and were broadly functional. These retain their ability for proliferation, secrete IFN- γ , and lyse target cells and recognition of naturally processed peptide on male fetal cells. Hence, fetal-specific adaptive cellular immune response is a normal consequence of human pregnancy, fetal-specific T cells are not deleted during human pregnancy²⁹.

Treg (the naturally arising regulatory cells) were found to be essential for surface expression of CD4 and CD25 (IR2R α chain)³⁰. In mouse model, it has been studied that fetal/ placental antigen presentation and recognition of fetal antigen are mediated by CD4⁺ T cells through indirect recognition. In human pregnancy, there is evidence of HLA-C mismatch leading to an increase of CD4⁺CD25^{dim} activated T cells and the presence of functional CD4⁺CD25^{bright} regulatory T cells.³¹ In human pregnancy also, similar results were obtained i.e. indirect recognition of HLAC allele.

The mammalian uterus is unique in that it possesses the ability to mount an immune response against pathogenic organisms while not usually responding to 'foreign' antigens on sperm and fetal allografts³². Mucosal sites inhabited by commensal flora which are responsible to generate protective immune responses in the lower female reproductive tract, whereas the upper reproductive tract is normally sterile³³.

The uterus contains populations of T cells sufficient to induce graft rejection^{34,35}. Proper adjustments in uterine lymphocyte function to inhibit anti-fetal responses are therefore critical for successful pregnancy and disruption of this regulation can lead to pregnancy loss³⁶.

Studies were conducted at genomic level through differential gene expression study of CD8 and CD4 gene. It was confirmed that both CD8 and CD4 gene expression was more in pregnant compared to that of non-pregnant uterus. Gene expression was also observed to be better in middle of the horn compared to that of tip of the horn for both genes. Since in sheep, implantation occurs in the horn, it is evident to have better CD8 and CD4 gene expression. This is the first report to study the expression level of CD8 in sheep, hence comparison was not possible. The study was further confirmed with immune histochemical studies which showed the reactivity of CD8 cells in gravid and non gravid ewes. This is the first report of CD4 gene expression in sheep with respect to pregnancy. However some preliminary studies revealed presence of CD8 mRNA expression in few other species as in human³⁷, in canine³⁸ and in cows³⁹. Preliminary study with CD4 gene expression in sheep had been studied in liver infected with cystic Echinococcus^{40,41}. Apart from the role on bacterial, protozoal, parasitic infestation, CD4 and CD8 were observed to have a positive role in viral infection⁴², thus multiple roles of CD4 and CD8 were depicted⁴³. Fetus specific T cell modulation was observed during fertilization, implantation and pregnancy⁴⁴, with positive influence of CD4 and CD8 coreceptor gene expression⁴⁵. Different molecular biology techniques were employed for studying CD4 and CD8 with differential mRNA expression profiling for Mycobacterium tuberculosis⁴⁶. Advanced NGS studies were employed to assess gene expression profiling of sheep ovary⁴⁷. We had earlier characterized CD14 gene in different species of livestock^{48, 49, 50}. CD8 and CD4 expression profile revealed that these may be used

as future marker for pregnancy detection in sheep, which always remain a challenge for sheep industry. The technique may even be standardized for detection of litter size, which is a economic trait and of great importance in sheep which is prolific.

Materials and methods:

Sample: To undertake the present investigations, apparently healthy six pregnant (gravid) and six non-pregnant (non-gravid) uterus of sheep were procured from the Haringhata meat slaughter unit, Nadia, Govt. of West Bengal, India. The samples were harvested immediately after slaughter and taken into the ice box after necessary cleaning. Tissues were collected from different parts of uterus and preserved in 10% neutral buffered formalin (NBF) for histological and immunohistochemical studies.

Simultaneously, tissue samples from different segment of uterus (middle horn uterus and tip of the horn) of pregnant and non-pregnant ewes were collected into 5 ml eppendorf tubes separately with trizol (Ambion, Cat.No. 155960) and preserved at 4°C for initial 24 hrs and subsequently at -20°C till estimation for the mRNA expression.

Real time PCR:

Total RNA was estimated from the middle horn of uterus and tip of the horn of both the gravid and non-gravid uterus by Trizol method^{48,49,50,51} and quantitative analysis of total RNA were performed using formaldehyde gel electrophoresis. The 28S rRNA and 18Sr RNA were demarcated the quality of RNA. First strand cDNA was synthesised by the process of reverse transcriptase polymerase chain reaction (rt-PCR) in the automated temperature maintained thermocycler machine. M-MLVRT (200 u/μl) was used as reverse transcriptase enzyme. All the primers were designed using primer 3 software (v. 0. 4.0) as per the recommended criteria. The primers used are listed in Table1. Equal amount of RNA (quantified by Qubit fluorometer, Invitrogen), wherever applicable, were used for cDNA preparation (Superscript III cDNA synthesis kit; Invitrogen). All qRT-PCR reactions were conducted on ABI 7500 fast system. Each reaction consisted of 2 μl cDNA template, 5 μl of 2X SYBR Green PCR Master Mix, 0.25 μl each of forward and reverse primers (10 pmol/μl) and nuclease free water for a final volume of 10 μl. Each sample was run in duplicate. Analysis of real-time PCR (qRT-PCR) was performed by delta-delta-Ct ($\Delta\Delta Ct$) method.

The entire reactions were performed in triplicate (as per MIQE Guidelines) and experiment repeated twice, in 20μl reaction volume, using FastStart Essential DNA Green Master (Himedia) on ABI 7500 system.

Immunohistochemistry

Paraffin tissue blocks were prepared by standard manual alcohol-acetone protocol. The 5-6 μm thick paraffin sections obtained from different segments of the uterus (middlehorn of uterus and tip of the horn) of both the pregnant and non-pregnant ewes, were taken on Millennia 2.0 adhesion slides (Cat. No. 71863-01, abcam). The tissue sections were de-

paraffinized and hydrated in distilled water. The tissues slides were covered with trypsin enzymatic antigen retrieval solution (Cat. No. ab970, abcam) and kept in incubator in humid environment at 37°C for 5-10 minutes. The sections were then incubated for 60 minutes in peroxidase blocking solution (Lot. No. 00065614, Dako) at room temperature to block non-specific antibody binding activity. After subsequent washing with phosphate buffer saline (PBS), the sections were incubated at 37°C for 2 hours in humid environment with mouse monoclonal anti CD8 antibody (Cat. No. MA1-80900, Thermo Fisher Scientific) and CD4 antibody (Mouse anti Sheep CD4 antibody Cat no. MCA2213GA, abCam) in 1: 200 dilution. Immunoreactivity was detected after one hour incubation at 37°C with secondary antibody, Rabbit anti-mouse IgG H&L (HRP Conjugated, Cat. No. ab6728; abcam) in dilution 1:200. Slides were then rinsed 3 times in PBS for 5 minutes each, followed by treatment with freshly prepared DAB solution for 3 minutes (DAB substrate, Cat. No. 34001, Thermo Fisher Scientific). The sections were counter stained with Mayer's haematoxylin, hydrated in ethanol, cleared in xylene and then mounted in DPX⁵².

Statistical analysis:

Researchers count the percentage of positive immune labeled cells over the total cells in each selected area . This method can be automated with the use of special plugins for computer counting of general amount of cells and positively stained cells in case of immunohistochemistry.

Descriptive statistics with mean and standard error were estimated through SYSTAT package for the expression level analyzed through real time PCR and presented accordingly in graph.

Conclusion:

The lymphocyte population was more at the middle horn of the uterus as compared to the tip of the uterus in both gravid and non-gravid ewes. The occurrence of CD8+ cells were found within the luminal and glandular epithelium of the caruncular and inter caruncular areas. In gravid uterus the population of CD8+ T cell was comparatively more. The middle horn of the uterus revealed maximum expression in both the gravid and non-gravid ewes. The current observations demonstrate that during pregnancy in ewe the immune system responds to changes in the maternal environment to maintain the size and function of the CD8 T-cell compartment, as revealed by immunohistochemistry studies. Studies at genomic level revealed an increased mRNA expression of CD8 and CD4 gene, which is the first report, confirmed the above findings. CD8 expression pattern was higher in middle part of the pregnant uterus with both the techniques studied, indicating the importance of CD8 in case of recurrent abortion in non-infective condition. CD8 expression was higher in gravid uterus compared to that of CD4 gene expression. CD8 and CD4 may be effectively used as marker for pregnancy detection in sheep.

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Author Contribution Statement:

MI, AP and PD has planned and designed the research. MI, SB and AP had conducted the research. AP, PD and MI had analyzed the data. MI, AP and PD had drafted the manuscript and prepared the figures. SB had critically reviewed the article.

Competing interest Statement

The author(s) declare no competing interests.

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Table 1: List of primers involves in Real time PCR of ovine cDNA

Gene	Primers
CD8F	GGTCCTCGGGTCGCTCTCG
CD8R	GAAGCTGGGTCTGGGGTCGTC
CD4F	TCGCCGACGCAGAAGGAGAC
CD4R	TTGGGCGGAGAGGTACATTAGGAA
18S RNA	TCCAGCCTTCCTTCCTGGGCAT
18S RNA	GGACAGCACCGTGTGGCGTAGA

Figure Legend:

- Fig 1 A. Differential mRNA expression profile of CD8 in middle and tip of the horn of uterus of ewe
- Fig 1B. Differential mRNA expression profile of CD4 in middle and tip of the horn of uterus of ewe (Middle of the horn had maximum expression compared with tip of the horn)
- Fig 2A. Differential mRNA expression profile of CD8 with respect to Pregnant and Non-pregnant uterus of ewe.
- Fig 2B. Differential mRNA expression profile of CD4 with respect to Pregnant and Non-pregnant uterus of ewe. (Gravid or pregnant uterus had increased expression than non-gravid uterus)
- Fig 3A. Differential mRNA expression level of CD8 and CD4 in different parts of uterus of ewe- in Middle and Tip of the horn.
- Fig 3B. Differential mRNA expression level of CD8 and CD4 in uterus of pregnant and non-pregnant ewes.
- Fig 4A. Photomicrograph of Immunohistochemistry showing CD4 lymphocytes distributed in stromal area of *middle horn* of gravid ewe in a cluster form (circle area). IHC 40X.
- Fig 4B. Photomicrograph of Immunohistochemistry showing CD8 lymphocytes localisation at the stroma of inter caruncular area in *tip of the horn* of gravid ewe. CD8 positive lymphocytes (arrows). IHC 40X (above) & 100X (below).
- Fig 5A. Photomicrograph of Immunohistochemistry showing CD4 positive cells (arrows) interspersed at the stromal region of caruncle of *middle horn* of uterus of gravid ewe. T. Serosa (S), T. Muscularis (M) and Caruncles (C). IHC 40X & 100X.
- Fig 5B. Photomicrograph of Immunohistochemistry showing CD4 positive cells (arrows) distributed at the stromal region of caruncle of *tip of the horn* of uterus of gravid ewe. IHC 100X
- Fig 6A. Photomicrograph of Immunohistochemistry showing CD8 lymphocytes distributed in stromal area of caruncular region of the middle horn of *gravid* ewe in a cluster form (circle area). IHC 40X.
- Fig 6B. Photomicrograph of Immunohistochemistry showing CD8 lymphocytes, interspersed at the stromal region of caruncular area of middle horn of uterus of non-gravid ewe. CD8 positive lymphocytes (arrows) at sub epithelial region. IHC 40X.
- Fig 7A. Photomicrograph of Immunohistochemistry showing CD4 positive cells (arrows) distributed at the stromal region of caruncle of tip of the horn of uterus of gravid ewe. IHC 100X.

533 Fig 7B. Photomicrograph of Immunohistochemistry showing CD4 positive cells (arrow)
534 interspersed at the stromal region of caruncle of tip of the horn of uterus of non-gravid
535 ewe.IHC 100X.

536

537

538

539

540

Fig 1:

A

B

Fig 2:

A

B

A

B

Fig 3:

Fig 4:

A

B

Fig 5:

A

B

Fig 6:

A

B

Fig 7:

A

B