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Investigation of IgG, vimentin, CD45 distribution, and density in mouse placenta at different periods of pregnancy

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Abstract: The aim of this study was to investigate the distribution and density of IgG, vimentin, and CD45 in mouse placenta on the fourth, tenth, and seventeenth days of pregnancy. Strept avidin-biotin-peroxidase complex (streptABC) staining method was used. On the fourth day of pregnancy, the expression of IgG, vimentin, and CD45 was more intense around the uterine glands. On the tenth day, IgG, vimentin and CD45 positive cells were demonstrated in decidua basalis and mesometrial parietal trophoblast giant cells (P-TGCs) and mesometrial lymphocyte groups (MLAp) portions. The formation of the placenta was completed on the seventeenth day of pregnancy. The expression of IgG in the labyrinth placenta as well as in the decidual spaces was evident on the seventeenth day of pregnancy. While the expression of vimentin was observed in the labyrinth placenta and the MLAp in a granular fashion, CD45 was expressed in a few P-TGCs as well as in MLAp along with vimentin.

Key words: CD45, histology, immunoglobulin G, placenta, vimentin

1. Introduction

The placenta, which is formed during pregnancy and ensures the development and maturation of the fertilized oocyte, is an extraembryonic tissue that provides the continuity of intrauterine life and is found only in mammals [1–3]. The placenta, which carries out the exchange of feto-maternal nutrients and substances during the gestation period, also acts as a comprehensive endocrine organ. Apart from these duties, the placenta also has duties such as removing waste and carbon dioxide from the fetus and helping the mother to develop some physiological changes related to pregnancy with its endocrine effect [1–4]. One of the most important functions of the placenta is to support the fetal allograft. With the placenta, the fetus is protected from the maternal immune system, preventing the rejection of the fetus, and the continuation of the pregnancy is ensured [5,6]. As the fetus is protected from maternal immunity, it is also protected from external factors thanks to the placenta. It is known that the fetus does not produce enough antibodies to protect itself from external factors in intrauterine life. Therefore, the fetus is protected from external factors by maternal antibodies. The only antibody that can pass through the placental barrier is immunoglobulin G (IgG). Thanks to the Fc (fragment crystallizable) receptors (FcRn) in the placenta, IgG transition is provided. It is reported that this transition varies between mammalian species. In ruminants, IgG is passed to offspring with colostrum, and in mice, it passes both through the placenta and in breast milk in the neonatal period [5–8]. CD45 is a receptor-dependent protein phosphatase that is expressed in all leukocytes and plays an important role in the function of these cells [9]. Precursor T and B-lymphocytes, granulocytes, monocytes, histiocytes, reticulum cells, and follicular dendritic cells have been reported as cells in which CD45 is expressed [9, 10]. It has been reported that CD45 identifies bone marrow-derived lymphoid and myeloid cells in the mouse uterus and is also used for immunohistochemical imaging of uterine leukocytes with dynamic populations [11].

Vimentin, which is the main component of intermediate filaments in many cells, is known to play a role in vital mechanical and biological events such as cell contraction, cell migration, creation of cell volume, and proliferation [12]. It has been reported that vimentin plays an important role in the realization of decidualization [13]. It was observed that it gave a strong reaction in stromal fibroblasts in the uterus of nonpregnant mice, and after implantation, it reacted in the uterine endothelial cells, stromal cells, and labyrinth [11].

The aim of this studywas to determine IgG, vimentin, CD45 localization, and density in mouse placenta on the

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fourth, tenth, and seventeenth days of pregnancy. Many studies have been done on the mouse and human placenta [5, 7, 8, 9, 13]. However, no study has been found in the mouse placenta, in which IgG, vimentin, and CD45 molecules belonging to the beginning, middle and late stages of pregnancy have been studied. This study will shed light on other studies on the placenta.

2. Materials and method

2.1. Material

In the study, 21 female and 7 male mice of 8–10 weeks old CD-1 breed obtained from Guinea Pig Experimental Animals Laboratory Industry (Ankara) were used. Every morning, the vaginal plug (plug) was checked and pregnant mice were taken into separate cages. Those with positive vaginal plugs were accepted on the first day of pregnancy. Seven healthy mouse placentas were obtained from animals euthanized by cervical dislocation under ether anesthesia on the fourth, tenth, and seventeenth days of pregnancy. All tissues were fixed with NBF (Neutral Buffer Formalin) solution. The whole procedure was carried out by ethical rules (Ethics Committee Approval Decision No: 64583101/2016/017). Serial sections of 5µ thickness were taken from the prepared paraffin blocks at 50µ intervals.

2.2. Method

To determine the expression of IgG, vimentin, and CD45 on the sections, the sABC method [14] was applied using anti-IgG (polyclonal rabbit antihuman IgG-DakoIR512), anti-CD45 (monoclonal mouse antihuman Dako-IR751), and antivimentin (monoclonal mouse antivimentin Dako-IR630) antibodies. The prepared sections were kept in xylol for 5 min, twice. The tissues waiting in xylol were passed through a series of decreasing alcohol and brought to distilled water (100% alcohol for 3 min, 96% alcohol for 3 min, 80% alcohol for 3 min, 70% alcohol for 3 min). It was rinsed twice in distilled water. For antigen retrieval, it was boiled 3 times for 5 min in a microwave oven in 0.01M sodium citrate pH 6 at 98 ºC. Then it was cooled at room temperature for 20 min and washed 2 times for 5 min with TBS (Tris Buffer Saline) pH 7.4. To remove endogenous peroxidase activity, the sections were kept in 3% H2O2 prepared with distilled water for 15 min. For the blocking application, the sections were kept in the blocking solution for 1 h. They have incubated in primary antibody diluted 1/200 with TBS overnight at +4 ºC. The next day, it was washed 2 times for 5 min with TBS. Sections were then incubated in a biotin-containing secondary antibody for 1 h at room temperature. Then it was washed with TBS twice for 5 min and then incubated for 1 h at room temperature in streptavidin HRP. It was washed 2 times for 5 min with TBS. Staining of cells showing antibody binding was performed using DAB (2 min). For core staining, it was kept in Harris hematoxylin for 10 s and quickly passed through 96% alcohol. It was passed through ascending alcohol series (3 min in 96% alcohol, 3 min in 100% alcohol I, 3 min in 100% alcohol-II).

2.3. Immunohistochemical evaluation

IgG, vimentin, and CD45 molecules were evaluated in placenta samples selected from all three periods of pregnancy. Brown precipitation was considered positive under the light microscope. The evaluation was graded according to whether the tissue was stained or not, the distribution and severity of the stain in the target tissue, and its character. For this purpose, lamina epithelialis, stroma, and serosa were examined on the fourth day of pregnancy. On the tenth day of pregnancy, the antimesometrial decidua, labyrinth, mesometrial decidua, and MLAp region were examined. On the seventeenth day of pregnancy, the mesometrial decidua, junctional zone, labyrinth, and yolk sac were examined and photographed.

3. Results

3.1. The fourth day of pregnancy

The reactions observed with immunohistochemical staining methods are given in Table 1.

3.1.1. Immunoglobulin G

IgG reaction was not observed in lamina epithelialis and gland epithelial cells. IgG-positive cells were observed around the glands in the endometrium (Figures 1A and 1B). These cells were especially close to the surface of the lamina epithelialis. Apart from the cells that showed positivity near the glands, cells showing individual IgG expression were detected in the lamina propria (Figure 1C). IgG expression was also observed in some cells located close to the serosa (Figure 1D).

3.1.2.Vimentin

In the examination, it was determined that vimentin expression was concentrated in the endometrial stromal cells around the uterine glands (Figures 2A and 2B). Cells showing vimentin expression were observed near the myometrium (Figure 2 A).

3.1.3.CD45

CD45 expression was found to be concentrated around the uterine glands (Figures 3A–3C). In addition, individual

Table 1. Immunohistochemical reaction on the fourth day of pregnancy.

	IgG	Vimentin	CD45
Lamina epitelyalis	-	-	-
Stroma	++	+++	++
Serosa	+++	+	+

None (-), Weak (+), Moderate (++), Strong (+++).

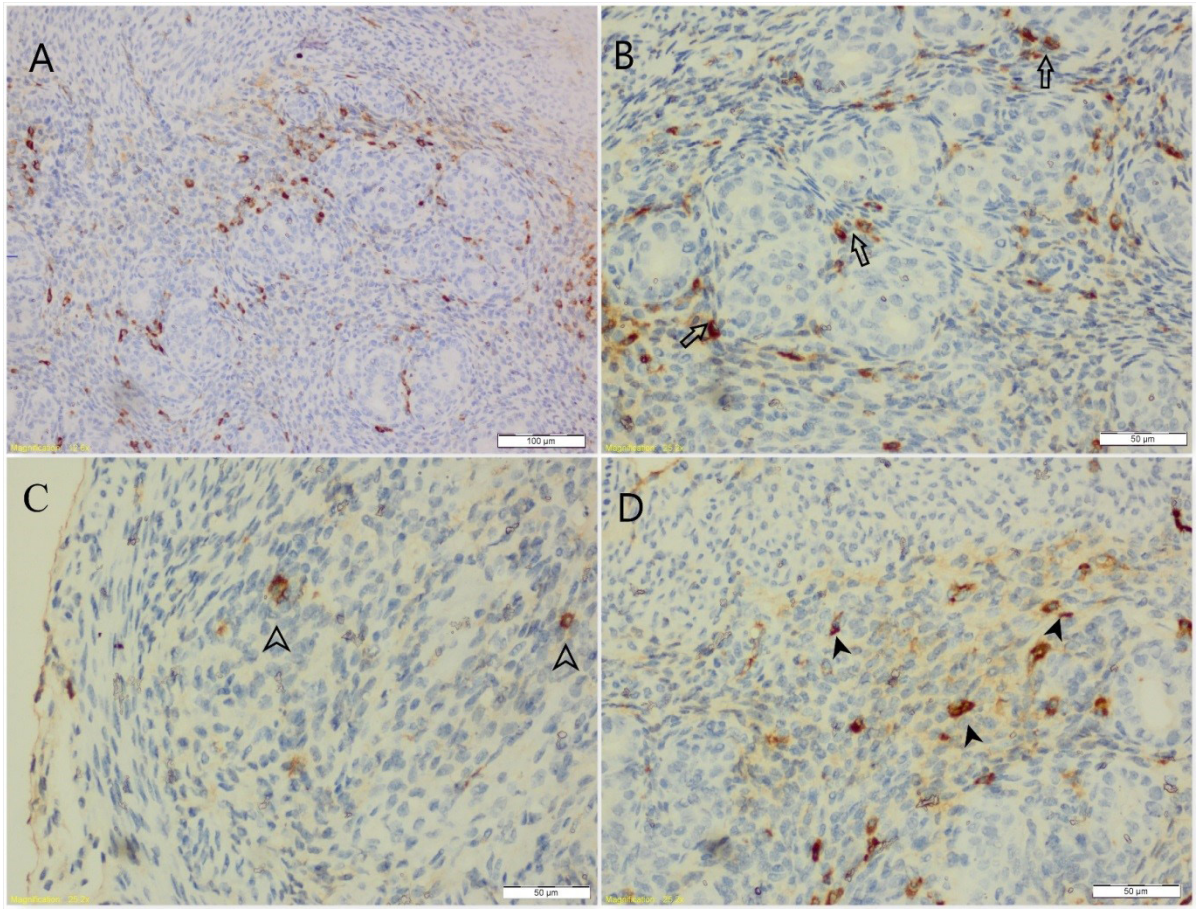

Figure 1. The fourth day of pregnancy. **A)** Cells showing IgG expression located around the glands. Bar 100 µm. **B)** Cells showing IgG expression located around the glands (arrows). Bar 50 µm. **C)** IgG positive cells located close to the serosa (arrowheads). Bar 50 µm. **D)** Cells showing IgG expression located singly in the lamina propria (arrowheads). streptABC staining method. Bar 50 µm.

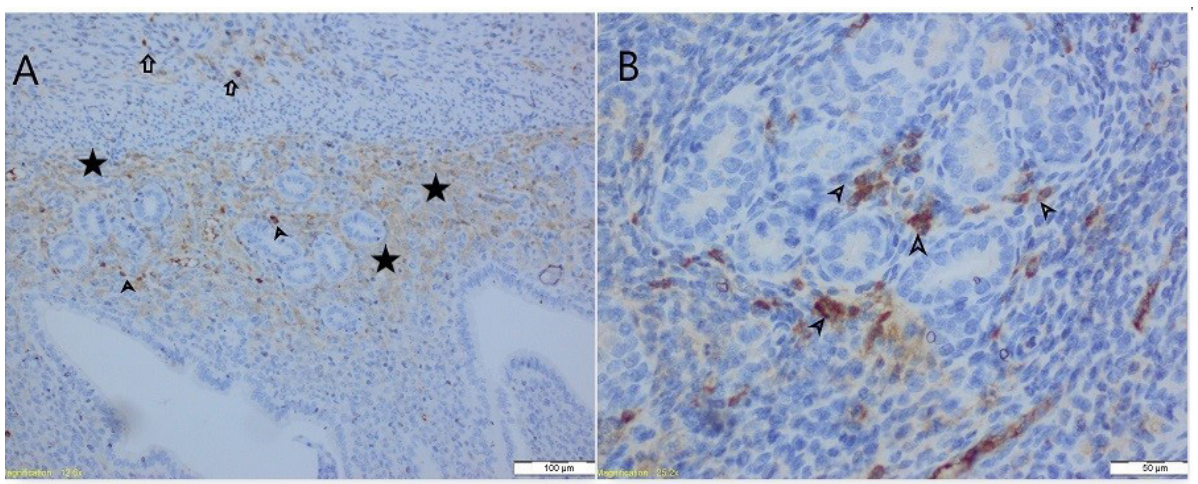

Figure 2. On the fourth day of pregnancy. **A)** Vimentin positivity in the endometrium. Image of positivity in the stroma (★) Expression of vimentin around the uterine glands (arrowheads), expression of vimentin near the myometrium (arrows). Bar 100 µm. **B)** Vimentin positive cells (arrowheads) around the uterine glands. sABC staining method. Bar 50 µm.

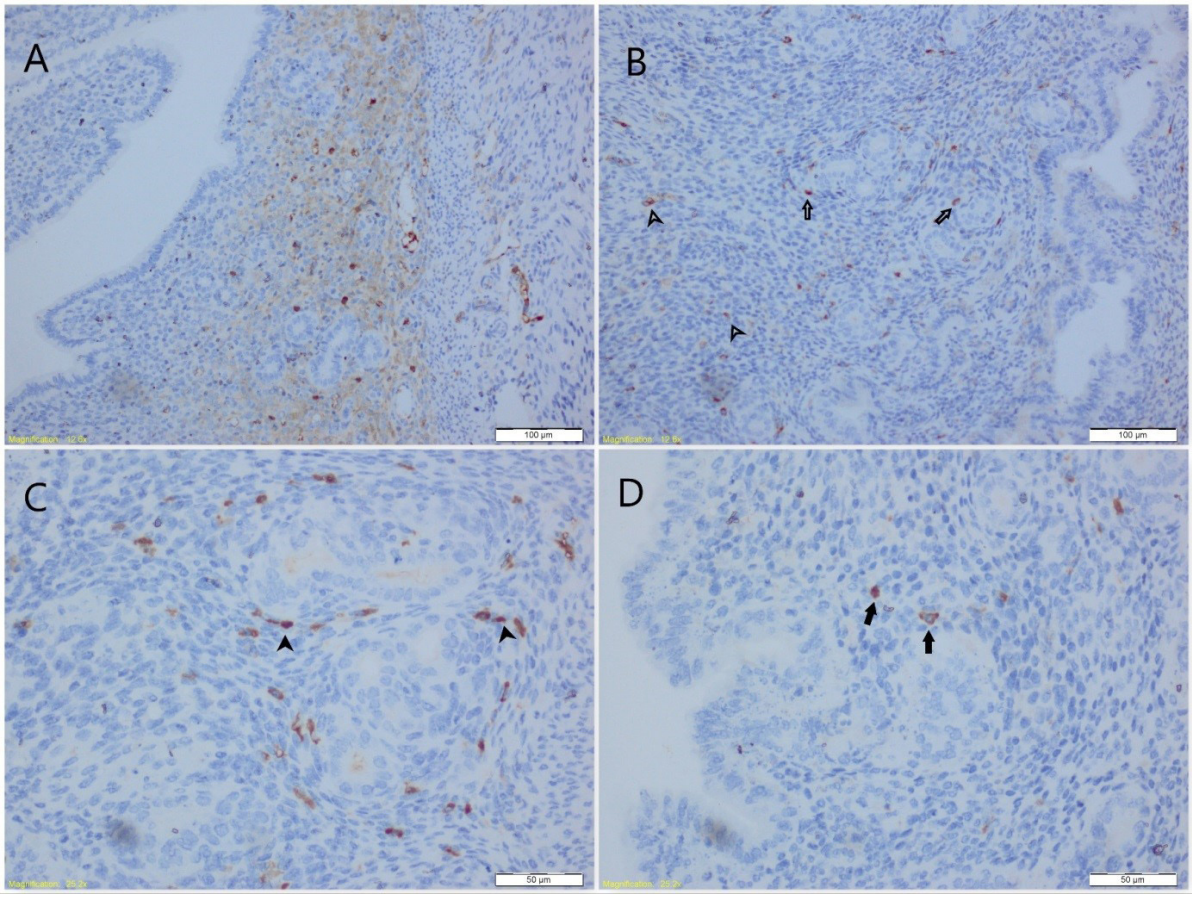

Figure 3. The fourth day of pregnancy. **A)** CD45 (+) cells in the endometrium. Bar 100 µm. **B)** (+) cells (arrowheads) in the basal parts of the endometrium and CD45 (+) cells (arrows) around the glands. Bar 100 µm. **C)** Positive cells (arrowheads) concentrated around the uterine glands. Bar 50 µm. **D)** CD45 (+) cells (arrows) in the lamina propria. sABC staining method. Bar 50 µm.

CD45 (+) expression was found in the basal parts of the endometrium (Figure 3B) and the lamina propria (Figure 3D).

3.2. The tenth day of pregnancy

The reactions observed with immunohistochemical staining methods are given in Table 2.

3.2.1. Immunoglobulin G

On the tenth day of pregnancy, large cell masses including decidua and glycogen cells were observed to express IgG in the decidua basalis. It was observed that this reaction occurred in clusters along the decidua basalis (Figures 4A and 4B). IgG positivity was observed in P-TGC (Parietal trophoblastic giant cell) in the mesometrium. A few cells were positive in MLAp (mesometrial lymphoid aggregate of pregnancy) (Figure 4C). Very few cells expressing IgG were observed in the antimesometrial serosa and antimesometrial decidua. No significant reaction was observed in the P-TGCs here either. Laterally, it was observed that lateral P-TGCs became IgG positive and gave a granular cytoplasmic reaction (Figure 4D).

3.2.2. Vimentin

On the tenth day of pregnancy, vimentin-positive cells were found individually located in the antimesometrium, serosa, and lamina propria. It was observed that the reaction occurring in the lamina propria also took place in the connective tissue around the glands. Vimentin-positive cells were seen in the antimesometrial decidua (Figure 5A). It was observed that the P-TGCs found here also gave a positive reaction to vimentin (Figure 5A). Vimentin-positive cells were detected in the region compatible with MLAp in the mesometrium. It was observed that glycogen cells in the decidua basalis and the cell masses where the decidua was together were vimentin-positive. It was observed that this positivity was strong in the neighborhood of MLAp, and its strength towards the labyrinth decreased. In the labyrinth, it was observed that P-TGCs gave a positive reaction to vimentin (Figure 5B).

3.2.3. CD45

In the antimesometrium, single and independent CD45 positive cells were detected in the serosa. Again, CD45

Table 2. Immunohistochemical reactions on the tenth day of pregnancy.

	IgG	Vimentin	CD45
Antimesometrial decidua	+	+	+
Antimesometrial P-TGC	-	+	+
Mesometrial P-TGC	++	++	+++
Labyrinth	+++	++	+++
Mesometrial decidua	+++	+++	+++
Glycogen cells	+++	+++	+++
MLAp	++	++	++

None (-), Weak (+), Moderate (++), Strong (+++).

positive cells were observed in the lamina propria, which was pushed in the antimesometrium, and located individually. In the antimesometrium, positivity was also observed in the P-TGCs that border the decidua and the part of this part facing the amniotic cavity (Figure 6A). In the mesometrium adjacent to MLAp, cell masses were found to give strong positivity in the decidua of the GlyC-decidua cell region (Figure 6B). CD45 positive cells were found in the mesometrium, as in the lamina propria and serosa in the MLAp region (Figure 6C). Very intense positivity was found in P-TGCs bordering the decidua in the mesometrium and adjacent trophoblast cells (Figure 6D).

3.3. The seventeenth day of pregnancy

The reactions observed with immunohistochemical staining methods are given in Table 3.

3.3.1. Immunoglobulin G

As a result of the applied sABC staining method, IgG expression was observed in the labyrinth in general (Figures 7A and 7B). While spongiotrophoblast cells did not react in the junctional zone, IgG-positive cells were found in glycogen cells (Figure 7C). IgG-positive cells were observed in areas compatible with MLAP in the decidua basalis (Figure 7D).

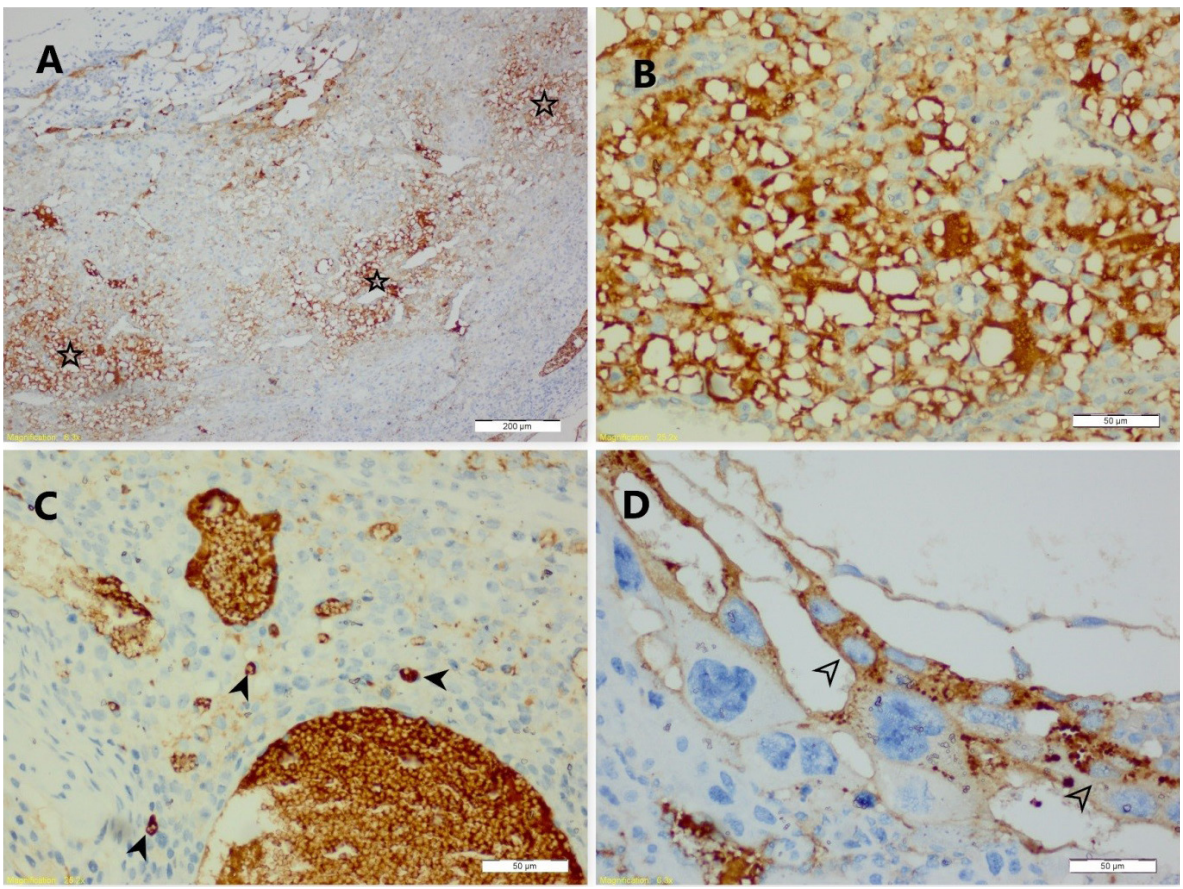

Figure 4. The tenth day of pregnancy. **A)** Mesometrium. Regions expressing IgG in decidua basalis (☆). Bar 200 µm. **B)** Glycogen and decidua cell mass showing IgG positivity. Bar 50 µm. **C)** IgG positive cells (arrowhead) seen adjacent to MLAP. Bar 50 µm. **D)** P-TGCs that give a granular cytoplasmic reaction in the labyrinth. sABC staining method. Bar 50 µm.

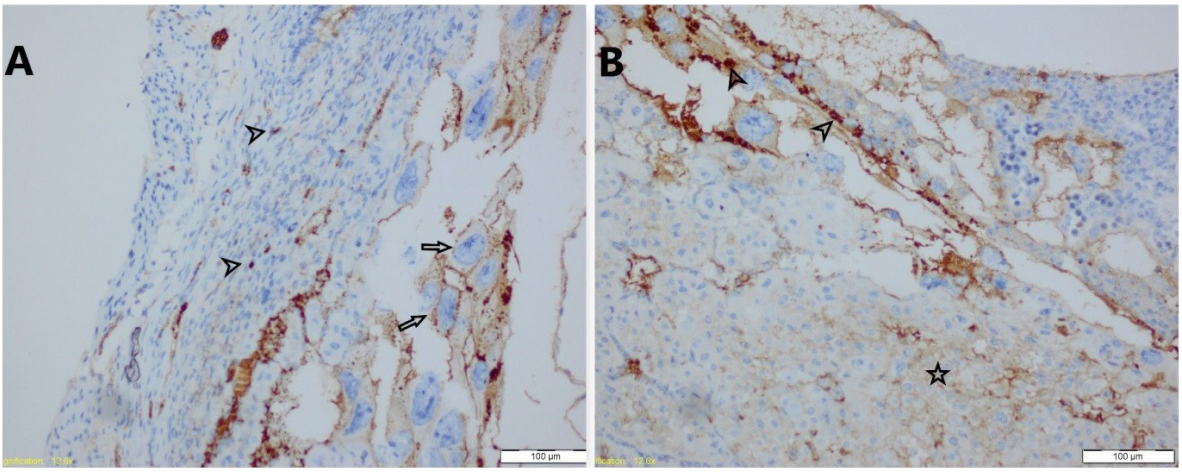

Figure 5. The tenth day of pregnancy. **A)** Antimesometrium. Vimentin-positive cells (arrowheads), vimentin-positive P-TGCs (arrows) in the antimesometrial decidua. **B)** Vimentin-positive P-TGCs (arrowhead) in the mesometrium, vimentin-positive areas in the decidua basalis (☆). sABC staining method. Bar 100 µm.

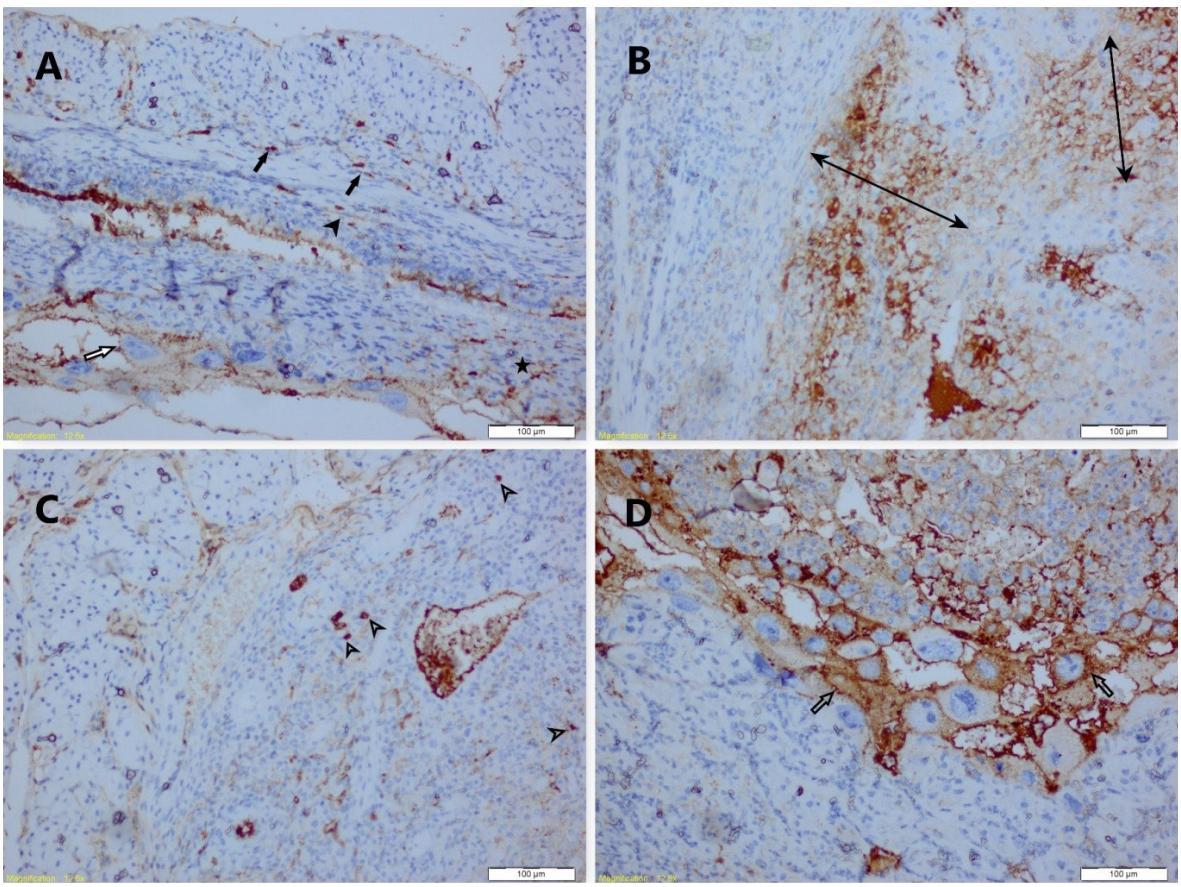

Figure 6. The tenth day of pregnancy. **A)** Antimesometrium. CD45 positive cells in serosa (black arrows), lamina propria (arrowhead), and antimesometrial decidua (★). CD45 positive P-TGCs (arrow). **B)** Decidua basalis areas showing intense CD45 positivity adjacent to MLAp (double-sided arrow). **C)** CD45 positive cells (arrowheads) seen in MLAp. **D)** Labyrinth part. P-TGC cells (arrows) showing CD45 positivity. sABC staining method. Bar 100 µm.

Table 3. Immunohistochemical reactions on the seventeenth day of pregnancy.

		IgG	Vimentin	CD45
Mesometrial decidua		++	+++	+++
Junctional zone	Glycogen cells	++	-	++
	Spongiotrophoblast	-	-	-
Labyrinth P-TGC		+	++	++
Labyrinth		++	++	+++
Yolk sac		-	-	-

None (-), Weak (+), Moderate (++), Strong (+++).

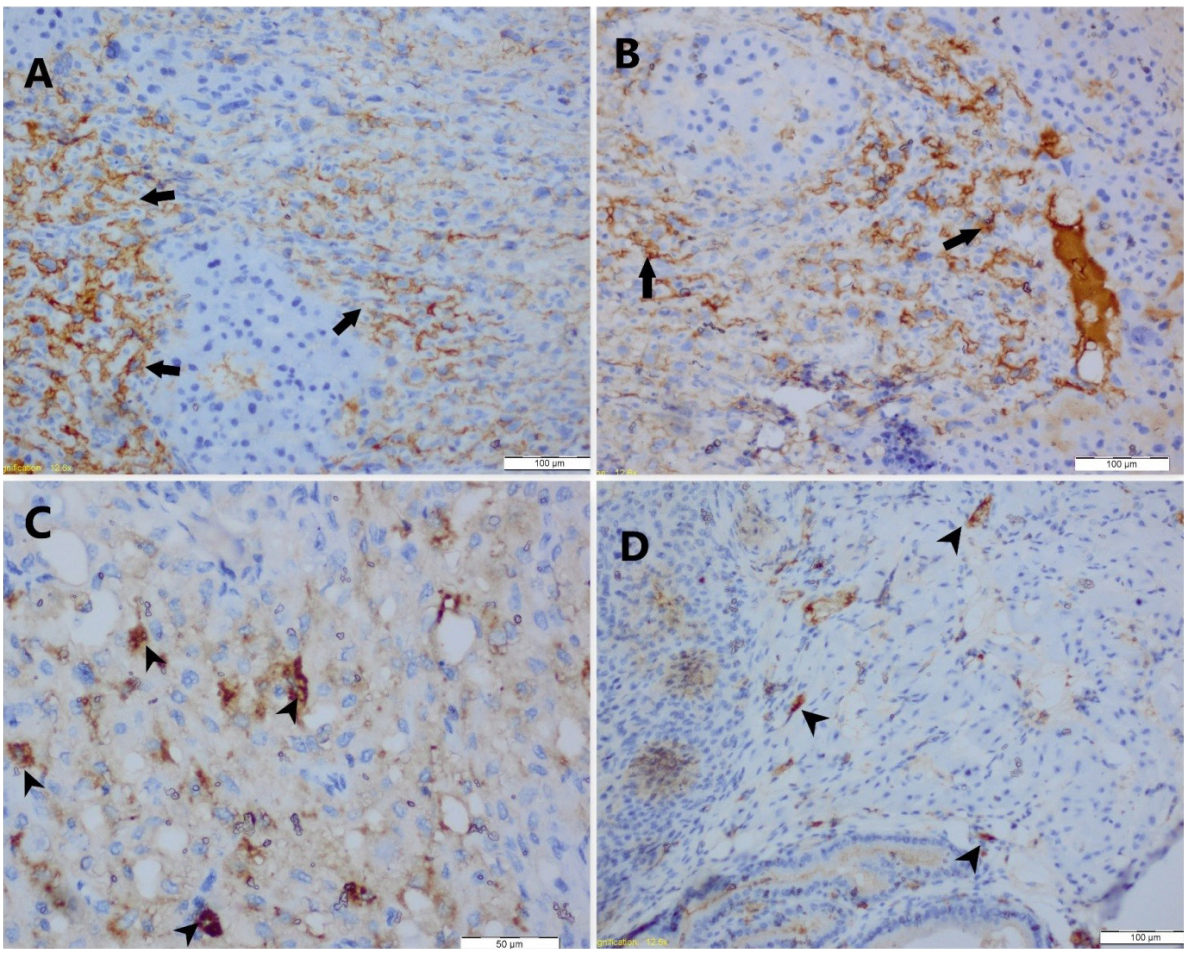

Figure 7. The seventeenth day of pregnancy. **A-B)** IgG positive areas (arrows) in the labyrinth zone. Bar 100 µm. **C)** Glycogen cells (arrowheads) reacting positively in the junction zone. Bar 50 µm. **D)** IgG positive cells (arrowheads) in the decidua basalis. sABC staining method. Bar 100 µm.

3.3.2.Vimentin

In sections belonging to the seventeenth day, cells showing vimentin expression were detected in the labyrinth zone as a result of the sABC staining method with an antivimentin antibody (Figure 8A). At the same time, vimentin-positive cells were detected in the cytoplasm of the decidua and MLAp in a granular fashion (Figure 8B).

3.3.3.CD45

As a result of the sABC staining method applied with an anti-CD45 antibody to the sections belonging to the

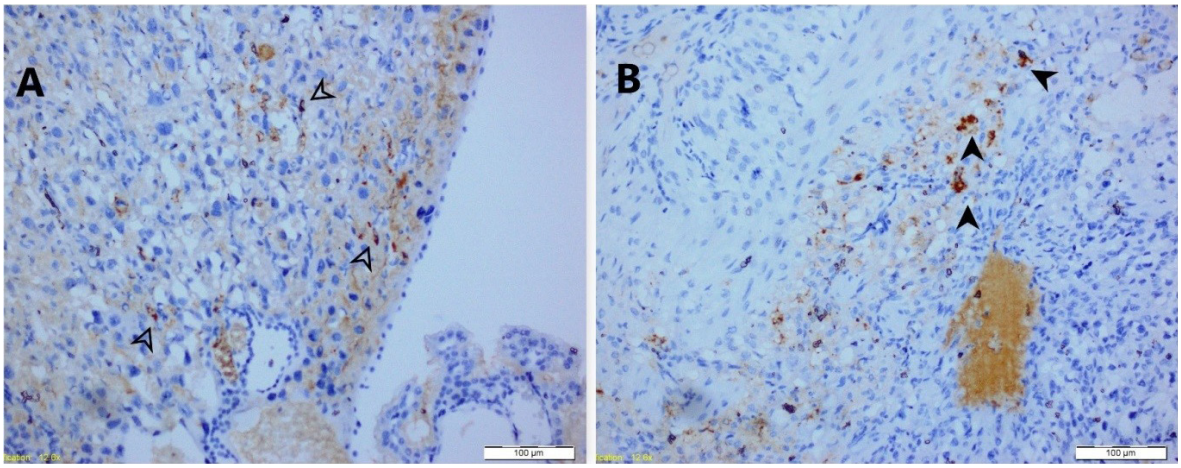

Figure 8. Seventeenth day of pregnancy. **A)** Vimentin positive cells (arrowheads) seen in the labyrinth part. **B)** Vimentin positive cells (arrowheads) reacting granularly in MLAp. sABC staining method. Bar 100 µm.

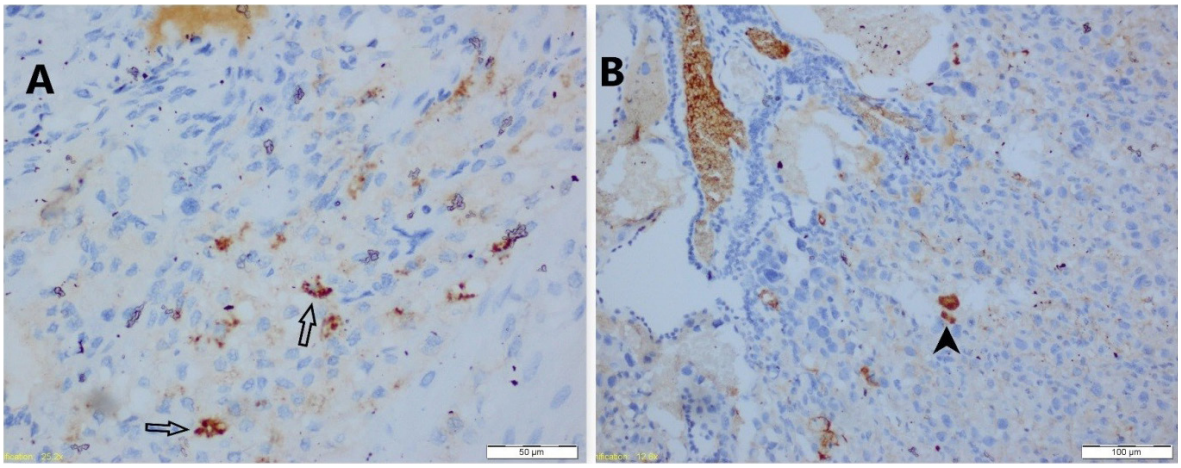

Figure 9. The seventeenth day of pregnancy. **A)** CD45 positive cells (arrows) in MLAp. Bar 50 µm. **B)** CD45 positive P-TGC (arrowhead) located close to the chorionic plate. sABC staining method. Bar 100 µm.

seventeenth day, no significant reaction was observed in the vitreous sac and labyrinth zone in general. In MLAp in the mesometrium, some CD45 positive cells showed granular positivity in the region compatible with the areas where vimentin expression was seen (Figure 9A). CD45 expression was found in several P-TGCs close to the chorionic plate (Figure 9B). While no positivity was observed in spongiotrophoblasts in the junctional zone, expression was observed in a small number of glycogen cells.

4. Discussion

Immunoglobulins are vital molecules that play an important role in organism defense. It is known that they also play an important role in pregnancy. In research on immunoglobulins and placenta types, it has been reported that the placenta type that allows the easiest passage is the hemochorial placentas [15]. It is known that IgG is the only immunoglobulin that can pass through the placenta [15, 16]. The localization of immunoglobulin A and G in the uterus, placenta, and embryo during pregnancy in mice was investigated. According to the research, IgA and IgG were observed in the endometrium, uterine glands, lumen, and a small amount in the vitreous sac at all stages of pregnancy, but IgA was not observed in the embryo and placenta at any time of pregnancy, only IgG was determined in the placenta and various parts of the embryo [17]. In a study examining the localization of IgG, IgA, and IgM in the first period of pregnancy in humans [18], these three immunoglobulins were found in the decidual tissue, while very weak staining of IgA in the fetal membranes and IgG in the placental tissues were observed. In the study, IgG expression was observed in all three gestational periods examined.

FcRn is an MHC class I-related molecule that plays a central role in the regulation of IgG homeostasis and IgG transport across polarized epithelial barriers. The main factor behind IgG transfer in the placenta is FcRn [19]. It is expressed by a variety of cells, including epithelial cells, endothelial cells, and myeloid-derived antigen-presenting cells [20].

In a study aimed at determining FcRn, which is the main molecule that provides IgG transport, it was reported that IgG is expressed by syncytiotrophoblasts in the human placenta [21]. In another study on placental IgG transport, it was stated that IgG expression was observed in the microvilli surface, apical and basal surfaces of the syncytiotrophoblasts in the chorionic villi of the human placenta, large granules on the apical and basal surfaces, and in the villus stroma [22]. Kiskova et al. [23] stated in a recent study that IgG and FcRn are expressed in the syncytiotrophoblast layer surrounding the chorionic villi, placental endothelial cells, and macrophages in the chorionic villi in the recent human placenta. In the study, IgG expression was observed in the placenta samples belonging to the seventeenth day, in the labyrinth zone, which is the equivalent of human chorionic villi.

In a study on FcRn in mouse placenta, it was reported that expression was seen in the vitellus endoderm, but the expression was not found in the placenta [24]. In the present study, IgG expression was observed in the labyrinth, decidua, and junctional zone.

In a study conducted to determine the transmission of IgG in the placenta between various species [25]. It was reported that FcRn was found in chorionic villi, syncytiotrophoblast cells, and fetal endothelial cells in humans and long-tailed macaques with the hemochorial placenta. In addition, in the same study, the presence of FcRn was observed in the endoderm cells of the yolk sac in rats and the labyrinth zone. In the presented thesis study, IgG positivity was found in the glycogen cells in the junctional zone and the labyrinth zone on the seventeenth day of pregnancy. The reactions are thought to be related to the presence of FcRns, which play a major role in IgG transfer, in chorionic villus trophoblast cells and labyrinth zone cells.

Vimentin is a protein expressed by intermediate filaments in the cytoskeleton. Vimentin is expressed only by cells of mesenchymal origin. It is considered to be a determinant of cells of mesenchymal origin [26]. Edwards et al. [11] reported that antivimentin antibody was expressed by stromal fibroblasts and endothelial cells in nonpregnant mouse uteri. In the same study, it was reported that expression was detected in the uterine endothelial cells and the labyrinth part of the developmental stages after implantation [11]. In another study, it was reported that trophoblasts, which show vimentin positivity in the placental development of mice, can invade the decidual tissue in the middle of pregnancy and have the ability to multiply rapidly [27].

In a study conducted in the human placenta at the end of pregnancy, it was reported that vimentin expression was found in some trophoblast cells and these cells were stromal-mesenchymal trophoblast cells. It is also stated that vimentin-positive cells also express CK5, 8, 10, 14, and 19 [28]. In the present study, vimentin expression was found in trophoblast cells in the labyrinth part, which can be considered homologous with chorionic villi in human placenta, in placenta samples belonging to the seventeenth day, which are considered as end-stage mouse placenta.

It has also been reported that vimentin positivity was observed in the endometrial stroma cells of the uterus before implantation in pregnant mice [11]. In the present study, vimentin positivity was observed in endometrial stromal cells in uterus samples belonging to the fourth day of pregnancy, which is considered the preimplantation period. It should be noted that the localization of the reaction is concentrated in the stroma around the uterine glands.

In a study conducted on the human placenta in the last period of pregnancy [29], it was reported that vimentin positivity was found in the chorionic villus stroma, while vimentin positivity was not observed in trophoblasts. In addition, in the same study, it was stated that vimentin expression was observed in the connective tissue of the myometrium, decidual cells, and stroma [29]. In this study, it was determined that there were cells showing vimentin positivity in the decidual stroma. However, unlike this study, cells showing vimentin expression were also seen in trophoblasts located in the labyrinth zone.

It has been reported that the spongiotrophoblast layer in the labyrinth and the decidua give a positive immune reaction to vimentin in the late pregnancy mouse placenta [30]. In this study, it was observed that trophoblasts in the labyrinth and some cells in the decidua showed vimentin positivity in the mouse placenta belonging to the seventeenth day.

In an immunohistochemical study performed to determine epidermal and mesenchymal cells in the placenta in guinea pigs [31], vimentin expression was reported in the cellular membrane surrounding the placenta. This membrane has been reported to be homologous to Reichert's Membrane in rats and mice. In addition, it has been reported that vimentin reaction is observed in trophoblasts located in the placenta facing the maternal-fetal face [31]. Consistent with these findings, a vimentin-positive reaction was observed in the trophoblasts located in the labyrinth part and surrounding the maternal-fetal face in the presented study.

In a study on trophoblasts between days 6.5 and 12.5 of pregnancy in mice [30], vimentin positivity was observed in trophoblastic giant cells at 7.5 gestational days. Afterward, it was stated that intense vimentin expression was observed in P-TGC in the giant cell zone and in SpA-TGC, C-TGC, S-TGC in the labyrinth, which is one of the specialized TGC types [30]. In this study, vimentin positivity was observed in P-TGCs on the tenth day of pregnancy and in P-TGC on the seventeenth day and in some cells in the labyrinth.

As a result of the vimentin immunohistochemical staining method applied to the placenta at 10.5 days of pregnancy in mice [11], it was reported that spongiotrophoblasts were vimentin-negative and vimentin-positive areas in the labyrinth. It was also stated that the MLAp portion showed intense vimentin positivity. In the presented study, similar findings were obtained in placenta samples belonging to the tenth day.

In a study conducted in rat uterus [13], widespread vimentin expression was observed in endometrial stromal cells on the fourth day of pregnancy. On the sixth gestational day, vimentin positivity was observed in the area where decidualization occurred near the luminal epithelium and around the implanted embryo, while vimentin expression was observed in all decidual areas on the eighth gestational day [13]. In the presented study, vimentin positivity was observed concentrated around the uterine glands in the mouse uterus on the fourth day of pregnancy. Expressions as single cells located close to the serosa were also found. On the tenth day of pregnancy, intense vimentin expression was observed in the serosa, decidua, and P-TGCs in the antimesometrium, in the part compatible with MLAp in the mesometrium, and in the parts adjacent to MLAp in the decidua basalis, which decreased towards the labyrinth. In the labyrinth, positivity was observed in P-TGCs.

In a study conducted in mouse placenta between the ninth and eleventh gestational days, vimentin expression was reported in P-TGCs in the antimesometrial, lateral and mesometrial parts [27]. In the study, vimentin positivity was observed in P-TGCs on the tenth gestational day, which supports the researchers.

CD45 is a pan-leukocyte-determining antibody encoded by the Ptprc gene. It is also known that CD45 determines bone marrow-derived lymphocytes and myeloid cells. It is reported that it also determines reactive leukocyte populations in the uterus [11].

In a study conducted on the human placenta, CD45 positivity was found in the decidual part at the tenth day week of pregnancy [32]. In this study, CD45 positivity was found in the MLAp part of the mouse placenta located in the decidual area on the tenth day and seventeenth days of pregnancy.

In a study on the determination of macrophages in the uterus in nonpregnant women, it was reported that CD45 positivity was found in the uterine stroma, concentrated around the uterine glands, as a result of immunohistochemical staining applied to the uterus during the proliferation, secretion, and pre-ovulation periods [33]. In the present study, similar results were obtained in the immunohistochemical application of the mouse uterus at the preimplantation stage.

It has been reported that CD45 positivity was observed in the uterus before implantation, along the junction of the endometrium and myometrium, and around the glands in pregnant mice [11]. In the study, CD45 positivity was observed around the uterine glands, which supports this study presented on the fourth day of pregnancy.

In a study investigating hemopoietic cell clusters in mouse placenta, it was stated that these cell clusters did not show CD45 positivity on the 10.5th gestational day as a result of immunofluorescent staining methods, but weak CD45 expression was observed on the 11.5th gestational day [34]. CD45 expression was detected in all of the fourth, tenth, and seventeenth gestational days observed in the presented study. Again, in a study investigating hemopoietic stem cells in mouse placenta, it was reported that CD45 positivity was not observed in decidua and umbilical vein cells isolated on the twelfth gestational day in Ly-6a GFP transgenic mice, but a weak CD45 positivity was found only in trophoblasts [35].

In a study investigating the expression of MHC antigens in cows [36]. It was reported that CD45(+) cells were found individually in the luminal epithelium of the uterine endometrium, around the uterine glands, and nonspecific cell staining was observed in the uterine stroma. In the same study, when the placenta was examined, it was stated that a few CD45 positive cells were observed on the allantois during late pregnancy, and no positivity was observed in the chorioallantoic membrane and other parts [36]. Compared to this study and other studies in humans and mice [32–35], Low et al. [36] concluded that cows are evolutionarily different. It is thought to be explained by the presence of a placenta.

It has been reported that the MLAp part of the CD45 staining applied on the 9.5 and 10.5 days of pregnancy in mice showed intense CD45 positivity, and CD45 positivity was found in the other parts of the decidua basalis [11]. Parallel results were also obtained in the study. In the same study, 15.5 and 19.5 days, a small number of CD45 positive cells were reported in the decidua basalis [11]. In the presented study, on the seventeenth day of pregnancy, a small number of CD45 positive cells were observed in the MLAp compatible area compared to the other days of pregnancy, in line with the stated study.

As a result, it was noted that on the fourth day of pregnancy, the expression of IgG, CD45, and vimentin concentrated around the uterine glands. Diffuse vimentin positivity was detected in the stroma of the endometrium, where decidual cells of mesenchymal origin were expected to form. On the tenth day of pregnancy, a granular IgG reaction was detected in the cytoplasm of the labyrinth-limiting P-TGC cells. And CD45 positivity was observed in mLAP as expected. On the seventeenth day of pregnancy, IgG reaction was widely observed in MLap in the labyrinth. The fact that IgG is common both in the maternal part and in the labyrinth part where the fetal parts are exchanged in the last period of pregnancy suggests that IgG passes from the maternal tissues to the fetal tissues at the end of the study.

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