CLINICAL RESEARCH / KLİNİK ARAŞTIRMA

Evaluation of JAK/STAT Signaling Pathway-associated Protein Expression at Implantation Period: An Immunohistochemical Study in Rats

İmplantasyon Döneminde JAK/STAT Sinyal Yoluyla İlişkili Protein İfadelerinin Değerlendirilmesi: Sıçanlarda İmmünohistokimyasal Bir Çalışma

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Abstract

Objective: The implantation period of gestation is a complex process in which numerous molecular pathways play a role. The Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway is one of the evolutionarily conserved cascades used to transduce a multitude signals for several biological events such as implantation and uterine receptivity. Previous studies have indicated that the implantation process is disrupted by the lack of proteins involved in this pathway. However, our literature knowledge showed that there is no study evaluated the expression of JAK/STAT signaling pathway-associated proteins during the implantation period. This study investigated the expression patterns of JAK/STAT signaling pathway-associated proteins in rats by immunohistochemical (IHC) staining according to gestational days.

Methods: In this study, thirty Wistar Albino rats weighing 250-300 g, without any problems in their menstrual cycles, were used. The pregnant animals were sacrificed on the 4th, 5th, and 6th days and histochemical and IHC analysis were performed on the uterine tissues taken from these animals.

Results: In this study, protein expression of JAK1, JAK2, JAK3, STAT2, STAT3, STAT4, STAT5, and STAT6 belonging to the JAK/STAT pathway was evaluated in the uterine surface epithelium, gland structures, antimesometrial region, cells of the immune system, myometrium, mesometrial region and decidual cells, which are associated with the implantation process. The result of this evaluation reveals that the expression levels of these proteins in the JAK/STAT pathway vary in different days of the implantation period in implantation-related structures.

Conclusion: This study indicates that JAK/STAT signaling pathway-associated proteins can function actively in the regulation of the immunological response of the uterus and embryo-uterus interaction during the implantation period in rats. However, the findings obtained from advanced research on JAK/STAT pathway can be used for treating recurrent pregnancy failures and in enhancing assisted reproductive technology.

Keywords: Implantation, JAK/STAT pathway, uterine receptivity, immunohistochemistry

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Öz

Amaç: Gebeliğin implantasyon periyodu, çok sayıda moleküler yolağın rol oynadığı karmaşık bir süreçtir. Janus kinaz/sinyal transdüserleri ve transkripsiyon aktivatörleri (JAK/STAT) yolağı, implantasyon ve uterus alıcılığı gibi çeşitli biyolojik olaylar için çok sayıda sinyali iletmek için kullanılan evrimsel olarak korunan kaskadlardan biridir. Önceki çalışmalar, implantasyon sürecinin, bu yolakta yer alan proteinlerin eksikliği nedeniyle bozulduğunu göstermiştir. Ancak literatür bilgimiz, implantasyon periyodu sırasında JAK/STAT sinyal yolu ile ilişkili proteinlerin ekspresyonunu değerlendiren bir çalışma olmadığını göstermiştir. Bu çalışma, sıçanlarda JAK/STAT sinyal yolu ile ilişkili proteinlerin ekspresyon paternlerini gebelik günlerine göre immünohistokimyasal (IHC) boyama ile araştırmayı amaçlamıştır.

Yöntem: Bu çalışmada, menstrual sikluslarında herhangi bir problem bulunmayan, ağırlıkları 250-300 gr arası otuz adet Wistar Albino sıçan kullanılmıştır. Gebe bırakılan hayvanlar 4. gün, 5. gün ve 6. günde sakrifiye edilmiştir ve bu hayvanlardan alınan uterus dokularında histokimyasal ve IHC analizler gerçekleştirilmiştir.

Bulgular: Bu çalışmada implantasyon süreciyle ilişkili yapılar olan uterus yüzey epiteli, bez yapıları, antimezometrial bölge, immün sisteme ait hücreler, miyometriyum, mezometriyal bölge ve desidual hücrelerde JAK/STAT yolağına ait JAK1, JAK2, JAK3, STAT2, STAT3, STAT4, STAT5 ve STAT6 protein ekspresyonları değerlendirilmiştir. Bu değerlendirmenin sonucu JAK/STAT yolağında görevli bu proteinlerin implantasyon döneminin farklı günlerinde implantasyonla ilişkili yapılardaki ekspresyon düzeylerinin değişiklik gösterdiğini ortaya koymaktadır.

Sonuç: Bu çalışma, JAK/STAT sinyal yolu ile ilişkili proteinlerin, sıçanlarda implantasyon periyodu sırasında uterusun immünolojik tepkisinin ve embriyo-uterus etkileşiminin düzenlenmesinde aktif olarak işlev görebileceğini göstermektedir. Öte yandan, JAK/STAT yolu ile ilgili ileri araştırmalardan elde edilen bulgular, tekrarlayan gebelik başarısızlıklarının tedavisinde ve yardımcı üreme teknolojisinin geliştirilmesinde kullanılabilir.

Anahtar Kelimeler: İmplantasyon, JAK/STAT yolağı, uterin reseptivite, immünohistokimya

Introduction

Reproduction is a "vital" evolutionary process for the continuity of life and the first step of reproduction is fertilization resulting in fusion of haploid oocyte and sperm[1]. In mammalian following to fertilization, the zygote (fertilized diploid oocyte) undergoes multiple mitotic cell divisions to form the blastocysts during its passage from the fallopian tube to the uterine cavity[2,3]. As the mitotic divisions continue, blastocysts begin to compaction and soon after formation of compact blastomer (blastocyst cells) clusters, blastocyst arrives in the uterine cavity and after then implantation period starts[4,5].

The implantation period of gestation is a complex process in which numerous molecules are produced because of endocrine, paracrine, autocrine, cell-cell and cell-matrix interactions plays a role[6]. Because of ethical limitations and difficulties in examining the interplay of the embryo and uterus at the molecular level in human implantation period caused the details of the interactions between these molecules involved in this process not yet been fully elucidated. Therefore, studies have focused especially on the molecular basis and physiology of the implantation periods in other mammals such as mice and rats[7]. Evidence from these studies reveals that the implantation in mammals consists of three stages: apposition, adhesion and invasion[8]. At the apposition stage of this three-stage process called "implantation window", cell fates of blastomeres (blastocyst cells) differentiate into inner cell mass or trophectoderm (TE)[9]. At this stage stromal cells surrounding the implanting blastocyst differentiate into a specialized cell type called decidual cells, via a process known as "decidualization" and apposition stage ends with the contact of TE cells to the uterine surface epithelium[9,10]. At the adhesion stage, TE adheres to the uterine surface epithelium via cell adhesion molecules such as integrins, cadherins, selectins, and immunoglobulins[5,10]. At the same stage, cell adhesion molecules secreted by TE cells and ligands expressed in the extracellular matrix of the decidua interact in a temporal and spatial manner and prepare the suitable environment for invasion[11]. At the invasion stage of the implantation window, TE cells migrate to the maternal decidua and form clusters of cytotrophoblasts and syncytiotrophoblasts at the implantation site[12]. Ultimately, trophoblastic cells degenerate the maternal spiral arteries, transforming them into flaccid sinusoidal sacs. The purpose of the invasion stage is to reconstruct the maternal spiral arteries, which will allow a high blood flow between the fetus and the mother, replacing small, high-resistance vessels with large, low-resistance vessels[10]. Because invasion stage determines placental efficiency and fetal viability in the late eras of pregnancy, deficiencies in trophoblastic cell-invasion cause adverse effects such as intrauterine growth restriction[13].

Two other phenomena associated with implantation in mammals are uterine receptivity and blockade of maternal immune response to "semi-allogeneic" fetus[14,15]. Uterine receptivity refers to changes that occur in the endometrium to facilitate opposition adhesion and invasion of the blastocyst

during implantation[16]. The main factors responsible for these endometrial changes, which soon disappear if implantation does not occur, are ovarian steroids, progesterone and estrogen[17]. Another important event during implantation is the immunological regulation necessary to protect against rejection and control the invasion of the semi-allogeneic fetus into the maternal decidua in the symbiotic relationship established between the mother and fetus[18].

Cytokines are regulatory proteins secreted by the cells of both the specific immune system and the innate immune system. These regulatory proteins play a cricual role in all stages of the immune response and inflammation such as antigen presentation, differentiation, maturation, activation of immune system cells, expression of adhesion molecules, and acute phase responses[19]. Growing evidence obtained from *in vitro* and *in vivo* studies demonstrated that cytokines secreted from trophoblast cells and maternal immune system cells are an important actor in implantation associated uterine receptivity and blockade maternal immune response events[20-22]. In fact, several studies have shown that implantation elicits an inflammatory reaction in subgroups of mammals by upregulation of inflammatory cytokines, including interleukin-6 (IL-6) and IL-1[23,24]. Also, Stewart et al.[25] reported that maternal leukemia inhibitory factor (LIF) expression is essential for implantation in mice. Besides, cytokines such as interferon-γ (IFN-γ) and tumor necrosis factor-α were found to be embryotoxic and disrupt the implantation[26]. In addition to cytokines, it has been demonstrated that proteins involved in signaling pathways regulated by cytokines may affect the implantation process. For example, in the absence of signal transducer and activator of transcription 1 (STAT1) protein related to the JAK/STAT pathway, one of the important signaling pathways downstream of cytokine receptors, spiral artery remodeling results in failure in mice[27]. Furthermore, two independent studies indicated that STAT3 knockout embryos degenerate and die in the early post-implantation period[28] and inhibition of STAT3 activation in the mouse endometrium also prevents the embryo implantation[29]. Morover, Cheng and Stewart[30] found that silencing janus kinaz 2 (JAK2), another member of the JAK/STAT pathway, disrupted the LIF signaling and caused failure in uterine receptivity.

The current study reveals the expression patterns of JAK/STAT signaling pathway-associated proteins, which we play a role in the implantation process by interacting with different molecules at various stages of implantation, according to the days of gestation.

Materials and Methods

Animals

Thirty female Wistar Albino rats weighing between 250 and 300 g, were used for this study. Rats caged in controlled rooms with 22±3 °C temperature and 12-h light/12-h dark cycle were fed by standard rat feed and water ad libitum. Experimental procedures used in this study were approved by Manisa Celal Bayar University, Local Ethics Committee for Animal Experiments. All procedures were carried out in strict compliance with the animal experiment guidelines prepared for the care and use of laboratory animals.

Experimental Design

After the menstrual cycle periods of the rats used in the study were determined by the vaginal smear method, the rats were followed for 3 cycles. Rats with normal menstrual cycles were used in the study. The last vaginal smear samples were evaluated by hematoxylin staining, and female rats in the estrus stage of the menstrual cycle allowed to mate with male rats in separate cages. After then, female rats whose pregnancies were detected by sperm detection method in vaginal swab were separated from the male rats and transferred to different cages. The rats taken to separate cages were accepted to be on the embryonic day 0.5 (E.05) of their pregnancy. On E.4, E.5, and E.6, rats were sacrificed. The tissues of sacrificed rats were divided into three groups, each group containing 10 uterine samples: day 4 (sacrificed on day 4), day 5 (sacrificed on day 5) and day 6 (sacrificed on day 6) groups. After determining the implantation sites of sacrificed rats by dissection microscope, uterine samples determined as implantation site was fixed in 10% formalin solution for 48 h.

Hematoxylin-eosin Staining

Uterine samples of all groups were fixed in 10% formalin and dehydrated with increasing concentrations of ethanol and embedded in paraffin. For hematoxylin-eosin (HE) staining, the uterine samples were deparaffinized in xylene and rehydrated in decreasing concentrations of ethanol. The samples were stained with HE using routine protocols[31].

Immunoexpression of the JAK/STAT Signaling Pathway-associated Proteins

The uterine tissue samples were fixed in 10% formalin, then dehydrated with increasing concentrations of ethanol and embedded in paraffin. Antigen retrieval was performed by

placing the sections in sodium citrate buffer for 30 min at 90 °C in a microwave oven. Sections were incubated with 10% H_2O_2 (Sigma-Aldrich, Inc., St Louis, Missouri, USA) for 30 min for endogenous peroxidase blockade. To prevent specific antibody-antigen binding, sections were incubated with Super Block (Scytec Consulting Inc., Greenwood Village, Colorado, USA) for 1 h at room temperature and washed with PBS. After this step, sections were incubated with 1:200 diluted primary antibodies (JAK1, JAK2, JAK3, STAT2, STAT3, and STAT5; Santa Cruz, California, USA, STAT4, and STAT6; Abcam, Cambridge, UK) for 24 h at 4 °C. At the end of this time, the sections were respectively incubated with biotinylated secondary antibody (Scytec Consulting Inc.) and horseradish peroxidase-conjugated streptavidin (Scytec Consulting Inc). Finally, the contrast staining of the sections incubated with diaminobenzidine was performed with Mayer Hematoxylin (Merck, Germany). Sections were cleaned with xylene and then mounted with the Entellan (Merck)[31]. Uterine sections stained by immunohistochemistry (IHC) were evaluated by light microscopy using three histologists blinded to each other and were defined as strong (3), moderate (2), weak (1) and none or indeterminate (0) according to their staining grade.

Statistical Analysis

Data analysis of IHC staining was performed with Statistics Package for Social Sciences (SPSS) 15.0 for Windows (SPSS Inc., Chicago, IL, USA). Comparisons were then made between groups using one-way analysis of variance followed by a Tukey post-hoc test. Values were presented with mean standard errors and $p<0.05$ was considered statistically significant.

Results

Histological Findings

When HE stained uterine tissue sections of day 4 group were examined, 3 separate layers were detected: the endometrium inside, myometrium in the middle and the perimetrium layers surrounding the organ from the outside. In this group, scattered stromal cells and immune competent cells were observed under the epithelium, as well as uterine glands separated from each other by connective tissue. The first decidual reaction and granulated metrial gland cells were observed in a small area on the mesometrial side. In the histological evaluation of day 5 group's uterine tissues, secondary decidual areas were found and primary decidual areas. Along with these areas, stromal cells and immunocompetent cells among them were observed in the uterus (Figure 1).

When HE stained uterine tissue sections belonging to day 6 group were examined, an increase was observed in both the number and volume of decidual cells of this group. Histological evaluation also showed that the embryo performed implantation by directing it to the antimesometrial side. Stromal cells at the implantation site were found to proliferate, expanded, took polyhedral shapes, and transformed into decidual cells. The decidual bed in which the embryo was embedded was divided into two: the primary decidual zone adjacent to the embryo and the secondary zone adjacent to the myometrium. The embryo was placed in the yolk sac and surrounded externally by the parietal layer of primary embryonic endoderm cells and internally by the visceral layer of primary embryonic endoderm cells (Figure 1).

Immunohistochemical Findings

In the IHC assessment, expression pattern of each JAK/STAT signaling pathway-associated protein was evaluated in uterine surface epithelium, gland structures, antimesometrial area, immunocomponent cells, myometrium, mesometrial area and decidual cells.

This assessment showed that the expression of JAK1 in the day 4 group was limited to immunocomponent cells and whereas in day 5 groups the endometrial stromal cells as well immunocompetent cells and gland structures expressed JAK1 (Figure 2). In the day 6 groups, however, a JAK1 expression merely in decidual cells. JAK3 expression pattern within all groups was found to be quite similar to JAK1 ($p>0.05$). In the evaluation of JAK2, it was noted that this protein was expressed in immune component cells in the day 4 group, as well as in the surface epithelium, gland structures, and antimesometrial area. Also, expression level of JAK2 in immune component cells decreased and its expression in the surface epithelium and gland structures increased in the day 5 groups (Figure 2). Interestingly, JAK2 expression in the day 6 group found significantly increased in decidual cells ($p<0.05$).

The evaluation of STAT proteins revealed that STAT2 immunoreactivation was quite high in especially in the antimesometrial area and surface epithelium of the day 4 groups (Figure 3). In this group, STAT2 was also expressed in gland structures, immunocomponent cells and
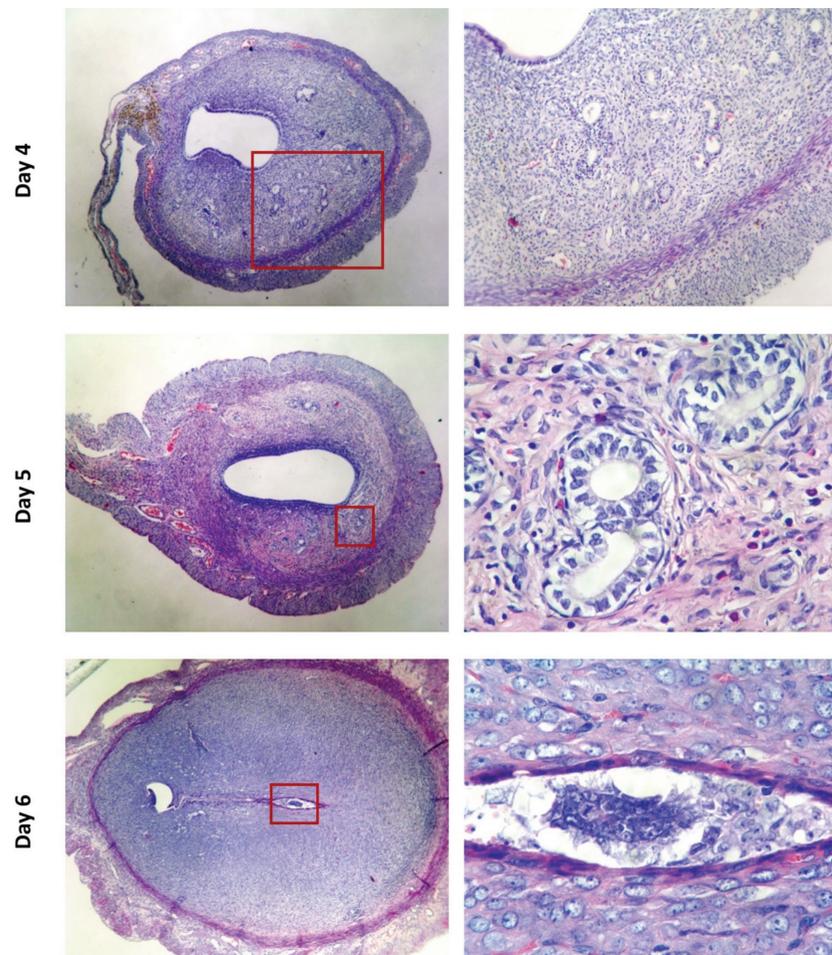
Figure 1. Hematoxylin-eosin staining of sections from all experimental groups. In day 4 group, the first decidual reaction and granulated metrial gland cells were observed in a small area on the mesometrial side. Secondary decidual areas detected and primary decidual areas in day 5 groups' uterine tissues. An increase was observed in both the number and volume of decidual cells in day 6 groups Histological evaluation of the same groups' uterine sections also showed that the embryo performed implantation at the antimesometrial side (the magnification of the left micrograph is 4x and the right micrograph is 40x)

myometrium. In day 5 groups, STAT2 immunoreactivity in the antimesometrial area and the surface epithelium significantly decreased compared to the day 4 group (p<0.05). In day 6 groups, only decidual cells leaky expressed STAT2 (Figure 3). STAT3 protein expression was detected in the antimesometrial area, immunocomponent cells, and myometrium in day 4 and day 5 groups (Figure 3). STAT3 expression observed in immunocomponent cells was significantly higher in the day 5 group compared to the day 4 group (p<0.05). In the day 6 groups, STAT3 expression was absent, except for the slight expression in decidual cells (Figure 3). Although there was a slight STAT4 expression only in immunocomponent cells in day 4 and day 5 groups, there was no STAT4 expression in the day 6 groups (Figure 3). STAT5 expression was observed in all areas except the myometrium and decidual areas in the day 4 and day 5 groups. Additionally, it was found that STAT5 expression in gland structures of day 5 group showed a significant increase compared to day 4 (p<0.05). The most striking result of STAT5 protein expression evaluation was the complete disappearance of STAT5 expression in the day 6 groups. Another interesting finding was that the STAT6 protein, which was not expressed in the day 4 groups, was expressed in all regions of the day 5 groups, and the STAT6 expression was again disappeared in the day 6 groups (Figure 3).

The immunoexpression levels of JAK/STAT signaling pathway-associated proteins and the p values of the groups are shown in the Table 1.
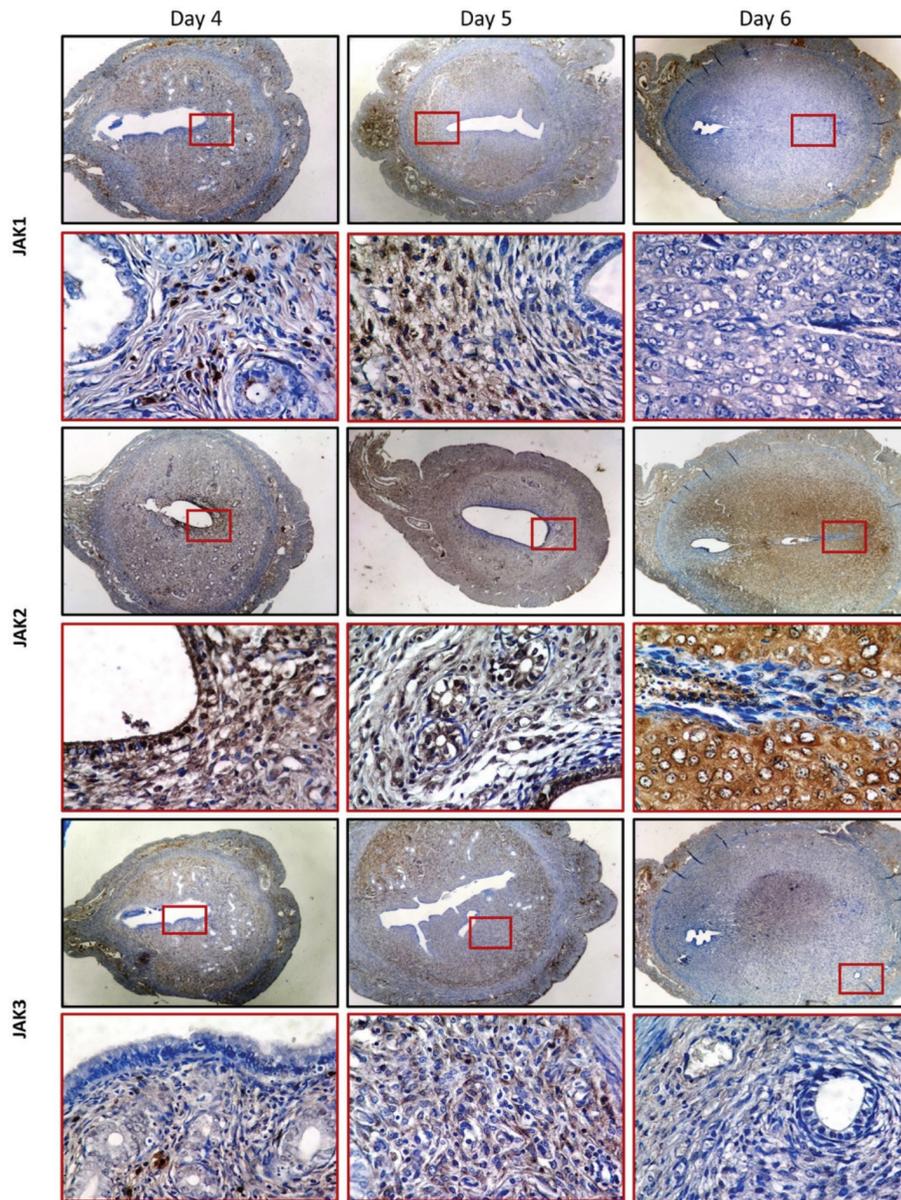
Figure 2. Immunoexpression patterns of JAK proteins in tissue sections from all groups (the magnification of the upper micrograph is 4x and the lower micrograph is 40x)

Discussion

The JAK/STAT signaling pathway is an evolutionarily conserved cascade that plays critical roles in orchestrating the immune system, especially cytokine functions[32]. Also, this pathway is essential for various biological events, such as cell fate decisions, cell proliferation, apoptosis, angiogenesis, hematopoiesis, lactation and development of the immune system and mammary glands[32,33]. JAK/STAT signaling pathway also plays on a role in reproduction via blastocyst implantation and uterine receptivity regulations[29]. Moreover, many preclinical studies shown that disruption of the JAK/STAT pathway, using a different from models, ultimately resulted in the failure of blastocyst implantation and uterine receptivity[29,34]. For example, Ernst et al.[35] demonstrated that mice with a mutation in the gp130 cytoplasmic domain, which selectively abolishes JAK/STAT signaling, were infertile because of implantation failure. Additionally, a study on LIF, a cytokine belonging to the gp130 family involved in the regulation of transcriptional activation in cells via JAK1 and STAT3, reported that implantation in LIF-deficient mice fails to proceed beyond the stage of apposition[25]. Several
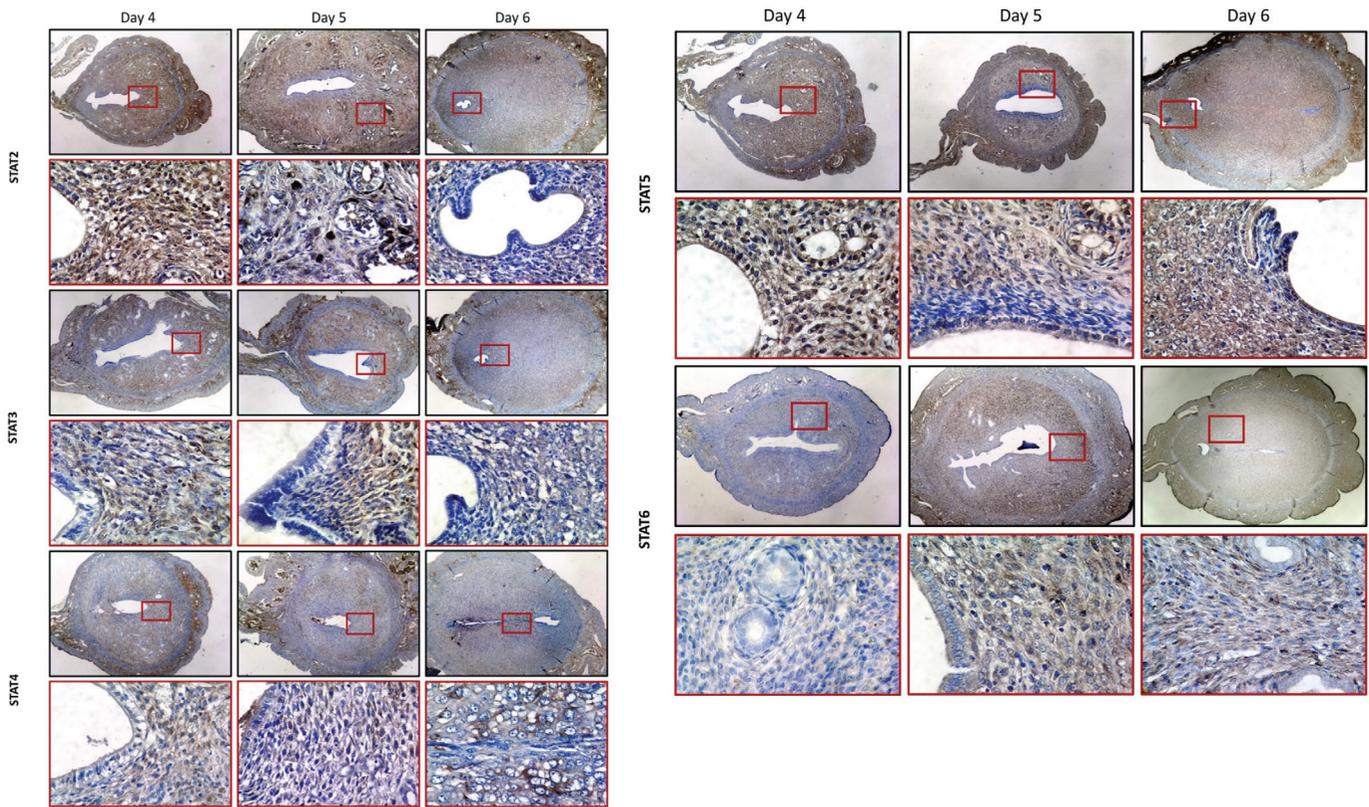
Figure 3. Immunoexpression patterns of STAT proteins in tissue sections from all groups (the magnification of the upper micrograph is 4x and the lower micrograph is 40x)

studies have confirmed that the abnormal expression of LIF is associated with embryo implantation failures[36,37]. For example, Takahashi et al.[38] suggested that LIF stimulates the differentiation of trophoblast giant cells via JAK1/STAT3 pathway. As mentioned in the Introduction section, Cheng and Stewart[30] found that silencing of JAK2 disrupts LIF signaling via demolish STAT3 phosphorylation and causes failure in uterine receptivity. Furthermore, the Yen et al.[37] revealed that the decrease in LIF expression caused implantation failures in adenomyosis patients by disrupting STAT3 signaling. Considering this literature information, cytokines such as LIF belonging to the gp130 family and cellular cascades induced by these cytokines play an important role in implantation and uterine receptivity. The findings obtained from this study reveal that JAK1, JAK2, and STAT3 proteins involved in the transcriptional activation regulated by LIF, are especially expressed in gland structures and immunocomponent cells in the day 4 groups. The fact that it was shown in the study by Bhatt et al.[39] on rodents that LIF expression started in the endometrial glands on the 4th day of pregnancy helps make sense to this finding obtained in the present study[40].

The necessity of controlled and mild inflammation during the implantation period may be the reason for the gradual decrease in JAK1, JAK2, and STAT3 immunoreactivations observed in day 5 and day 6 groups. Moreover, Parganas et al.[41] revealed that JAK2 is necessary for generating responses to hematopoietic factors such as erythropoietin, thrombopoietin, IL-3, and IL-5. However, erythropoietin triggers mitotic activity and angiogenesis[42]. High prevalence of JAK2 expression in the day 4 and day 5 groups may be due to increased mitotic activity and erythropoietin secretion that induces angiogenesis. The intense staining pattern of JAK2 immunoreactivity in the decidual area on the day 6 can be evaluated as an indication that JAK2 function continues after implantation.

In early gestation, the flow of immunocomponent cells such as dendritic cells (DC), macrophages and natural killer (NK) cells into the endometrium is regulated by blastocyst[43]. DCs are important actors in not only in induction of primary immune responses but also in induction of immunological tolerance and development of T regulatory (Treg) cells[44].

Table 1. The immunoexpression scores of the experimental groups

	Days	Surface epithelium	Gland structures	Antimesometrial area	Immunocomponent cells	Myometrium	Mesometrial area	Decidual cells	p-value	F
JAK1	4	0.33±0.255	2.66±0.352	0.33±0.255	1±0.258	1±0.258	0.33±0.255	0.33±0.255	0.000	596.7
	5	0	0	1.66±0.266	1±0.258	0	0.33±0.255	0.33±0.255	0.000	576.2
	6	0	0	0	0	0	0	1±0.258	0.012	526.8
JAK2	4	2±0.32	1±0.258	1±0.258	3±0.375	0	0	0	0.000	537.8
	5	2.66±0.352	3±0.375	1.33±0.26	1±0.258	1±0.258	0	0	0.000	603.6
	6	0	0	0	0	0	0	3±0.375	0.012	588.3
JAK3	4	0.33±0.255	0	0.33±0.255	2±0.32	1±0.258	0.33±0.255	0.33±0.255	0.000	596.9
	5	0	0	1.66±0.266	1±0.258	0	0	0	0.000	576.2
	6	0	0	0	0	0	0	1±0.258	0.012	566.8
STAT2	4	2±0.32	1±0.258	3±0.375	2±0.32	1±0.258	0	0	0.000	603.7
	5	1±0.258	1±0.258	2±0.32	2±0.32	0	0	0	0.000	536.1
	6	0	0.33±0.255	0	0.33±0.255	0	0	1±0.258	0.012	567.2
STAT3	4	0	2±0.32	1±0.258	1±0.258	1±0.258	1±0.258	0	0.000	535.9
	5	0	0.33±0.255	1±0.258	2±0.32	0.66±0.256	0	0	0.000	594.0
	6	0	0	0	0	0	0	0.66±0.256	0.012	525.3
STAT4	4	0	0	0.66±0.256	1±0.258	0.66±0.256	0.66±0.256	0	0.000	570.7
	5	0	0	0.66±0.256	1±0.258	0	0.66±0.256	0	0.000	571.5
	6	0	0	0	0.33±0.255	0	0.33±0.255	1±0.258	0.012	528.4
STAT5	4	1±0.258	1±0.258	2±0.32	2±0.32	0	0.66±0.256	0	0.000	589.1
	5	1.33±0.26	2±0.32	2±0.32	2±0.32	0	1.66±0.261	0	0.000	604.3
	6	0	0	0	0.33±0.255	0	0	0	0.012	511.8
STAT6	4	0	0	0	0.33±0.255	0	0	0	0.000	511.8
	5	0.66±0.256	0.66±0.256	1±0.258	1.33±0.26	0.33±0.255	0	0.66±0.256	0.000	534.7
	6	0	0	0	0.66±0.256	0	0	0	0.012	512.3

Today, it known that decreased expression of Treg in the endometrial tissue is associated with primary unexplained infertility in humans[45]. Macrophages are the major antigen presenting cells in the endometrium and these cells play a key role in the counteract nitric oxide (NO) synthesis and immune tolerance toward the implanting blastocysts[43,44]. At the beginning of gestation, the percentage of endometrial NK cells increases rapidly, reaching up to 70% of the total uterine leukocyte population. Furthermore, following implantation, endometrial NK cells differentiate into decidual NK cells, which begin secreting cytokines[46]. However, studies have reported that JAK3-driven STAT phosphorylation is important in the differentiation and maturation of DCs, macrophages, Treg and NK cells, which are critical for the maintenance of pregnancy. In their study, Vento-Tormo et al.[47] found that activation of the JAK3/STAT6 pathway, downstream of IL-4, is required for the DNA demethylation leading to DC differentiation. Additionally, Bingisser et al.[48] emphasized that JAK3/STAT6 signaling pathway is involved in T cell activation driven by NO produced by macrophages. Also, JAK1 and JAK3 act in the differentiation and maturation of NK cells through a pathway involving STAT5[49]. Considering this literature information, we thought that the JAK3, STAT5, and STAT6 protein expression observed, especially in immunocomponent cells in the day 4 groups, may be associated with the suppression of the immune response to implanting embryo. The fact that STAT4 protein expression, which is involved in the differentiation and maturation of Th1 cells involved in immune system activation, is at the basal level in all groups support this idea. However, the high expression of STAT5 in the surface epithelium and antimesometrial area in the day 4 and day 5 groups indicated that this protein could be used for different pathways than immune suppression. The relative increase in STAT4 protein expression in the day 6 group can be explained by the role of this protein in the transformation of villous trophoblast cells into extravillous trophoblast cells[50,51].

IFNs are important in establishing uterine receptivity to implantation in mammals[52]. Mice that lack IFN-γ and/or IFN-γ receptor do not have spiral artery remodeling, indicating an essential role for IFN-γ in this process[27]. Mice lacking type 1 IFN receptor (IFNAR) also lack spiral artery remodeling, indicating a non-redundant role of type 1 and type 2s[53]. However, STAT2 is an intracellular transcription factor, which is activated by the type 1 IFN family[54]. However, the role of STAT2 during implantation and gestation has not been fully explained yet. Nevertheless, the high expression of STAT2 in all groups in the study indicates that this protein may affect the implantation process with the effect of IFNs.

Study Limitations

The limitation of the present study is that the data obtained by IHC cannot be supported by molecular methods such as real-time polymerase chain reaction and Western blotting due to technical and economic inadequacies.

Conclusion

In conclusion, the results obtained from this study suggest that JAK/STAT signaling pathway-associated proteins can function actively in the regulation of the immunological response of the uterus and embryo-uterus interaction during the implantation period. In the future, advanced research may reveal the biological processes that the JAK/STAT pathway regulates during the implantation period. The findings obtained from this advanced research can be used for treating recurrent pregnancy failures and in improving assisted reproductive technology.

Ethics

Ethics Committee Approval: The study were approved by the Manisa Celal Bayar University of Experimental Animal Studies Ethics Committee (protocol number: 0151, date: 07.07.2005).

Informed Consent: Animal study.

Authorship Contributions

Concept: K.Ö., C.K., Design: K.Ö., C.K., Data Collection or Processing: S.İ., S.V., C.K., Analysis or Interpretation: C.K., Literature Search: K.Ö., S.İ. S.V., Writing: Ç.G., D.E., G.C.K., C.K.

Conflict of Interest: No conflict of interest was declared by the authors.

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