Differential Expression and Activation of Stat3 During Mouse Embryo Implantation and Decidualization

CHUN-BO TENG,1,2 HONG-LU DIAO,1 XING-HONG MA,1 LI-BIN XU,1 AND ZENG-MING YANG1*

1College of Life Sciences, Northeast Agricultural University, Harbin, China
2Heilongjiang Fishery Research Institute, Chinese Academy of Fishery Science, Harbin, China

ABSTRACT

Signal transducer and activator of transcription (STATs) can be activated by many cytokines and growth factors. Stat3, a member of STAT family, is essential for embryonic development. Stat3 is specifically activated during mouse embryo implantation. This study was to investigate the expression, activation, and regulation of Stat3 in mouse uterus during early pregnancy, pseudopregnancy, delayed implantation, artificial decidualization, and hormonal treatments using in situ hybridization and immunohistochemistry. There was a strong level of Stat3 phosphorylation in the luminal epithelium only at the midnight of day 4 pregnancy, which coincides with attachment reaction between the blastocyst and luminal epithelium. However, there was no detectable Stat3 phosphorylation at the corresponding period during pseudopregnancy. On day 5 of pregnancy, Stat3 phosphorylation was strongly observed in the luminal epithelium and the stroma surrounding the implanting blastocyst at implantation sites, but not at the interimplantation sites. Stat3 phosphorylation was also not detected on day 5 of pseudopregnancy. Stat3 phosphorylation was at a high level in the decidual cells on days 6–8 of pregnancy. Under artificial decidualization, Stat3 was also phosphorylated in the decidual cells. In the ovariectomized mice, there was no Stat3 expression and activation in the uterus. Progesterone had no obvious effects. However, Stat3 mRNA expression and phosphorylation were significantly stimulated by estrogen treatment. Our data suggest that Stat3 phosphorylation may be important for mouse embryo implantation and decidualization, and may also be regulated by maternal estrogen.


Key Words: Stat3; uterus; implantation; decidualization

INTRODUCTION

Signal transducer and activator of transcription (STATs) can be activated by receptor-associated cytoplasmic tyrosine kinases (Jak) through phosphorylation on a single tyrosine residue. Phosphorylated Stat proteins can form dimers and translocate into the nucleus, where they participate in transcriptional gene activation by binding to specific response elements in the promoter of target genes (Darnell, 1997; Pellegrini and Dusanter-Fourt, 1997). STAT molecules are stored in the cytoplasm in an inactive state until they are phosphorylated and translocated into nucleus. Stat3, a member of STAT family, can be activated by a variety of cytokines, including leukemia inhibitory factor (LIF), interleukin-6 (IL-6), IL-11, oncostatin M, cardiotrophin-1, ciliary neurotrophic factor, leptin, granulocyte colony-stimulating factor, and epidermal growth factor (Takeda et al., 1997). This family of signaling molecules has been implicated in growth, differentiation, survival, and apoptosis (Chapman et al., 2000).

Stat3-deficient embryos showed a rapid degeneration between embryonic days 6.5 and 7.5, although they developed into the egg cylinder stage until embryonic day 6.0, demonstrating that Stat3 is essential for the early development of mouse embryos (Takeda et al., 1997). gp130 is a signal-transducing receptor component of LIF, IL-6, IL-11, oncostatin M, cardiotrophin-1, and ciliary neurotrophic factor receptors, but by itself, does not bind LIF (Gearing et al., 1992; Davis et al., 1993). Embryos homozygous for the gp130 mutation progressively die between 12.5 days postcoitum and term (Yoshida et al., 1996). However, mice carrying a mutation of gp130 which deleted all STAT-binding sites are viable, but infertile in female mice because of implantation failure (Ernst et al., 2001). Moreover, LIF-deficient or IL-11 receptor α-deficient mice were unable to support pregnancy due to defective blastocyst implantation and decidua formation, respectively (Stewart et al., 1992; Bilinski et al., 1998; Robb et al., 1998).

The phosphorylation and nuclear translocation of Stat3 can be activated in the isolated epithelium of mouse uterus only by LIF, not by IL-6, ciliary

© 2004 WILEY-LISS, INC.
neurotrophic factor, and epidermal growth factor. In vivo Stat3 activation is induced by LIF alone, resulting in the localization of Stat3 specifically to the nuclei of the luminal epithelium coinciding with the onset of uterine receptivity (Cheng et al., 2001). Nuclear localization of Stat3 in the day 4 luminal epithelium was not detected in any LIF-deficient mice, indicating that LIF is the principal mediator of Stat3 activation in vivo (Cheng et al., 2001). In mouse uterus, Stat3 is also present and becomes phosphorylated in response to oil infusion (Hewitt et al., 2002). Although Stat3 activation in mouse uterus during embryo implantation was examined (Cheng et al., 2001), the expression, activation, and regulation of Stat3 in mouse uterus during early pregnancy were still unknown. The aim of this study was to investigate the expression, activation, and regulation of Stat3 in mouse uterus during early pregnancy, pseudopregnancy, delayed implantation, artificial decidualization, and hormonal treatments by in situ hybridization and immunohistochemistry.

MATERIALS AND METHODS

Animals and Treatments

Mature mice (Kunming white, outbred) were caged in a controlled environment with a 14 hr light:10 hr dark cycle. All animal procedures were approved by the Institutional Animal Care and Use Committee of Northeast Agricultural University. To confirm reproducibility of results, at least three mice per group were used in each stage or treatment in this study.

Adult females were mated with fertile males of the same strain to induce pregnancy (day 1 = day of vaginal plug). Pregnancy on days 1–4 was confirmed by recovering embryos from the reproductive tracts. The implantation sites on days 5–6 were identified by intravenous injection of 0.1 ml of 1% Chicago sky blue (Sigma, St. Louis, MO) in 0.85% sodium chloride. Additionally, vasectomized males of the same strain were mated with adult females to get pseudopregnancy (day 1 = day of vaginal plug).

The pregnant mice were ovariectomized under ether anesthesia at 08:30–09:00 hr on day 4 of pregnancy to induce delayed implantation. Progesterone (2 mg/mouse) was injected subcutaneously to maintain delayed implantation from days 5 to 7. To terminate delayed implantation, estradiol-17β (25 ng/mouse) was given to progesterone-primed delayed-implantation mice. The mice were sacrificed to collect uteri 24 hr after estrogen treatment. The implantation sites were identified by intravenous injection of 0.1 ml of 1% Chicago sky blue (Sigma, St. Louis, MO) in 0.85% sodium chloride. Additionally, vasectomized males of the same strain were mated with adult females to get pseudopregnancy (day 1 = day of vaginal plug).

The pregnant mice were ovariectomized under ether anesthesia at 08:30–09:00 hr on day 4 of pregnancy to induce delayed implantation. Progesterone (2 mg/mouse) was injected subcutaneously to maintain delayed implantation from days 5 to 7. To terminate delayed implantation, estradiol-17β (25 ng/mouse) was given to progesterone-primed delayed-implantation mice. The mice were sacrificed to collect uteri 24 hr after estrogen treatment. The implantation sites were identified by intravenous injection of Chicago sky blue solution. Delayed implantation was confirmed by flushing the blastocysta from the uterus.

Artificial decidualization was induced by intraluminally infusing 50 μl sesame oil into one uterine horn on day 4 of pseudopregnancy, while the contralateral un.injected horn served as a control. The uteri were collected on day 8 of pseudopregnancy. Decidualization was confirmed by weighing uterine horn and histological examination of uterine sections (Psychoyos, 1986).

Immunohistochemistry

Mouse uteri were immediately cut into small pieces, fixed in Bouin’s solution, dehydrated, and embedded in paraffin. Sections (7 μm) were cut, deparaffinized, and rehydrated. Antigen retrieval was performed by incubating the sections in 1 mM EDTA (pH 9.0) at 90°C for 10 min and by cooling naturally at room temperature for 20 min. Nonspecific binding was blocked in 10% normal horse serum in PBS for 1 hr. The sections were incubated with rabbit anti-human Stat3 polyclonal antibody (SC-7179, Santa Cruz Biotechnology, Inc., CA) or goat anti-human phospho-Stat3 polyclonal antibody [Tyr-705, SC-7993; rabbit anti-human phospho-Stat3 (SC-7993-R) was also used in this study, Santa Cruz] in 10% horse serum overnight at 4°C, respectively. After washing in PBS three times for 5 min each, the sections were incubated with corresponding biotinylated goat anti-rabbit IgG or biotinylated rabbit anti-goat IgG, followed by an avidin–alkaline phosphatase complex and Vector Red according to the manufacturer’s protocol ( Vectastain ABC-AP kit, Vector Laboratories, Burlingame, CA). The positive staining was visualized as a red color. Endogenous alkaline phosphatase activity was inhibited by supplementing 1 mM levamisole (Sigma) into Vector Red substrate solution.

In some sections, rabbit anti-human Stat3 was replaced with normal rabbit IgG as a negative control. The sections were counterstained with hematoxylin and mounted. Additionally, goat anti-human phospho-Stat3 polyclonal antibody was replaced by normal goat IgG to incubate some sections as a negative control. The sectioned incubated with goat anti-human phospho-Stat3 polyclonal antibody were counterstained with 0.1% fast green containing 10% glacial acetic acid and 30% ethanol. The degree of staining was assessed subjectively by blinded examination of the slides by two investigators.

In Situ Hybridization

Total RNAs from the mouse uterus on day 7 of pregnancy were reverse-transcribed and amplified with forward primer 5’-AGGAGGCGAGTTTGAGTC and reverse primer 5’-AGTTGAAGTGACAGAGCC designed according to mouse Stat3 (2,312–2,799 bp, Genbank Accession number U06922). The amplification of Stat3 cDNA was done for 35 cycles at 94°C for 30 sec, 60°C for 30 sec, and 72°C for 45 sec. The amplified fragment (486 bp) of Stat3 was recovered from the agarose gel and cloned into pGEM-T plasmid (pGEM-T Vector System 1, Promega, Madison, WI). The orientation of Stat3 fragment in pGEM-T plasmid was determined by a combination of the primers for T7, SP6, and mouse Stat3. The cloned Stat3 fragment was further verified by sequencing. The plasmid containing the Stat3 fragment was linearized with the appropriate enzymes and labeled. DIGoxigenin (DIG)-labeled antisense and sense cRNA probes were transcribed in vitro using a DIG RNA labeling kit (Roche Diagnostics GmbH, Mannheim, Germany).
STAT3 EXPRESSION AND ACTIVATION IN MOUSE UTERUS

Uteri were cut into 4–6 mm pieces and flash frozen in liquid nitrogen. Frozen sections (10 μm) were mounted on 3-aminopropyltriethoxy-silane (Sigma)-coated slides and fixed in 4% paraformaldehyde solution in PBS. The sections were washed in PBS twice, treated in 1% Triton-100 for 20 min and washed again in PBS three times. Following the prehybridization in the solution of 50% formamide and 5 × SSC (1 × SSC is 0.15 M sodium chloride, 0.015 M sodium citrate) at room temperature for 15 min, the sections were hybridized in the hybridization buffer (5 × SSC, 50% formamide, 0.02% BSA, 250 μg/ml yeast tRNA, 10% dextran sulfate, 1 μg/ml denatured DIG-labeled antisense or sense RNA probe for mouse Stat3) at 55 °C for 16 hr. After hybridization, the sections were washed in 50% formamide/5 × SSC at 55 °C for 15 min, 50% formamide/2 × SSC at 55 °C for 30 min, 50% formamide/0.2 × SSC at 55 °C twice for 30 min each, and 0.2 × SSC at room temperature for 5 min. After nonspecific binding was blocked in 1% block mix (Roche) for 1 hr, the sections were incubated in sheep anti-DIG antibody conjugated with alkaline phosphatase (1:5,000, Roche) in 1% block mix overnight at 4 °C. The signal was visualized with 0.4 mM 5-bromo-4-chloro-3-indolyl phosphate and 0.4 mM nitroblue tetrazolium in the buffer containing 100 mM Tris-HCl, pH 9.5, 100 mM NaCl and 50 mM MgCl2. Endogenous alkaline phosphatase activity was inhibited with 2 mM levamisole (Sigma). All of the sections were counterstained with 1% methyl green in 0.12 M glacial acetic acid and 0.08 M sodium acetate for 30 min. The positive signal was visualized as a dark brown color.

RESULTS

Stat3 mRNA Expression During Early Pregnancy

There was a low level of Stat3 mRNA signals in the luminal epithelium on day 1 of pregnancy (Fig. 1A). A basal level of in situ signals was seen in the uteri from days 2 to 3 (Fig. 1B). In the morning of day 4, there was no detectable Stat3 mRNA signal in the whole uterus (Fig. 1C). However, Stat3 mRNA expression was detected in the luminal epithelium in the afternoon of day 4 (Fig. 1D). On day 5, Stat3 mRNA expression was observed in the luminal epithelium and slightly in the glandular epithelium at implantation site (Fig. 1E). From days 6 to 8 of pregnancy, there was a high level of Stat3 expression in the decidua (Fig. 1F).

Stat3 Immunostaining in Mouse Uterus During Early Pregnancy

A basal level of Stat3 immunostaining was seen in the luminal and glandular epithelium on day 1 of pregnancy (Fig. 2A). There was a low level of Stat3 immunostaining in the luminal epithelium on days 2 and 3 of pregnancy (Fig. 2B,C). In the morning of day 4, only a basal level of Stat3 immunostaining was seen in the glandular epithelium (Fig. 2C). In the day 4 afternoon, a low level of Stat3 immunostaining was seen in the glandular epithelium (Fig. 2D). However, a strong level of Stat3 immunostaining was observed in the glandular and luminal epithelium in the midnight of day 4 (Fig. 2E). On day 6 of pregnancy, Stat3 immunostaining was obviously detected in the decidualized cells near the lumen at implantation site (Fig. 2F). Stat3 immunostaining was still located in the primary decidua on day 6 of pregnancy (Fig. 2G). However, Stat3 immunostaining was distributed in the whole decidua on days 7 and 8 of pregnancy (Fig. 2H,I).

Phospho-Stat3 Immunostaining During Early Pregnancy

Phospho-Stat3 immunostaining was strongly detected in the glandular epithelium, and weakly seen in the luminal epithelium and stroma on day 1 of pregnancy (Fig. 3A). On day 3, there was a low level of phospho-Stat3 immunostaining in the luminal epithelium (Fig. 3C). However, there was no detectable immunostaining in the morning of day 4 (Fig. 3D). In the afternoon of day 4, an intermediate level of phospho-Stat3 immunostaining was seen in the luminal epithelium and the stroma near the lumen (Fig. 3E). In the midnight of day 4, a strong level of phospho-Stat3 immunostaining was detected in the nuclei of luminal epithelial cells, and a low level in the stroma near the lumen (Fig. 3F). After embryo implantation, phospho-Stat3 immunostaining was strongly seen only in the luminal epithelium surrounding the implanting blastocyst and in the glandular epithelium, and weakly seen in the stroma near the lumen (Fig. 3G). But only a basal level of immunostaining was detected in the luminal epithelium of the inter-implantation area on day 5 of pregnancy (Fig. 3H). From days 6 to 8, phospho-Stat3 immunostaining was strongly detected in the whole decidua (Fig. 3I,J).

Stat3 Expression and Phosphorylation in Mouse Uterus During Pseudopregnancy

There was a low level of Stat3 mRNA expression in the luminal epithelium on day 1 of pseudopregnancy (Fig. 4A). No expression was detected in the uterus from days 2 to 3 of pseudopregnancy (data not shown). Stat3 mRNA signals were also not observed in the day 4 morning and afternoon of pseudopregnancy (Fig. 4B,C). However, Stat3 mRNA expression was detected in the luminal epithelium on day 5 of pseudopregnancy (Fig. 4D). A low level of Stat3 immunostaining was seen in the glandular and luminal epithelium on day 1 of pseudopregnancy (Fig. 4E). No Stat3 immunostaining was seen in the uteri from days 2 to 3 of pseudopregnancy (data not shown). Although there was no immunostaining in the uterus in the morning of day 4, a basal level of Stat3 immunostaining was observed in the glandular epithelium and stroma in the afternoon of day 4 (Fig. 4G). No Stat3 immunostaining was seen in the uterus on day 5 of pseudopregnancy (Fig. 4H). There was a low level of phospho-Stat3 immunostaining in the glandular epithelium on day 1 of pseudo-
pregnancy (Fig. 4I). No phospho-Stat3 immunostaining was seen on days 2 and 3 of pseudopregnancy (data not shown). Phospho-Stat3 immunostaining was weakly observed in the luminal epithelium and stroma near the lumen in the day 4 morning and afternoon of pseudopregnancy (Fig. 4J,K). On day 5 of pseudopregnancy, only a basal level of phospho-Stat3 immunostaining was seen in the stroma near the lumen (Fig. 4L).

**Stat3 Expression and Phosphorylation During the Delayed Implantation and Activation**

There was no Stat3 mRNA expression in the uterus under delayed implantation (Fig. 5A). When delayed implantation was terminated by estrogen treatment and embryo implanted, Stat3 mRNA expression was strongly detected in the luminal epithelium surrounding the implanting blastocyst (Fig. 5B).
A basal level of Stat3 immunostaining was seen in the stroma under delayed implantation (Fig. 5C). After the activation of delayed implantation, Stat3 immunostaining was seen in the luminal epithelium (Fig. 5D).

Under delayed implantation, a low level of phospho-Stat3 immunostaining was seen in the stroma (Fig. 5E). After the activation of delayed implantation, phospho-Stat3 immunostaining was at a low level in the luminal epithelium surrounding the implanting blastocyst and the stroma near the lumen, and at a high level in the glandular epithelium (Fig. 5F).

**Stat3 Expression and Phosphorylation During Artificial Decidualization**

Stat3 mRNA signals were at a basal level in the control uterus (Fig. 6A), while strongly detected in the decidualized cells under artificial decidualization (Fig. 6B).

There was a low level of Stat3 immunostaining in the stroma in the control uterus (Fig. 6C). Under artificial decidualization, Stat3 immunostaining was strongly observed in the decidualized cells (Fig. 6D).

No phospho-Stat3 immunostaining was seen in the control uterus (Fig. 6E). Under artificial decidualization, phospho-Stat3 immunostaining was strongly detected in the decidualized cells (Fig. 6F).

**Hormonal Regulation of Stat3 Expression and Phosphorylation**

There was no Stat3 mRNA expression in the uterus of ovariectomized mouse (Fig. 7A). Stat3 mRNA expression was also not detected after progesterone treatment (Fig. 7B). After the ovariectomized mice were treated with estrodiol-17β, Stat3 mRNA signals were strongly detected in the luminal epithelium (Fig. 7C). Stat3 mRNA expression was also strongly detected in the luminal epithelium after the ovariectomized mice were treated with estrodiol-17β and progesterone (Fig. 7D).

There was no detectable Stat3 immunostaining in the uterus of ovariectomized mouse (Fig. 7E). Stat3 immunostaining was also not seen after progesterone treatment (Fig. 7F). There was a low level of Stat3 immunostaining in the glandular and luminal epithelium after the ovariectomized mice were treated with either estrodiol-17β (Fig. 7G) or estrodiol-17β and progesterone (Fig. 7H).

There was no phospho-Stat3 immunostaining in the uterus of ovariectomized mice (Fig. 7I). After ovariectomized mice were treated with progesterone, a basal level of phospho-Stat3 immunostaining was seen in the luminal epithelium and the stroma (Fig. 7J). However, phospho-Stat3 immunostaining was strongly detected in the luminal epithelium and weakly in the glandular epithelium after estrodiol-17β treatment (Fig. 7K). After ovariectomized mice were treated with a combination of estrodiol-17β and progesterone, phospho-Stat3 immunostaining was strongly detected in the luminal epithelium and weakly in the stroma (Fig. 7L).

**DISCUSSION**

In this study, phospho-Stat3 immunostaining reached its highest level in the nuclei of the luminal epithelium of mouse uterus at the day 4 midnight of pregnancy, when only the Stat3 protein in the luminal epithelium was phosphorylated although the Stat3 immunostaining was highly detected in both glandular
Fig. 3. Phospho-Stat3 immunostaining in mouse uteri during early pregnancy on days 1 (A), 2 (B), 3 (C), 4 morning (D), 4 afternoon (E), 4 midnight (F), 5 (G), 5 (inter-implantation site, H), 6 (I), and 7 (J), respectively. Arrow: Embryo; *: Uterine lumen. Bar = 15 μm.
and luminal epithelium. In the mouse, the attachment reaction between the blastocyst and uterine luminal epithelium occurs around 2,300–2,400 hr on day 4 of pregnancy (Das et al., 1995). We found that there was a basal level of phospho-Stat3 immunostaining at the morning (around 0800 hr) of day 4 pregnancy and a low level in the luminal epithelium at the afternoon (around 1,600 hr). However, Cheng et al. (2001) reported that on day 4 (around 0800 hr), Stat3 localization was predominantly to the nuclei of the entire luminal epithelium. Because they did not check Stat3 immunostaining at the midnight of day 4, it is impossible to compare with their results. The reason for causing this difference may be from the antibodies used in our study. In our study, we used phospho-Stat3 antibody (Tyr 705) specific for phosphorylated Stat3, while rabbit anti-Stat3 antibody in their study detected both inactivated and activated Stat3 (Cheng et al., 2001).

It seems that there was a regulation on the phosphorylation of Stat3 protein during the peri-implantation. At the midnight on day 4 of pregnancy, Stat3 immunostaining was strongly observed in both glandular and luminal epithelium, while phospho-Stat3 immunostaining was strongly detected only in the luminal epithelium. Moreover, in the implantation sites on day 5 of pregnancy, phospho-Stat3 immunostaining was strongly seen only in the luminal epithelium and the stroma surrounding the blastocyst although Stat3 immunostaining was observed in a wider area. Our data suggest that Stat3 is only phosphorylated in a limited area during uterine receptivity. Furthermore, phospho-Stat3 levels in the luminal epithelium from day 4 of pregnancy uteri were induced maximally, whereas luminal epithelium from day 5 and day 3 uteri were refractory to LIF stimulation (Cheng et al., 2001), suggesting that Stat3 is only activated in a limited period. Additionally, the phosphorylation and nuclear translocation of Stat3 can be activated in the isolated epithelium only by LIF, not by IL-6, ciliary neurotrophic factor and epidermal growth factor. In vivo Stat3 activation is induced by LIF alone, resulting in the localization of Stat3 specifically to the nuclei of the

---

Fig. 4. Stat3 mRNA expression (left column), Stat3 immunostaining (middle column), and phospho-Stat3 immunostaining (right column) in mouse uteri during early pseudopregnancy on days 1 (A, E, I), 4 morning (B, F, J), 4 midnight (C, G, K), and 5 (D, H, L), respectively. The days of pseudopregnancy were also labeled at the left side of this figure. Bar = 25 μm.
luminal epithelium coinciding with the onset of uterine receptivity (Cheng et al., 2001). Upon cytokine stimulation, Stat3 rapidly translocates to the nucleus. During the subsequent period of signal decay, Stat3 is re-exported back to the cytoplasm in preparation for the next round of signaling (Bhattacharya and Schindler, 2003).

On day 5 of pregnancy, phospho-Stat3 immunostaining was localized to the stromal cells and the luminal epithelium surrounding the implanting blastocyst. However, there was only a basal level of phospho-Stat3 immunostaining in the luminal epithelium at the inter-implantation sites on day 5 of pregnancy. Moreover, the pattern of phospho-Stat3 immunostaining in the uterus after activation of delayed implantation was similar to that at implantation sites on day 5 of pregnancy. Additionally, there was only a basal level of phospho-Stat3 immunostaining in the luminal epithelium of mouse uterus at the midnight of day 4 pseudopregnancy although phospho-Stat3 immunostaining was highly detected in the luminal epithelium at the midnight of day 4 pregnancy. Furthermore, it has been shown that nuclear localization of Stat3 in day 4 luminal epithelium was not detected in any LIF-deficient mice (Cheng et al., 2001), suggesting that Stat3 phosphorylation occurs only during the implantation window.

In our study, phospho-Stat3 immunostaining was strongly detected in the decidual cells on days 6–8 of pregnancy. Under artificial decidualization, phospho-Stat3 immunostaining was also strongly observed in the decidua. Stat3 is present and becomes phosphorylated in both the wild-type and estrogen receptor α-deficient uterine tissues in response to oil infusion for inducing decidualization (Hewitt et al., 2002). Moreover, Stat3 was abundantly expressed in the cytosolic fraction of decidual homogenates throughout rat pregnancy. Stat3 in the decidua was most abundant during the active period of stroma cell proliferation and decidualization (days 8–10) (Liu and Ogle, 2002). These data shows that Stat3 is phosphorylated during decidualization.
However, the significance of Stat3 phosphorylation during decidualization remains to be determined. Stat3 can also be activated by IL-11. IL-11 receptor α signaling at the implantation site appears to be required for decidual development. Female mice with a null mutation of the interleukin-11 receptor alpha chain are infertile because of defective decidualization (Bilinski et al., 1998; Robb et al., 1998). Stat3 may play a role during the decidualization through the activation by IL-11.

Our data suggest that Stat3 may play an essential role for embryo implantation and decidualization. LIF is highly expressed in mouse uterus just before implantation and has been shown to be required for embryo implantation in the mouse (Bhatt et al., 1991; Stewart et al., 1992; Yang et al., 1995). Nuclear localization of Stat3 in day 4 luminal epithelium was not detected in any LIF-deficient mice, indicating that LIF is the principal mediator of Stat3 activation in vivo (Cheng et al., 2001). Stat3-deficient embryos showed a rapid degeneration between embryonic days 6.5 and 7.5, although they developed into the egg cylinder stage until embryonic day 6.0, demonstrating that Stat3 is essential for the early development of mouse embryos (Takeda et al., 1997). However, mice carrying a mutation of gp130 which deleted all Stat-binding sites are viable, but infertile in female mice because of implantation failure, phenocopied mice deficient for LIF (Ernst et al., 2001). Nevertheless, embryos homozygous for the gp130 mutation progressively die between 12.5 days postcoitum and term. On 16.5 days postcoitum and later, they show hypoplastic ventricular myocardium without septal and trabecular defect (Yoshida et al., 1996). This also shows that the signal pathway mediated by Stat3 is essential for embryo implantation just like what LIF acts.

In the ovariectomized mice, there was no detectable Stat3 expression and activation. Progesterone had no obvious effects on Stat3 expression and activation. However, Stat3 mRNA expression and phosphorylation was significantly induced in the luminal epithelium by estrogen treatment. The strong level of phospho-Stat3 immunostaining in the luminal epithelium after estrogen treatment of ovariectomized mice was similar to that at the midnight on day 4 of pregnancy, which was consistent with the high level of maternal estrogen (Yoshinaga and Adams, 1966; Psychoyos, 1973). It seems that Stat3 phosphorylation is stimulated by maternal estrogen. However, IL-6-induced activation of Stat3 activity and Stat3-mediated gene expression were suppressed by 17β-estradiol in breast cancer cells. E2-mediated inhibition of Stat3 activation can be reversed by tamoxifen, an estrogen receptor antagonist (Yamamoto et al., 2000).

In conclusion, Stat3 phosphorylation was highly induced at the midnight on day 4 of pregnancy, coinciding with the attachment reaction between the

![Fig. 7. Stat3 mRNA expression (left column), Stat3 immunostaining (middle column), and phospho-Stat3 immunostaining (right column) in the ovariectomized mouse uteri treated with sesame oil (A, E, I), progesterone (B, F, J), 17β-estradiol (C, G, K), and a combination of progesterone and 17β-estradiol (D, H, L), respectively. Bar = 20 μm.](image-url)
blastocyst and luminal epithelium. There was a high level of Stat3 phosphorylation in the decidual cells from days 6 to 8 of pregnancy and under artificial decidualization. In the ovariectomized mice, Stat3 phosphorylation was strongly stimulated in the luminal epithelium by estrogen treatment.

REFERENCES


