Do circulating blood cells contribute to maternal tissue remodeling and embryo–maternal cross-talk around the implantation period?†

Hiroshi Fujiwara

Department of Gynecology and Obstetrics, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto 606-8397, Japan

Abstract: In early pregnancy, human chorionic gonadotrophin (HCG) stimulates the corpus luteum (CL) of pregnancy to produce progesterone, which in turn maintains human embryo implantation in the uterus. In addition to this embryo–maternal cross-talk via the endocrine systems through blood circulation, accumulating evidence suggests that circulating blood cells also play an important role in embryo implantation. Peripheral blood mononuclear cells (PBMC) derived from pregnant women increased the progesterone production by luteal cells and promoted the invasion of embryos in vitro. Recombinant-HCG increased chemokine production by PBMC through lectin–glycan interaction and enhanced the effects of PBMC on embryo invasion. Later, it was shown that not only PBMC, but also circulating platelets were possible sources of these chemokines that promote extravillous trophoblast invasion to reconstruct maternal endometrial artery. Circulating platelets were also proposed to induce neovascularization during CL formation. Furthermore, intrauterine administration of autologous PBMC effectively improved live birth, pregnancy and implantation rates in patients with repeated (four or more) implantation failures during in vitro fertilization therapy. These findings suggest that circulating blood cells positively contribute to maternal tissue remodeling and embryo–maternal cross-talk around the implantation period in cooperation with the endocrine system.

Key words: corpus luteum / cross-talk / embryo implantation / endometrial differentiation / trophoblast invasion

Introduction

Since mammalian developing embryos must be implanted in the maternal uterus for development to continue, mothers must interact with the embryo in utero during this parasitic phase. Maternal tissues also have to undergo extensive reconstruction to maintain embryo implantation during the corpus luteum (CL) and placental formation. The pathophysiological significance of embryo–maternal cross-talk in the genital tract has received attention (Simón et al., 2001; Hamatani et al., 2004), and in addition to this local cross-talk, the importance of systemic embryo–maternal cross-talk by soluble factors, in other words, via the endocrine system through the blood circulation is well accepted (Yen, 1991). However, little attention has been paid to the roles of circulating blood cells in systemic embryo–maternal cross-talk. Accumulating evidence suggests that immune cells play an important positive role in embryo implantation from a very early stage of pregnancy (Wegmann, 1988). Previously, we proposed a hypothesis that after the immune system has recognized the presence of an embryo in the female genital tract, peripherally circulating immune cells act on the reproductive organs to facilitate embryo implantation (Fujiwara, 2006). On the basis of recent findings, I have further extended this concept. In this article, I will introduce a new concept that peripheral blood cells including platelets contribute to maternal tissue remodeling and embryo–maternal cross-talk around the implantation period.

Regulation of systemic embryo–maternal cross-talk by the endocrine system

During the human menstrual cycle, gonadotrophin-releasing hormone produced by hypothalamic neurons stimulates secretion of pituitary FSH and LH hormones, inducing follicular growth in the ovary and estrogen production. This leads to endometrial proliferation in the uterus. After LH surge induces ovulation, the CL is formed from the
ovulated follicle, to produce progesterone under LH control, which induces adequate endometrial differentiation for embryo implantation. When pregnant, human chorionic gonadotrophin (HCG), which shares a receptor with LH, is secreted from the embryo. Through blood circulation, HCG stimulates the maternal CL to produce progesterone, which in turn acts on the endometrium to maintain embryo implantation in the uterus (Yen, 1991). In this manner, there is cross-talk between the embryo and mother through systemic blood circulation.

During the human menstrual cycle, CL function persists for only 14 days. However, when pregnancy occurs, HCG stimulates CL of the menstrual cycle to transform into CL of pregnancy in order to maintain embryo implantation. Accordingly, CL of pregnancy is an essential organ for embryo implantation and it is thought that HCG is the main regulator of CL of pregnancy. However, there are several lines of clinical evidence to suggest other mechanisms. For example, in patients with ectopic pregnancy or natural abortion, despite an elevated HCG level in blood, progesterone production decreases and spontaneous miscarriage proceeds. In addition, it was reported that exogenous HCG cannot maintain progesterone or relaxin production, and CL regression proceeds in non-pregnant women (Quagliarello et al., 1980). Although it has been proposed that a 2-fold increase of HCG concentration is necessary to maintain CL of pregnancy (Yen, 1991), such a high concentration is not required to activate the LH/HCG receptor system. However, there is no explanation of why such a dramatic increase in HCG is necessary to support CL function. On the other hand, in women with missed abortion, progesterone production is maintained despite a low level of HCG, and there is no CL regression or spontaneous miscarriage.

Humans appear to differ from most other mammals. Chorionic gonadotrophin is detected in the horse and primates only (Murphy and Martinuk, 1991), and the regulatory mechanisms for CL of pregnancy are therefore completely different among mammals. These findings naturally lead to a simple question of whether CL of pregnancy, which is essential for human reproduction, is regulated by HCG alone. However, there is no soluble factor other than HCG that has been identified to date (Kratzer and Taylor, 1990), and the precise regulatory mechanisms remain unknown (Johnson et al., 1993).

Possible regulatory mechanisms for embryo–maternal cross-talk by the immune system

To clarify the regulatory mechanism(s) for CL of pregnancy, we raised monoclonal antibodies that reacted with molecules in human CL and found that membrane-bound peptidases and cell adhesion-related molecules were expressed on human luteal cells as cell surface markers (Fujiiwara et al., 1998, 1999). Interestingly, HLA-DR and CD56/LFA-3, which mediate interactions with immune cells, were expressed on the luteal cell surface in human CL of pregnancy (Fujiiwara et al., 1993; Hattori et al., 1995). Later, it was also reported that intercellular adhesion molecule-1 was expressed on human luteal cells and could induce the binding of luteal cells to lymphoid cells (Vigano et al., 1997). In general, immune cells are considered to enhance CL regression (Auleta and Flint, 1988; Pate and Keyes, 2001). However, the expression of these molecules was prepared during CL formation, suggesting their involvement in subsequent transformation from CL of the menstrual cycle to CL of pregnancy.

At the embryo implantation site, the human embryo has already buried in the maternal endometrium within 12 days after ovulation and been surrounded by maternal blood, which contains peripheral blood mononuclear cells (PBMC) (Boyd and Hamilton, 1970). These PBMC can move to CL in the ovary through blood circulation. Therefore, it was speculated that not only HCG, but also immune cells contribute to the cross-talk between mother and embryo via blood circulation. Accordingly, we hypothesized that information regarding the presence of the developing embryo in the genital tract is transmitted to the ovary by not only the endocrine system, but also the immune system, in other words, not only soluble factors, but also circulating cells (Fujiiwara et al., 1993; Hattori et al., 1995).

To examine this hypothesis, we investigated the effects of PBMC derived from women in early pregnancy on progesterone production in luteal cell culture. As a result, PBMC from pregnant women promoted more progesterone production compared with those from non-pregnant women, suggesting that peripheral blood immune cells in early pregnancy stimulate CL function. In the same culture, the production of Th-2-related cytokines, interleukins IL-4 and IL-10, was increased in the co-culture of PBMC and luteal cells derived from pregnant women. Furthermore, IL-4 and IL-10 stimulated progesterone production as much as HCG. These findings supported a novel concept that PBMC contribute to embryo–maternal cross-talk via systemic circulation. In other words, it is suggested that the transmission of information from a superior endocrine organ (trophectoderm/chorionic villi) to an inferior endocrine organ (CL) can be mediated by circulating immune cells. We further expanded this idea to the concept that these immune cells transmit information on the presence of an embryo to various organs throughout the whole body including the uterus and maternal vessels in order to prepare or maintain embryo implantation (Hashii et al., 1998) (Fig. 1).

On the basis of this hypothesis, we examined the effects of circulating immune cells on the uterus during an embryo implantation in early pregnancy using mouse implantation experiments. When blastocysts were transferred into the uterine cavity of a pseudo-pregnant mouse, successful implantation was achieved only within 3–5 days after ovulation when the endometrium had been adequately differentiated. This period is the so-called implantation window (Psychoyos, 1993; Dey, 1996). However, when spleen cells derived from mice on pregnant day 4 (before implantation) were intravenously administered to pseudo-pregnant mouse, embryo implantation was induced in recipient mice on pseudo-pregnant day 1 or 2 when the embryo normally cannot implant (Takabatake et al., 1997a). However, splenocytes derived from pseudo-pregnant day 4 mice did not show significant effects on extending the implantation window, suggesting that the presence of pre-implanted embryos in the genital tract is important to influence splenocyte function in the mice donating spleen cells. Furthermore, direct intraendometrial injection of a T lymphocyte-rich preparation also induced embryo implantation (Takabatake et al., 1997b). In a delayed implantation model, intravenous administration of splenocytes derived from pregnant day 4 mice induced leukemia inhibitory factor (LIF) expression in the uterus and restarted embryo implantation as was reported with estrogen administration (Bhatt et al., 1991). These findings indicate that
immune cells can induce early endometrial differentiation required for subsequent embryo implantation. On the other hand, thymocytes derived from immature non-pregnant female mice significantly promoted embryo implantation rates on pseudo-pregnant day 2. Intravenous administration of thymocytes also induced LIF expression in the uterus in the delayed implantation model mice (Fujita et al., 1998). Furthermore, intraendometrial administration of CD4(+)CD8(-) cells from thymocytes enhanced embryo implantation, demonstrating that even under non-pregnant conditions, certain immune cell populations can induce endometrial differentiation and promote embryo implantation.

Two decades ago, Wegmann proposed the immunotrophic hypothesis. He showed that T cells promote placental growth and prevent spontaneous miscarriage (Wegmann, 1988) and that the Th-2 cytokine IL-10 prevented the induction of abortion in mice (Chaouat et al., 1995), suggesting that successful allopregnancy is a Th-2 phenomenon in the local balance between Th-1 and Th-2 environment. Later, at the embryo implantation site in the uterus, adequate relationships among trophoblasts, decidual cells and immune cells were shown to be regulated by cytokine and growth factor networks, promoting materno-fetal immunotolerance and placentation (Guimond et al., 1998; Saito, 2001; Chaouat et al., 2002). Recently, CD4(+)CD25(+) regulatory T cells were reported to play a very important role in regulating immune tolerance at the implantation site to support implantation and successful pregnancy in mice and humans (Aluvihare et al., 2004; Saito et al., 2007). In addition, uterine NK cells play an important role at the implantation site for placentation and angiogenesis (Hanna et al., 2006; Saito et al., 2008).

On the basis of a different aspect of this concept, I have proposed that circulating immune cells additionally support the endocrine system to mediate embryo–maternal cross-talk through systemic circulation (Fig. 1). At present, I speculate that immune cells that recognize the presence of developing embryos migrate to lymphoid organs and amplify this information. Thereafter, other effector immune cells are mobilized into systemic blood circulation to travel to target organs such as the endometrium, ovary and so on and then induce tissue differentiation and/or functional changes (Fujiwara, 2006) that are considered mainly regulated by endocrine system. Considering the results of mouse implantation experiments, functional changes in immune cells are supposed to have already occurred when developing embryos stay in the Fallopian tube. Consequently, on the basis of this hypothesis, the immune system can induce differentiation of human CL of pregnancy prior to HCG stimulation, an endocrine signal. Although the precise mechanisms remain unknown, dendritic cells in the Fallopian tube are one of the candidates as effector cells that recognize the presence of developing embryos in the maternal genital tract, because it was proposed that residual dendritic cells were activated by the embryo in the uterus and migrated into local lymph nodes, regulating immune cell reaction (Blois et al., 2007). To support this speculation, it was reported that dendritic cell depletion reduced the embryo implantation in mice (Krey et al., 2008). On the other hand, CD4(+)CD8(-) T cells or NK cells are candidates for effector cells that induce tissue differentiation and/or change the organ functions (Fujiwara, 2006).

An implantation window is also supposed to exist in humans. To confirm its presence, we developed an attachment assay using
human choriocarcinoma-derived BeWo cell mass and human endometrial epithelial cell culture. In this assay, high attachment rates were observed in endometrial culture derived from the midluteal phase, suggesting that human endometrial receptivity increases during the implantation period. Importantly, when co-cultured with PBMC, attachment rates were increased, showing that PBMC promote endometrial cell receptivity in vitro (Kosaka et al., 2003).

A considerable population of infertile patients treated with in vitro fertilization (IVF) therapy shows repeated implantation failure, especially when they cannot adequately respond to endocrine stimulation. On the basis of our findings, we have proposed a novel therapy using autologous PBMC. In this therapy, PBMC are isolated from the patients and incubated with HCG in order to activate PBMC as described in the following section. Thereafter, activated PBMC are administered into the uterine cavity to induce adequate endometrial differentiation and then blastocysts are transferred into the uterine cavity. We applied this treatment to patients (n = 35) with four or more repeated failures in IVF therapy and found that PBMC treatment effectively improved the live birth, pregnancy and implantation rates (Yoshioka et al., 2006). Since this therapy is simple and can be safely applied to patients treated with intrauterine insemination, we hope that it will be investigated further.

Cooperative effects of endocrine and immune systems on embryo–maternal cross-talk

After attachment to endometrial epithelial cells, the trophoderm of the human embryo is activated, which increases its invasive property. Then, the human embryo invades the endometrium, becoming buried within endometrial stromal tissue (Boyd and Hamilton, 1970). Previously, we proposed that activated leukocyte adhesion molecule/CD166 is one of the candidates for a key molecule inducing human trophoderm activation (Fujiwara et al., 2003). Although the precise mechanisms involved in the stepwise invasion processes of human embryos are still unknown, it is well known that the initial change around the implantation site is an increase in vascular permeability (Rockwell et al., 2002, 2003), recruiting a specific population of immune cells at this site (McMaster et al., 1993; Bulmer, 1996). Interestingly, circulating mononuclear cells derived from women in early pregnancy were shown to enhance trophoderm invasion of murine embryo (Nakayama et al., 2002). These PBMC were also demonstrated to promote BeWo cell invasion by producing chemoattractive factors (Egawa et al., 2002). Strictly speaking, BeWo cells are derived from choriocarcinoma and differ from the activated trophoderm of invading human embryo just after endometrial attachment. However, these findings obtained using BeWo cells suggest that circulating mononuclear immune cells are involved in the initial step of human embryo invasion at the edematous implantation site. More importantly, when PBMC derived from non-pregnant women were incubated with HCG, HCG-treated PBMC increased the production of chemoattractive factors to promote murine embryo and BeWo cell invasion. These findings suggest that HCG alters PBMC functions to facilitate embryo invasion.

Several decades ago, crude HCG purified from urine was reported to suppress immune reactions (Adcock et al., 1973). However, it was later shown that highly purified HCG had no effect on lymphocyte function (Muchmore and Blaese, 1977; Morse et al., 1982). Therefore, the effects of HCG on immune cells have long been controversial. By screening of mRNA expressions in HCG-treated PBMC, we found that recombinant-HCG enhanced IL-8 production by human monocytes at a high concentration. This reaction was evoked by NFκB activation, but not through protein kinase A and protein kinase C pathways, which are well known to be involved in signal transmission via the LH/HCG receptor. Although labeled-HCG could bind to monocytes, the expression of LH/HCG receptor was not detected on the cell surface of monocytes (Kosaka et al., 2002). Therefore, it was speculated that there is a different pathway other than the LH/HCG receptor system, which could respond to a high HCG concentration.

HCG is an evolutionally new hormone in primates that shares a receptor with LH. The most important difference between LH and HCG is the presence of abundant sugar chains at the C-terminal of HCG b-chain. Actually, HCG-induced IL-8 production was inhibited by exogenous excess of sugars. These findings suggest that HCG affects PBMC function through sugar chain receptors, which is a primitive mechanism in the immune system (Kosaka et al., 2002). It should be noted that sugar chains of purified HCG were mostly cleaved before urinary production. Recent studies demonstrated that human invading trophoblasts at the implantation site produce hyperglycosylated HCG, and the more glycosylated HCG up-regulates trophoblast invasion (Handschi et al., 2007). Trophoblast invasion mimics that of cancer cells (Bischof and Campagna, 2000). In accordance with that thesis, it was reported that more invasive choriocarcinoma produce hyperglycosylated HCG b subunit (Cole, 2007). These findings support a novel concept that locally high concentration of HCG produced at the implantation site by an invading human embryo stimulates recruited or resident immune cells to produce chemoattractants through sugar chain receptor, and then this in turn induces embryo invasion toward endometrial stromal tissues (Fujiwara, 2006) (Fig. 2). We preliminarily observed that r-HCG also promoted vascular endothelial growth factor (VEGF) production by PBMC, which may
contribute to the increase in vascular permeability at the implantation site. Consequently, we proposed that HCG stimulation may cause a remarkable increase in the local production of vascular activating substances by immune cells, which leads to pathological conditions such as ovarian stimulation syndrome (Kosaka et al., 2007).

It has been proposed that progesterone is involved in the regulation of the immune system during embryo implantation, through the progestosterone–immune–cytokine relationship (Lea and Sandra, 2007; Yoshinaga, 2008). Taken together with our data, the cross-talk between the endocrine and immune systems may occur parallel to the direct local cross-talk between mother and embryo in the uterus during early pregnancy (Fig. 3).

**Figure 3** Cross-talk between the endocrine and immune systems. Cross-talk between the endocrine (HCG and progesterone) and immune systems may occur parallel to the direct local cross-talk between mother (endometrium) and embryo in the uterus during early pregnancy. Both systems cooperatively contribute to embryo implantation and maintenance.

**Contribution of circulating platelets to maternal tissue remodeling around the implantation period**

After the human embryo has invaded the endometrium, placental formation progresses rapidly. At the embryo–maternal interface, extravillous trophoblasts proliferate in the cell column of the anchoring villi and then invade the maternal endometrium. Extravillous trophoblast migrates toward the maternal arteries and replaces endothelium and muscle layer, remodeling artery to become dilated. This contributes to an adequate blood supply into the intervillous space in the placenta (Fig. 4). However, if this mechanism is inhibited, the subsequent insufficient blood supply will cause placental dysfunction in the late stage. Thus, remodeling of the maternal arteries by embryo-derived cells is an important mechanism to maintain fetal development. However, since this phenomenon is specific to humans, it cannot be examined by animal experiments. Accordingly, the precise mechanisms that direct extravillous trophoblast invasion to the arteries remain unknown.

Recently, we found that extravillous trophoblasts that had already ceased invasion expressed dipeptidyl peptidase IV/CD26 on the cell surface. This membrane-bound enzyme can degrade several chemokines such as RANTES to inhibit the access of chemokines to their specific receptors and was shown to be involved in the regulation of trophoblast invasion (Sato et al., 2002). Then, we observed that chemokine receptor CCR1, which is a representative receptor for RANTES, was expressed on extravillous trophoblasts that migrated toward maternal arteries. In matrigel invasion assay using primarily isolated human extravillous trophoblast, RANTES significantly enhanced trophoblast invasion. Similar effects were observed in other CCR1 ligands. Accordingly, we initially proposed the concept that extravillous trophoblast invasion is induced by chemokines (Sato et al., 2003), which was gradually supported by subsequent studies (Red-Horse et al., 2005; Hannan et al., 2006). It was well known that there is recruitment from the vascular spaces of immune cells, including large granular cells, T lymphocytes and monocytes, during embryo implantation and placenta in the decidual tissue (Bulmer, 1996).

Therefore, we considered that immune cells that have been activated by the signals from the implantation site move to the endometrium through blood circulation and migrate into the endometrial stroma, influencing the invasion of extravillous trophoblasts in the decidual tissue by producing chemoattractive substances (Egawa et al., 2002). However, in contrast to BeWo cells and murine embryo, the invasion of primarily isolated human extravillous trophoblast was not enhanced by PBMC derived from pregnant women in early pregnancy (Sato et al., unpublished work). This may be because human invading extravillous trophoblasts from anchoring villi are further differentiated trophoblasts and are apparently different from the activated trophoderm of invading embryo in the initial stage of implantation. Later, it was reported that decidual NK cells, but not peripheral blood-derived NK subsets, regulate trophoblast invasion both in vitro and in vivo by production of the interleukin-8 and interferon-inducible protein-10 chemokines (Hanna et al., 2006). These findings suggest that additional induction of immune cell function and/or differentiation in the decidual tissues is necessary to regulate invasion of human extravillous trophoblasts.

Although CCR1 expression on the invading extravillous trophoblasts was reduced under a hypoxic environment, its expression was increased under high oxygen conditions in vitro. In addition, its expression was also suppressed by decidua-derived soluble factors, being compatible with the immunohistochemical observation that invading extravillous trophoblasts from the cell column to spiral arteries through the shell expressed CCR1. However, by immunohistochemical examination, RANTES was detected on decidual lymphocytes, whereas another CCR1 ligand, monocyte chemotactic protein-2, was observed in decidual cells. In addition, macrophage inflammatory protein-1α was observed in extravillous trophoblasts. Thus, we did not obtain any definitive evidence about key cells that induce extravillous trophoblast invasion specifically toward the maternal artery (Sato et al., 2003).

Later, we found that contaminated platelets with isolated PBMC were more potent than PBMC in promoting human extravillous trophoblast invasion. This finding led us to further discovery that CD-41-positive platelets were deposited and activated among extravillous trophoblasts that had invaded the maternal artery. Recently, it was reported that activated platelets secrete chemotactants. In the invasion assay, platelet-derived soluble factors including RANTES increased extravillous trophoblast invasion (Sato et al., 2005). Consequently, we proposed that platelets that are deposited among
Figure 4 The possible roles of platelets in the reconstruction of the maternal spiral artery. In spiral arteries that are undergoing vascular remodeling, platelets and extracellular matrix are deposited among the endovascular extravillous trophoblasts (EVTs). These activated platelets are likely to have released various soluble factors to form a local chemokine gradient around the remodeling spiral arteries, encouraging further arterial EVT infiltration.

Figure 5 The possible roles of platelets in the human corpus luteum (CL) formation. Platelets are mainly deposited at the central areas facing the central cavity. These activated platelets may create a gradient of angiogenic factor concentration from the central cavity to the peripheral stromal layer, inducing endothelial cell migration toward the central cavity. Until fine vascular networks are established, extravascular fluid is speculated to circulate around granulosa cells and recruit progesterone hormone to the blood circulation although it remains unknown how anti-coagulant mechanisms are operating in CL during the tissue remodeling process.
extravillous trophoblasts in the maternal artery form a chemokine gradient and further induce extravillous trophoblast invasion toward the artery (Fig. 4).

On the basis of this concept, we found that circulating platelets also play important roles in the remodeling process during human CL formation. At the ovulation site, the basement membrane has been destroyed and granulosa cells are surrounded by extravasated blood containing red cells (Corner, 1956; Woodruff et al., 1988). White blood cells were proposed to play a role in the regulation of granulosa cell luteinization (Matsuyama et al., 1987; Adashi, 1990; Emi et al., 1991). Blood cells are still abundantly observed among luteinizing granulosa cells 3 or 4 days after ovulation. These findings indicate that during CL formation, there is an extravascular circulation that transports progesterone back to the systemic circulation (Furukawa et al., 2007) (Fig. 5). Although blood cells in the extravascular spaces were histologically recognized more than a half of century ago (Brewer, 1942), the possible existence of extravascular circulation has not been recognized and the precise mechanism by which the coagulation system is controlled remains unknown for a long time. In this period, new vascular development toward the central cavity is observed until the midluteal phase when peripheral anastomosis is achieved and the vascular network has been established. Although granulosa cells are known to produce angiogenic factors such as VEGF and so on (Yan et al., 1993; Sugino et al., 2005), it is also unclear how endothelial migration is induced toward the cavity.

Recently, we observed that platelets were deposited near the central cavity and microvessels were extending there. These platelets were activated and isolated platelets induced much greater endothelial migration than granulosa cells (Furukawa et al., 2007). These findings suggest that platelets deposited on the central area of the CL produce a chemokine gradient, which in turn induces endothelial migration toward the central cavity (Fig. 5). This novel concept will also provide direction for many researchers studying the wound-healing process, including hematologists (Nurden, 2007). Platelets were also shown to enhance luteinization of granulosa cells. Thus, it was proposed that circulating platelets are novel regulators of neovascularization and luteinization in the tissue remodeling process during CL formation (Furukawa et al., 2007).

Conclusion and future perspectives

In conclusion, I described here a novel mechanism for systemic crosstalk via the immune system, which regulates the CL function, endometrial differentiation and embryo invasion (Fig. 1). Accordingly, it has been proposed that not only soluble factors, but also circulating blood cells are important to maintain embryo implantation in early pregnancy. This concept can provide new insights into the regulatory mechanisms involved in functional alteration of the maternal organs during pregnancy. On the basis of this theory, for example, PBMC in pregnant women are further speculated to regulate vascular resistance by producing vasodilating substances (Fujinara, 2006). Cooperative effects of endocrine and immune systems on embryo–maternal cross-talk should also be noted (Fig. 3). Importantly, when the endocrine mechanism does not operate adequately, we can clinically utilize alternative mechanisms for infertility therapy (Yoshioka et al., 2006). In addition, circulating platelets were shown to play a role in maternal tissue remodeling processes such as CL formation and placental formation (Fig. 6). On the basis of these findings, a novel global concept that peripherally circulating blood cells contribute to maternal tissue remodeling and embryo–maternal cross-talk around the implantation period can be proposed here. Since little attention has been paid to the systemic embryo–maternal cross-talk through
blood circulation, this hypothesis will contribute to clarifying various issues in reproductive medicine in the future.

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**References**

Adashi EY. The potential relevance of cytokines to ovarian physiology; the emerging role of resident ovarian cells of the white blood cell series. Endocr Rev 1990;11:454–464.


Hannan NJ, Jones RL, White CA, Salamonsen LA. The chemokines, CX3C1L1, CCL14, and CCL4, promote human trophoblast migration at the feto-maternal interface. Biol Reprod 2006;74:896–904.
