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A Novel Concept of Fundus-Ovary-Salpinx-Para-Aorta Implantation Promoting Unit during Human Embryo Implantation

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Additional information is available at the end of the chapter

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Abstract

Human embryo implantation is mainly regulated by the endocrine system. Since the ovary, fallopian tube, and fundus can directly communicate through the mesosalpinx and ovarian ligament, the local concentration of progesterone in the pathway of the developing embryo is considered to be higher than in systemic blood circulation. The immune system promotes embryo implantation by stimulating progesterone production of the ovary and by inducing endometrial differentiation. The recognition of the developing embryo in the fallopian tube by the immune system is achieved through the para-aortic lymph nodes. On the basis of the above evidence, the autologous immune cells activated in vitro were demonstrated to improve clinical pregnancy rates in patients with repeated implantation failures. In addition, the autonomic nerve system that innervates the fundus, the ovary, and the fallopian tube from the para-aortic region is proposed to regulate the environment of the pathway of the developing embryo. From these findings, we suppose that a unique unilateral functional unit to promote human embryo implantation exists in the pathway of the developing embryo including the para-aortic regions and propose naming this novel functional unit the Fundus-Ovary-Salpinx-Para-aorta Implantation Promoting unit (FOSP-a-IP unit).

Keywords: embryo, FOSP-a-IP unit, implantation

1. Introduction

In humans, the corpus luteum, which is formed from the ovulated follicle, produces progesterone that induces adequate endometrial differentiation for embryo implantation. During pregnancy, the embryo trophoblast cells secrete human chorionic gonadotropin (HCG) that stimulates the maternal corpus luteum to sustain progesterone production. In turn, it acts on the endometrium to maintain embryo implantation in the uterus. Thus, human embryo implantation is mainly regulated by the endocrine system.

In addition to this endocrine system, we have demonstrated that the immune system is involved in the process of promoting embryo implantation by stimulating progesterone production of the ovary and by inducing endometrial differentiation [1]. It is also suggested that recognition of the developing embryo in the fallopian tube by the immune system is achieved through the para-aortic lymph nodes from a very early stage of pregnancy. The intrauterine administration of autologous immune cells that was activated by HCG in vitro was demonstrated to improve embryo implantation rates in patients with repeated failure of in vitro fertilization and embryo transfer treatment [2].

These lines of evidence led us to conceive a novel concept that there is a unique unilateral functional unit to promote human embryo implantation among the fundus, the ovary, the fallopian tube, and the para-aortic regions. In this chapter, we propose naming this novel functional unit as the Fundus-Ovary-Salpinx-Para-aorta Implantation Promoting unit (FOSP-IP unit) and describe its estimated characteristics.

2. The implantation pathway of the developing embryo

In humans, the ovulated oocyte is picked up by the fimbria of the fallopian tube and then the fertilized oocyte is transferred to the uterine cavity through the fallopian tube, causing embryogenesis to proceed toward the blastocyst stage (Figure 1). Recently, it has been widely accepted that a synchronized dialog between the developing embryo and the temporally and coordinately differentiated maternal endometrium is necessary for successful embryo implantation [3]. Accordingly, the adequate preparation of endometrial receptivity as well as the quality of the embryo affects the success of the outcome of in vitro fertilization-embryo transfer (IVF-ET) therapy.

To support the significance of this period, the phenomenon of delayed implantation is well known in rodents and it has also been reported in humans [4]. Furthermore, in cows, this process continues for at least a few days and bovine early embryos become elongated during the pre-implantation period. Consequently, it can be speculated that embryonal signals locally induce further endometrial differentiation and/or an environment suitable for subsequent embryo implantation. We previously proposed that the Eph-ephrin system, which can induce a repulsive force between the epithelial cell layers, contributes to maintaining these crosstalk phases [5]. Several other systems may be involved in the molecular mechanisms of regulation of embryo-maternal crosstalk [4].

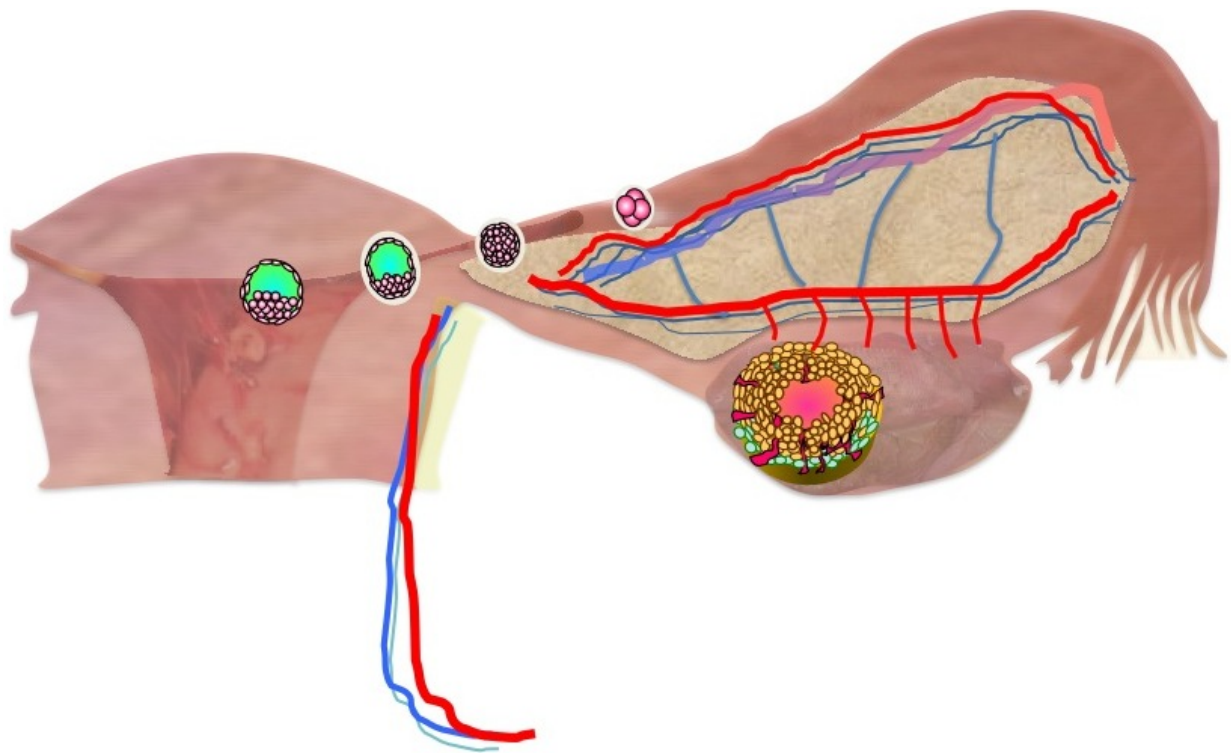


Figure 1. The implantation pathway of the developing embryo In humans, the ovulated oocyte is picked up by the fimbria of the fallopian tube and then the fertilized oocyte is transferred to the uterine cavity through the fallopian tube, causing embryogenesis to proceed toward the blastocyst stage.

In contrast to the majority of mammals with uterus bicornate bicollis, in humans, women have a single fused uterus derived from bilateral paramesonephric (Müllerian) ducts. From this perspective, the uterine fundus is a structure specific to primates among mammals. Shiotani et al. showed that the human uterus possesses a latent fluid-retaining space along the transversely communicating line (TCL) between the bilateral utero-tubal angles on the fundus [6]. To build on and confirm their findings, when we injected a small amount of contrast dye (70 μ l) into the upper portion of the cavity, the dye spontaneously migrated toward the ceiling of the cavity, spread bilaterally to the utero-tubal angles, and formed along cylindrical space (TCL space) that was gently expanded by the dye (Figure 2A). This space communicated directly with the bilateral fallopian tubes (Figure 2B). When we observed the uterine cavity from a sagittal perspective using a surgically resected uterus, the TCL space was macroscopically manifested by innate tissue pressure of the muscle layer (Figures 3A and B). In contrast, macroscopic TCL space was not formed in the uterus with diffusion and firm enlargement by adenomyosis lesions (Figure 3C).

From these findings, we speculate that the main site of crosstalk between the human embryo and maternal tissues before implantation is the upper site of the uterine cavity in the fundus, that is, the TCL space. In support of this theory, Minami et al. reported that gestational sacs of patients in the early stage of spontaneous normal pregnancy were mainly observed on the right or left side of the upper third of the uterine cavity. They also reported that patients with gestational sacs in the upper region had a significantly lower miscarriage rate than those in

the middle and lower regions, proposing that the endometrium at the uterine fundus, especially near the utero-tubal junction, is suitable for human blastocyst implantation under physiological conditions [7].

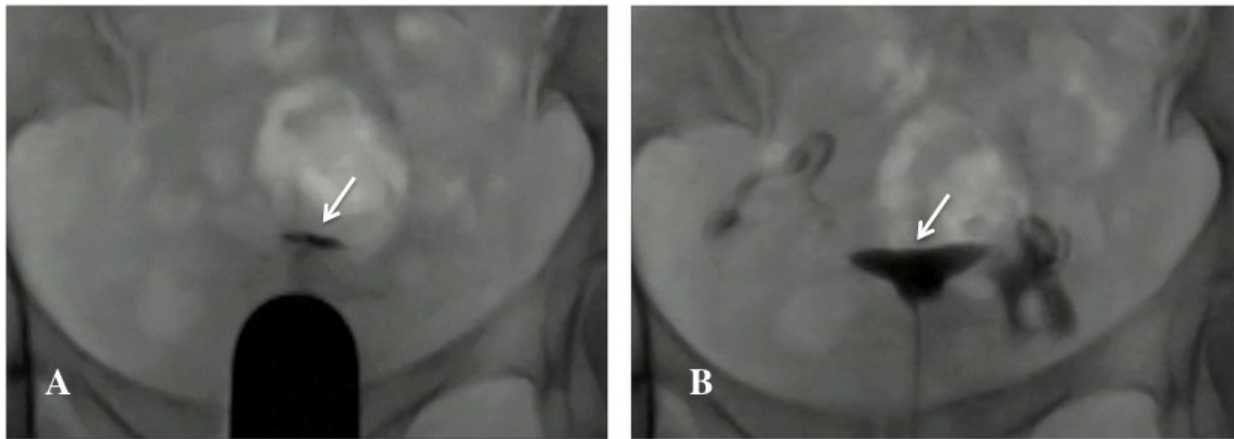


Figure 2. TCL space detected by hysterosalpingography A and B: The human uterus possesses a latent fluid-remaining space along the transversely communicating line (TCL, arrows) between the bilateral utero-tubal angles at the top of the cavity in the fundus. A: When a small amount of contrast dye was injected into the upper portion of the cavity, the dye spontaneously migrated toward the ceiling of the cavity, spread bilaterally to the utero-tubal angles, and formed along cylindrical space (TCL space) that was gently expanded by the dye. B: By subsequent conventional hysterosalpingography, this space communicated directly with the bilateral fallopian tubes.

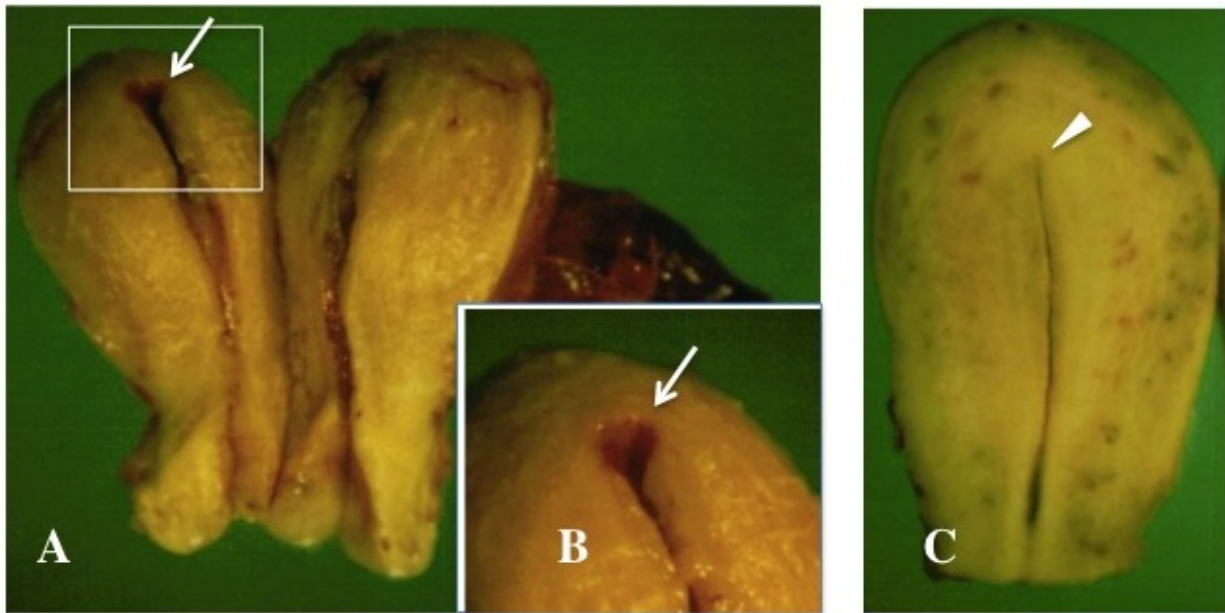


Figure 3. Macroscopic observation of TCL space A: When the uterine cavity was observed from a sagittal perspective using a surgically resected uterus due to carcinoma in situ lesion in the cervix, the TCL space was macroscopically manifested by innate tissue pressure of the muscle layer. B: A magnified figure of the square area of A. C: A uterine specimen that was resected due to adenomyosis. Macroscopic TCL space was not formed in the uterus with diffusion and firm enlargement by adenomyosis lesions.

3. The endocrine network around the implantation pathway

3.1. Local concentration of progesterone in the implantation pathway

Corpus luteum in the ovary is the main endocrine organ that produces progesterone. It has been widely accepted that progesterone prepares the uterus for embryo implantation, induces endometrial differentiation and decreases the contractility of uterine smooth muscle cells. Human endometrial decidualization is also induced by progesterone. The ovary is anatomically connected with the ipsilateral fallopian tube and the corneal region of the uterus. The vascular network is present among the fallopian tube as well as the ovary through the mesosalpinx and ovarian ligament. Therefore, it is speculated that the local concentration of progesterone is very high in the ipsilateral fallopian tube adjacent to the ovary that has an ovulated follicle.

Clinically, vaginal administration of progesterone is usually performed for luteal support for infertile patients receiving in vitro fertilization therapy. After vaginal administration, the uterine tissue concentration of progesterone has been found to exceed more than 10-fold the levels achieved by systemic administration. To explain this phenomenon, the "first uterine pass effect," that is, direct preferential vagina-to-uterus transport, was proposed [8]. By drug delivery analysis using tritiated progesterone, Bulletti et al. obtained experimental data to support this hypothesis [9]. Consequently, similar to the direct preferential vagina-to-uterus transport system, estrogen and progesterone produced by the corpus luteum in the ovary can be delivered to the corneal region of the uterus by a direct ovary-to-uterus transport system.

Using slice computed tomography (CT) scanning and vascular casting, it was demonstrated that both the intramuscular uterine artery and the ovarian artery had a typical ovarian branch connected as an arterial arch, that is, the ovarian artery-to-uterine artery anastomoses [10]. Importantly, these ovarian artery-to-uterine artery anastomoses are located in the mesosalpinx region. In mammals, the anatomical structure among the utero-ovarian vein and the ovarian artery is considered to be important to regulate the counter-current system of exchange from the uterus to the ovary and back again. In humans, the utero-ovarian vein forms a plexus around the ovarian artery. Therefore, it has been suggested that counter-current transfer facilitates local communication between the ovary, the fallopian tube, and the uterus [11]. Later, it was also reported that serum levels of estradiol and progesterone in the uterine vessels were more than 2–4 times higher than those in the systemic circulation, demonstrating the preferential transport of sex steroids produced in the ovary to the uterus [12]. Interestingly, the same group also suggested that the main blood supply to the uterine corneal region from uterine and ovarian arteries is shifted following ovulation [13]. This suggests the possibility that progesterone regulates counter-current blood flows in the mesosalpinx. Consequently, the local concentration of progesterone along the implantation pathway of the human embryo is speculated to be considerably high during its developmental process (Figure 4).

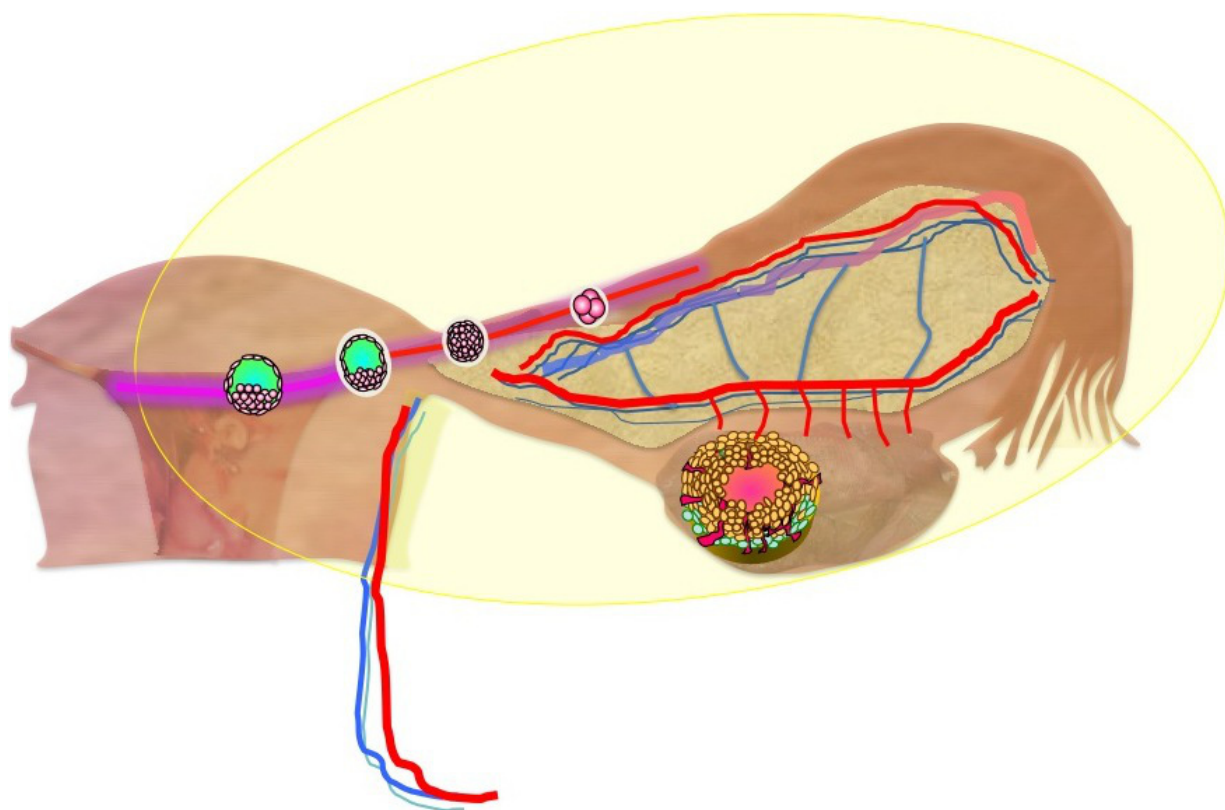


Figure 4. The local concentration of progesterone along the implantation pathway The local concentration of progesterone along the implantation pathway of the human embryo is speculated to be considerably high during its developmental process.

3.2. Hormonal regulation of the contractility around the implantation pathway by ovarian steroid hormones

Strict regulation of contractility in the uterus and the fallopian tube is essential for various reproductive functions including expulsion of menstrual debris, sperm transport, and adequate embryo placement during implantation [14]. More than half a century ago, the precise profiles of contractile activity of the non-pregnant uterus throughout the menstrual cycle were reported using an intrauterine pressure recording system [15, 16]. Recently, it has become possible for uterine contractility to be directly and non-invasively assessed using ultrasound scans and ultrafast magnetic resonance imaging techniques [17]. Accordingly, the inherent contractility of the uterus is classified into two patterns: a focal and sporadic bulging of the myometrium and a rhythmic and subtle stripping movement in the subendometrial myometrium, known as uterine peristalsis. Using these direct and non-invasive techniques, the precise profiles of several wavelike activity patterns throughout the menstrual cycle have been thoroughly analyzed, and it has been widely accepted that ovarian steroid hormones regulate contractions of the non-pregnant uterus. Clinically, uterine contractility has been demonstrated to influence the human embryo implantation process in both spontaneous cycles and assisted reproduction [18, 19].

Waves from fundus to cervix are dominant in the follicular phase, but diminish after ovulation. In contrast, waves from cervix to fundus were observed in the late follicular and luteal phases [20, 21], supporting implantation of the embryo at the upper region of the uterine cavity [22]. During the luteal phase, the movement of the upper fundal region is relatively quiescent facilitating embryo implantation [23]. By the sequential administration of estradiol (days 1–28) and progesterone (days 14–28), waves from fundus to cervix were induced by estradiol, but were immediately diminished after the administration of progesterone, whereas waves from cervix to fundus were observed in both the estradiol-only and the estradiol and progesterone combined phases [18]. It was also reported that there is horizontal movement at the fundus, often to and fro, not unidirectional [24]. Importantly, this horizontal movement can theoretically induce the migration of pre-implanted embryo back to the fallopian tubes along the TCL through the fluid by endometrial secretion (Figure 5).

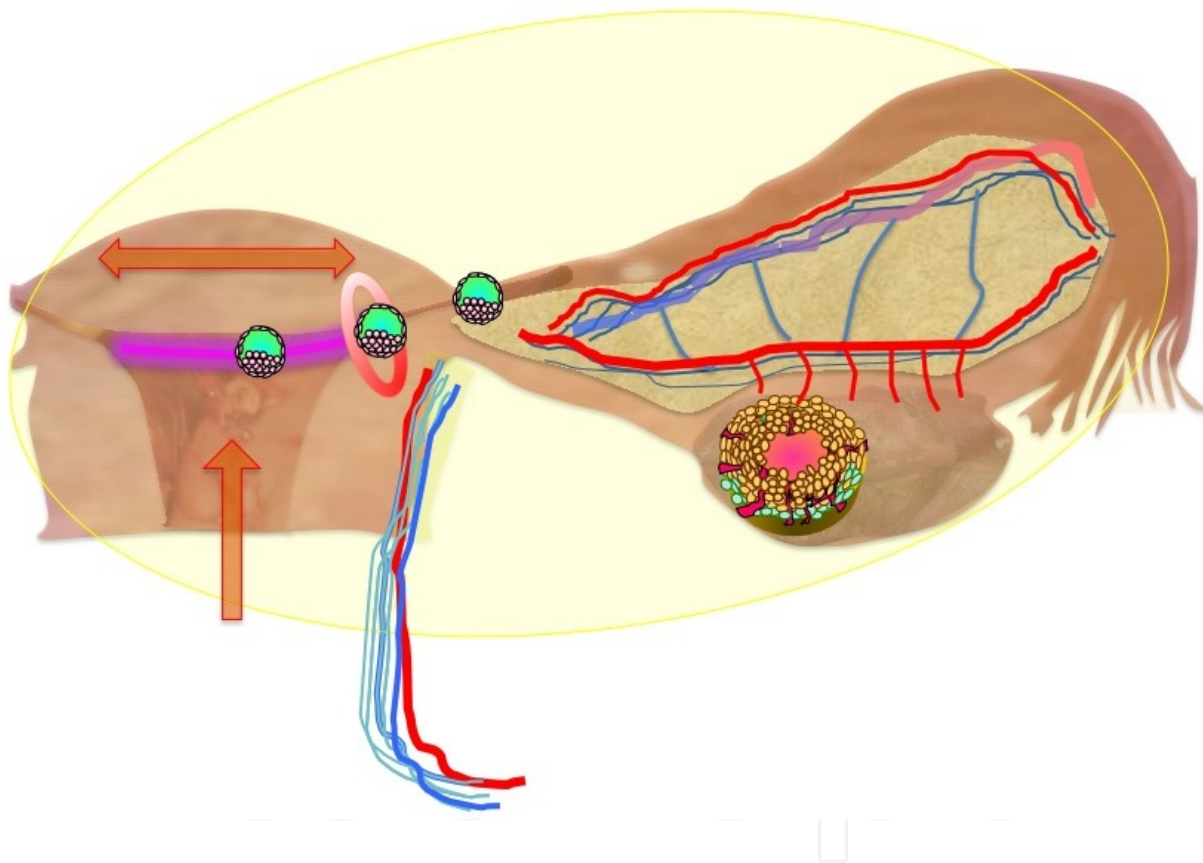


Figure 5. Hormonal regulation of the contractility around the implantation pathway by ovarian steroid hormones. Ovarian steroid hormones regulate contractions of the uterus. Waves from cervix to fundus were observed in the late follicular and luteal phases. There is also horizontal movement at the fundus, often to and fro, not unidirectional, which can theoretically induce the migration of pre-implantation embryo back to the fallopian tubes along the TCL.

Recently, it has been reported that a new population of c-kit-positive cells, interstitial Cajal-like cells, now called telocytes, had been found on the borders of smooth muscle bundles in human myometrium. These cells resemble interstitial cells of Cajal in the gut, which generate electrical slow waves to control peristalsis [14]. Telocytes in the myometrium are double-positive for c-kit and CD34, and have very long cellular extensions called telopodes that release

extracellular vesicles, sending macromolecular signals to neighboring cells. It was proposed that they modulate spontaneous contractions of the non-pregnant human uterus, through a tyrosine-kinase-independent signaling pathway [25, 26]. Although the precise effects of ovarian steroid hormones on telocyte functions remain unclear, immunoreactive estrogen and progesterone receptors localized at the nucleus level of uterine telocytes suggested their involvement in the hormonal regulation of uterine contractility [27].

3.3. Hormonal regulation of immune environment around the implantation pathway by ovarian steroid hormones

During the implantation process, the semi-allogeneic embryo is not rejected by the maternal immune system. The mechanisms regarding how the fetus is tolerated by the maternal immune system are still not well understood. It is generally accepted that ovarian sex steroids regulate the function and population of endometrial and/or decidual immune cells in the uterus [28] and these immune–endocrine interactions contribute to fetal survival within the maternal uterus, suppressing adverse maternal immune responses and promoting immunotolerance pathways [29].

Progesterone regulates immune function by producing mediators such as the progesterone-induced blocking factor that induces Th2-dominant cytokine production [30] and glycodelin A that protects the embryo from maternal immune attack by inhibiting the proliferation of T cells and B cells and the activity of natural killer cells, or by deleting the monocytes [31]. The physiological effects of progesterone are mediated by its specific nuclear progesterone receptor (PR) that activates transcription factors. Nuclear PR has two main isoforms: PR-A and PR-B. PR-B acts as an activator of progesterone-responsive genes, while PR-A can inhibit the activity of PR-B. Using nuclear PR knockout mice, it has been shown that progesterone antagonizes estrogen-induced recruitment of macrophages and neutrophils into the uterus [32]. Recently, it has been demonstrated that progesterone at a relatively high concentration also acts on cells by a non-genomic mechanism through progesterone binding membrane proteins such as progesterone membrane components 1 and 2, and the membrane progestin receptors [33, 34]. Considering a local high concentration of progesterone, these non-genomic mechanisms may operate in the implantation pathway.

CD56(high+)CD16(–) uterine natural killer cells are the predominant population in the decidual tissues during the late secretory phase of the menstrual cycle and early pregnancy. They may be derived from natural killer cell progenitors and/or peripheral natural killer cells and are considered to contribute to the remodeling of maternal uterine vasculature in interaction with extravillous trophoblasts [29, 35]. Although the level of the expression of PR on uterine natural killer cells is very low [36], the progesterone-induced endometrial environment is an important factor for the *in situ* proliferation or differentiation of uterine natural killer cells in human endometrium, inducing reprogramming of their chemokine receptor profiles [37, 38]. Progesterone is also reported to reduce the antigen-presenting capacity of dendritic cells, monocytes, and macrophages and induce the recruitment of regulatory T (Treg) cells, contributing to local accumulation of pregnancy-protective cells [29]. These lines of evidence

suggest the relationship between the endocrine and immune systems for establishing the embryo implantation environment.

4. The autonomic nerve network around the implantation pathway

In pigs, it was demonstrated that the oviduct is innervated by various efferent autonomic neurons such as the inferior mesenteric ganglion, ovarian ganglion, and celiac-superior mesenteric ganglion complex, forming discrete “oviductal centers” and implying that these nerve fibers regulate oviductal blood flow, non-vascular smooth muscle contraction, transmission of sensory information, and epithelial secretion [39, 40]. In monkeys, noradrenaline of the sympathetic nerves innervating the smooth musculature of the oviduct was demonstrated to change cyclically during the menstrual cycle, suggesting that the system of adrenergic nerves in the primate oviduct is under the control of endogenous estrogen and progesterone [41]. It was also reported that estrogen and progesterone affect not only the noradrenaline content of adrenergic nerves in the uterus and oviduct but also the turnover of noradrenaline, the activity of the enzymes that synthesize it, and the release of noradrenaline from nerve terminals [42]. By electron microscopic examination, non-vascular adrenergic nerves were found in smooth muscle bundles of human fallopian tube and electrical field stimulation of adrenergic nerves in the isthmic smooth muscle induced an alpha-receptor-mediated contractile response [43]. In rats, the sympathetic nerve fibers of the upper part of the uterus arise from the ovarian plexus nerve that mainly originates from neurons of the suprarenal ganglia and of the T10 to L3 ganglia of the paravertebral sympathetic chain, whereas most of the sympathetic innervation of the lower uterus arises from neurons of the paravertebral ganglia T13 to S2, principally at the L2–L4 levels, suggesting that regulation of myometrial activity by the sympathetic nerve system is functionally different between the oviduct and the cervical ends of the uterus [44].

Accordingly, the influence of the sympathetic nerve response on the female genital tract should be considered clinically. In fact, it was demonstrated that mock embryo transfer stimulation (injection of 20 µl of ultrasound contrast agent alone) evoked uterine peristalsis that could cause embryo migration and extrude the transferred embryo with fluid [45]. The density of nerve fibers in the oviduct isthmus in women with hydrosalpinx was revealed to be low compared with that in women without hydrosalpinx, suggesting the involvement of autonomic nerve system in the mechanism of hydrosalpinx-associated infertility [46]. A recent study showed that transcutaneous electrical acupoint stimulation significantly improved the clinical outcome of embryo transfer [47].

5. The immune network around the implantation pathway

5.1. Circulating immune cell contribution to embryo implantation

Mammals are a unique group of species in terms of accepting embryos within the maternal uterus (embryonal parasitic strategy). In this respect, maternal recognition of the developing

embryo in the genital tract is a very important process to prepare a favorable maternal environment for subsequent embryo implantation. In humans, HCG secreted by the implanting embryo stimulates the corpus luteum of pregnancy to produce progesterone, maintaining embryo implantation in the uterus. Previously, we found that the immune system also contributes to this process and proposed that “corpus luteum function is maintained not only by HCG (endocrine system), but also by lymphocytes (immune system)” [48]. In mouse, implantation experiments, intravenous or intrauterine administration of splenocytes derived from early pregnant mice induced endometrial differentiation and successful implantation in pseudopregnant recipient mice [49, 50]. On the basis of these results, we proposed a new concept that “The immune system recognizes some information on the presence of the developing embryo around the implantation period and, thereafter, circulating immune cells transmit this information to the ovary and the uterus through blood circulation to induce adequate differentiation of pregnancy CL and endometrium to facilitate embryo implantation.” Furthermore, we found that peripheral blood mononuclear cells (PBMC) promoted endometrial receptivity *in vitro*, while HCG affected PBMC function not through authentic HCG receptor, but sugar chain receptors, which is a primitive mechanism in the immune system [51, 52]. These experimental facts led us to pay attention to sugar chain moieties as candidate key structures of embryonal signals to the maternal immune system. These findings also suggest the important roles of circulating immune cells in embryo implantation from a very early stage [53].

5.2. Direct and functional communication between para-aortic lymph nodes and the implantation pathway of the developing embryo

What is the main immune organ for the first recognition of the developing embryo in the implantation pathway? From insight obtained from gynecologic oncology, para-aortic lymph nodes are classified as regional lymph nodes in patients with uterine corpus cancer. When we used a fluorescent indocyanine green to confirm the sentinel lymph nodes from the fundus lesion, rapid drainage into para-aortic lymph nodes, especially around the proximal site of the branch of the inferior mesenteric artery, from the uterine fundus through the suspensory ligament of the ovary and the meso-oviductal space, was initially detected using a PDA camera system (Figure 6). Theoretically, this supports the presence of direct communication between para-aortic lymph nodes and the implantation pathway of the developing embryo through the immune system.

Recently, Treg cells have been shown to facilitate maternal immune tolerance of the semiallogeneic conceptus and proposed to play a crucial role in embryo implantation and fetal development. During the pre-implantation period, factors in the seminal fluid delivered at coitus cause expansion of a CD4(+)CD25(+) putative Treg cell population in the para-aortic lymph nodes [54]. They were also reported to be rapidly recruited to para-aortic lymph nodes and activated in the first days after embryo implantation [55]. In mouse, implantation experiments, splenocytes derived from early pregnant mice (post-ovulation day 4) when the embryo had not yet attached to the endometrium could induce endometrial differentiation and successful implantation in the early stage of pseudopregnant recipient mice that had been

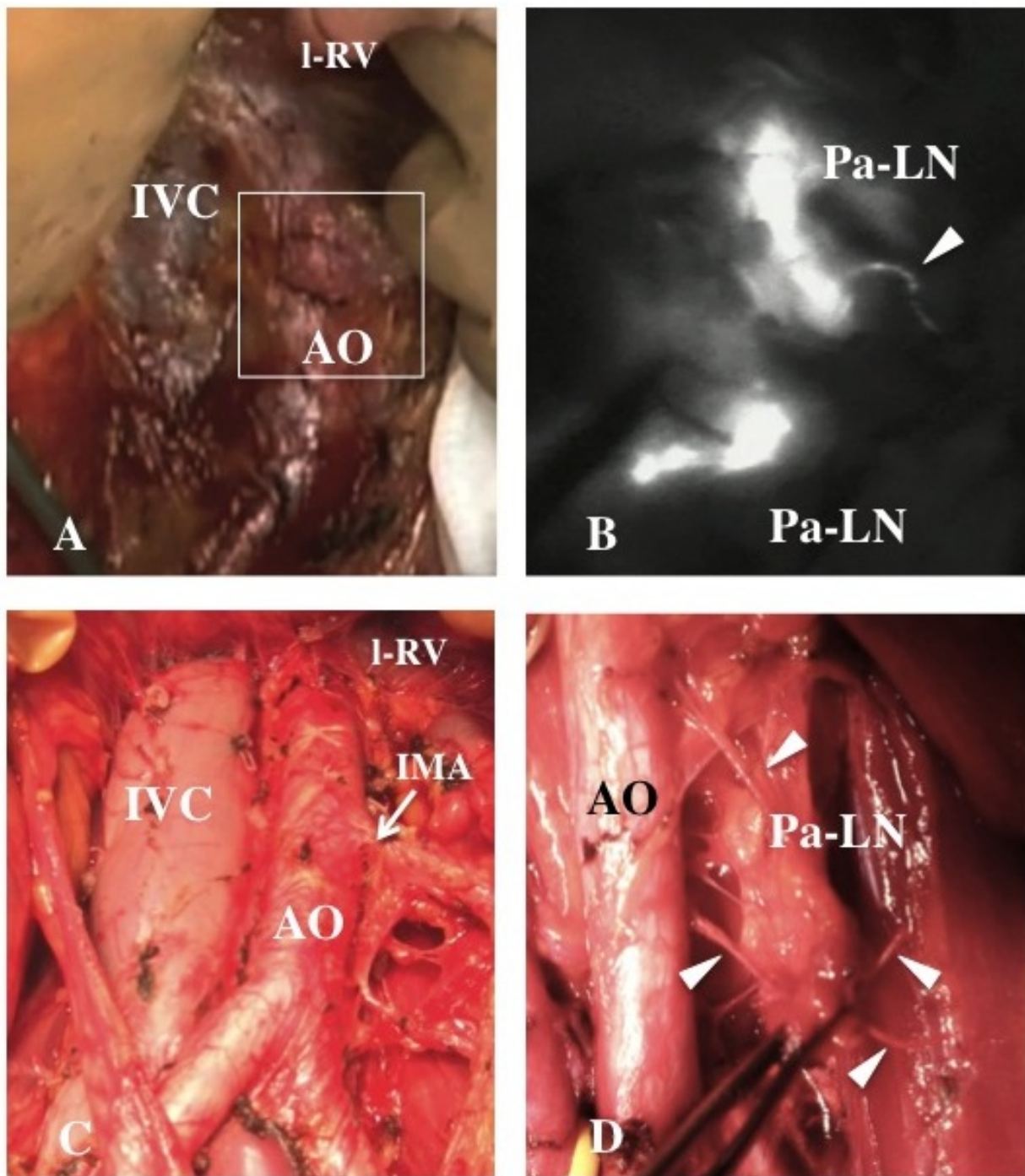


Figure 6. Direct communication between para-aortic lymph nodes and the embryo through the immune system. When fluorescent indocyanine green was injected into the uterine fundus that was affected by endometrial cancer cells in order to confirm the sentinel lymph nodes from the fundus lesion, rapid drainage into para-aortic lymph nodes, especially around the proximal site of the branch of the inferior mesenteric artery was detected by the PDA camera system. A: The retroperitoneal para-aortic region was opened. B: A magnified figure of the square area of A. Indocyanine green-positive para-aortic lymph nodes and an afferent lymph vessel (arrowhead) were clearly detected. C: A figure from after lymph node dissection. D: Para-aortic lymph nodes communicated by vessels and nerves (arrows). Pa-LN, para-aortic lymph nodes; Ao, aorta; IVC, inferior vena cava; IMA, inferior mesenteric artery; I-RV, left renal vein.

mated with vasectomized male mice, indicating that functional change of peripheral immune cells has already occurred before embryo implantation [49, 50]. Importantly, since the immune system of pseudopregnant recipient mice mated with vasectomized male mice was already sensitized with seminal plasma component of seminal fluid, the changes in splenocyte function were induced by the presence of developing embryos [56]. In addition, it was reported that functional changes in the endometrium could be induced in pregnant mice even when the uterotubal transition sites were ligated and entry of the developing embryos into the uterine cavity was inhibited [57], indicating that the developing embryo in the fallopian tube can influence maternal endometrial differentiation. Collectively, it is speculated that mothers can recognize the developing embryo during this early phase through the para-aortic lymph nodes.

The human para-aortic lymph nodes are rich in not only vascular but also automatic nerve networks (Figure 6D). Importantly, all primary and secondary immune organs receive substantial sympathetic innervation from sympathetic post-ganglionic neurons. This sympathetic nervous system either enhances or inhibits the activity of both acquired and adaptive immune systems [58]. Adrenergic nerve fibers were found following both afferent and efferent blood vessels as well as T areas, supporting a regulatory role of the sympathetic nervous system in human lymph nodes [59]. Intriguingly, amputation of autonomic nerves innervating the uterus was reported to cause on-time implantation failure in rats, increasing the population of uterine mast cells and facilitating the release of histamine by mast cells [60]. These findings support the concept that the neuro-immune network plays an important role in embryo implantation.

6. Fundus-Ovary-Salpinx-Para-aorta Implantation Promoting Unit (FOSP a-IP unit)

On the basis of the above evidence, we suppose that there is a unique unilateral functional unit to promote human embryo implantation among the fundus, the ovary, the fallopian tube, and the para-aortic regions (Figure 7). From an anatomical viewpoint, we here propose naming this novel functional unit as the Fundus-Ovary-Salpinx-Para-aorta Implantation Promoting Unit, that is, the FOSP a-IP unit. This functional unit seems to be co-operatively regulated by the endocrine, immune, and nerve systems.

Recently, increasing attention has been paid to patients with repeated implantation failures under IVF-ET treatment. It should be noted that the process of maternal recognition by the immune system in the FOSP a-IP unit is largely skipped in the treatment of IVF-ET. Considering the intrinsic function of the FOSP a-IP unit, we developed a novel therapy for patients with repeated implantation failures to complement the functions of the unit. Concretely speaking, PBMC are isolated from patients and incubated for two days with HCG in order to activate them. Thereafter, activated PBMC are administered into the uterine cavity to induce adequate endometrial differentiation. Three days later, blastocysts are transferred into the uterine cavity.

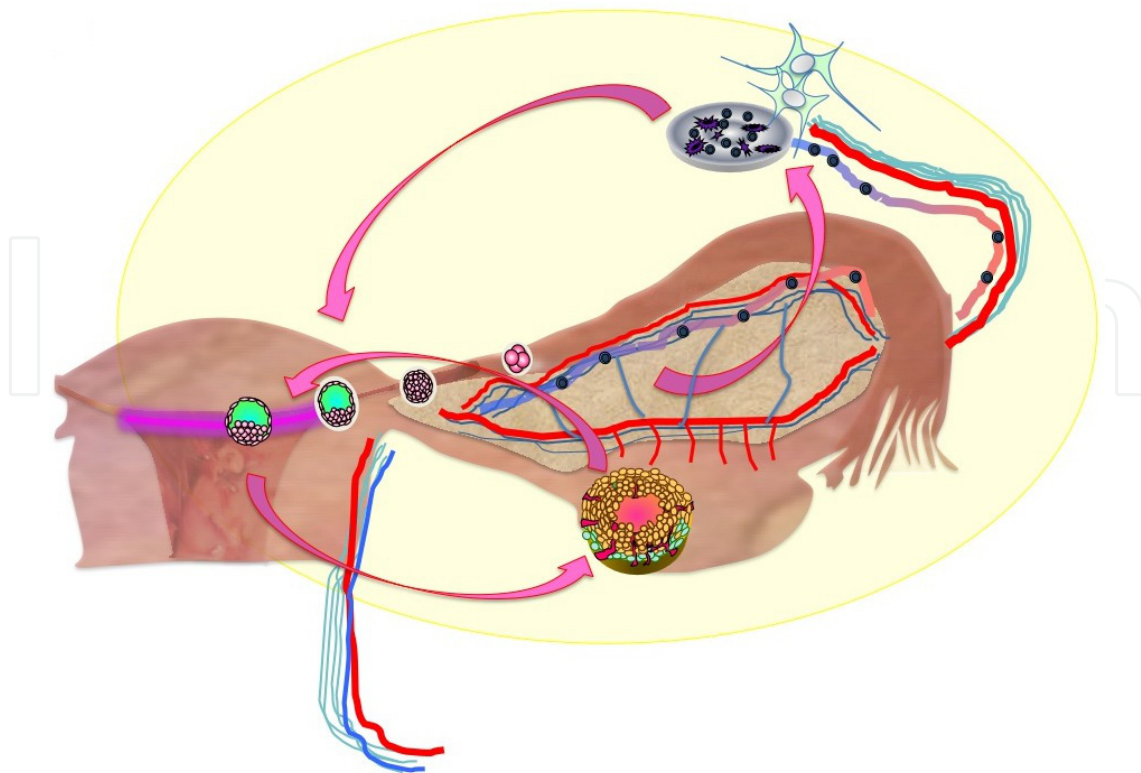


Figure 7. Proposal of the FOSP a-IP unit. We propose a unique unilateral functional unit that promotes human embryo implantation among the fundus, the ovary, the fallopian tube, and the para-aortic regions. This functional unit seems to be co-operatively regulated by the endocrine, immune, and nerve systems and can be named the Fundus-Ovary-Salpinx-Paraaorta Implantation Promoting Unit, that is, FOSP a-IP unit.

We applied this treatment to patients with 4 or more repeated failures in IVF therapy and it effectively improved the clinical pregnancy and implantation rates [2, 61].

7. Conclusion

In humans, the TCL space at the top of the uterine cavity in the fundus may be the main site of crosstalk between embryo and mother before implantation. Including this space, we propose the presence of a unique unilateral functional unit, named the FOSP a-IP unit, among the fundus, the ovary, the fallopian tube, and the para-aortic regions, which promotes human embryo implantation. The local concentration of progesterone along the implantation pathway of the human embryo is considered high, regulating uterine contractility and influencing human embryo implantation. On the other hand, the immune-endocrine interactions along the implantation pathway of the embryo generate an environment that promotes embryo implantation, contributing to fetal survival within the maternal uterus, suppressing adverse maternal immune responses and promoting immunotolerance pathways. In addition, circulating immune cells were shown to contribute to embryo implantation in a very early stage, probably after being activated in para-aortic lymph nodes. Furthermore, the influence of the sympathetic nerve response on the female genital

tract has been clinically noticed, based on the concept that the neuro-immune network plays an important role in embryo implantation. Considering the intrinsic function of the FOSPα-IP unit, we developed a novel therapy for patients with repeated implantation failures to complement functions of this unit. Further understanding of reproductive organs from the viewpoint of the FOSPα-IP unit is expected to contribute to the development of new therapies, especially in the field of reproductive medicine.

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References

- [1] Fujiwara H: Do circulating blood cells contribute to maternal tissue remodeling and embryo-maternal cross-talk around the implantation period? *Mol Hum Reprod.* 2009;15:335-43. doi: 10.1093/molehr/gap027.

- [2] Yoshioka S, Fujiwara H, Nakayama T, Kosaka K, Mori T, Fujii S: Intrauterine administration of autologous peripheral blood mononuclear cells promotes implantation rates in patients with repeated failure of IVF-embryo transfer. *Hum Reprod.* 2006;21:3290-4.
- [3] Cakmak H, Taylor HS: Implantation failure: molecular mechanisms and clinical treatment. *Hum Reprod Update.* 2011;17:242-53. doi: 10.1093/humupd/dmq037.
- [4] Grinsted J, Avery B: A sporadic case of delayed implantation after in-vitro fertilization in the human? *Hum Reprod.* 1996;11:651-4.
- [5] Fujii H, Tatsumi K, Kosaka K, Yoshioka S, Fujiwara H, Fujii S: Eph-ephrin A system regulates murine blastocyst attachment and spreading. *Dev Dyn.* 2006;235:3250-8.
- [6] Shiotani M, Taniwa H, Hattori N, Nakamura K, Komatsu T, Hosino T, Shimada H, Ikeuchi M, Ihara Y: A specific uterine lumen for human embryo implantation deduced from its configuration and implantation sites. *Nihon Sanka Fujinka Gakkai Zasshi.* 1999;51:S331. Japanese, Abstract.
- [7] Minami S, Ishihara K, Araki T: Determination of blastocyst implantation site in spontaneous pregnancies using three-dimensional transvaginal ultrasound. *J Nippon Med Sch.* 2003;70:250-4.
- [8] De Ziegler D, Bulletti C, De Monstier B, Jääskeläinen AS: The first uterine pass effect. *Ann N Y Acad Sci.* 1997;828:291-9.
- [9] Bulletti C, de Ziegler D, Flamigni C, Giacomucci E, Polli V, Bolelli G, Franceschetti F: Targeted drug delivery in gynaecology: the first uterine pass effect. *Hum Reprod.* 1997;12:1073-9.
- [10] Ouyang Z, Liu P, Yu Y, Chen C, Song X, Liang B, Zhong G, Liu C, Li Z: Role of ovarian artery-to-uterine artery anastomoses in uterine artery embolization: initial anatomic and radiologic studies. *Surg Radiol Anat.* 2012; 34:737-41.
- [11] Einer-Jensen N, Hunter RH: Physiological and pharmacological aspects of local transfer of substances in the ovarian adnexa in women. *Hum Reprod Update.* 2000;6:132-8.
- [12] Cicinelli E, Einer-Jensen N, Cignarelli M, Mangiacotti L, Luisi D, Schonauer S: Preferential transfer of endogenous ovarian steroid hormones to the uterus during both the follicular and luteal phases. *Hum Reprod.* 2004;19:2001-4.
- [13] Cicinelli E, Einer-Jensen N, Barba B, Luisi D, Alfonso R, Tartagni M: Blood to the cornual area of the uterus is mainly supplied from the ovarian artery in the follicular phase and from the uterine artery in the luteal phase. *Hum Reprod.* 2004;19:1003-8.
- [14] Hutchings G, Williams O, Cretoiu D, Ciontea SM: Myometrial interstitial cells and the coordination of myometrial contractility. *J Cell Mol Med.* 2009;13:4268-82. doi: 10.1111/j.1582-4934.2009.00894.x.

- [15] Hendricks CH: A new technique for the study of motility in the nonpregnant human uterus. *J Obstet Gynaecol Br Commonw.* 1964;71:712-5.
- [16] Hendricks CH: Inherent motility patterns and response characteristics of the non-pregnant uterus. *Am J Obstet Gynecol.* 1966;96:824-43.
- [17] Togashi K: Uterine contractility evaluated on cine magnetic resonance imaging. *Ann N Y Acad Sci.* 2007;1101:62-71.
- [18] Van Gestel I, IJland MM, Hoogland HJ, Evers JL: Endometrial wave-like activity in the non-pregnant uterus. *Hum Reprod Update.* 2003;9:131-8.
- [19] Fanchin R, Ayoubi JM: Uterine dynamics: impact on the human reproduction process. *Reprod Biomed Online.* 2009;18 Suppl 2:57-62.
- [20] Lyons EA, Taylor PJ, Zheng XH, Ballard G, Clifford CS, Kredentser JV: Characterization of subendometrial myometrial contractions throughout the menstrual cycle in normal fertile women. *Fertil Steril.* 1991;55:771-4.
- [21] Kunz G, Beil D, Deininger H, Wildt L, Leyendecker G: The dynamics of rapid sperm transport through the female genital tract: evidence from vaginal sonography of uterine peristalsis and hysterosalpingography. *Hum Reprod.* 1996;11:627-32.
- [22] IJland MM, Evers JLH, Dunselman GAJ, Van Katwijk C, Lo CR, Hoogland HJ: Endometrial wavelike movements during the menstrual cycle. *Fertil Steril.* 1996;65:746-9.
- [23] Kunz G, Kissler S, Wildt L, Leyendecker G: In: Filicori M. editors, *Endocrine Basis of Reproductive Function.* Monduzzi. 2000.
- [24] Fukuda M, Fukuda K: Uterine endometrial cavity movement and cervical mucus. *Hum Reprod.* 1994;9:1013-6.
- [25] Cretoiu SM, Simionescu AA, Caravia L, Curici A, Cretoiu D, Popescu LM: Complex effects of imatinib on spontaneous and oxytocin-induced contractions in human non-pregnant myometrium. *Acta Physiol Hung.* 2011;98:329-38. doi: 10.1556/APhysiol.98.2011.3.10.
- [26] Cretoiu SM, Cretoiu D, Marin A, Radu BM, Popescu LM: Telocytes: ultrastructural, immunohistochemical and electrophysiological characteristics in human myometrium. *Reproduction.* 2013;145:357-70. doi: 10.1530/REP-12-0369.
- [27] Cretoiu D, Ciontea SM, Popescu LM, Ceafalan L, Ardeleanu C: Interstitial Cajal-like cells (ICLC) as steroid hormone sensors in human myometrium: immunocytochemical approach. *J Cell Mol Med.* 2006;10:789-95.
- [28] Lea RG, Sandra O: Immunoendocrine aspects of endometrial function and implantation. *Reproduction.* 2007;134:389-404.
- [29] Schumacher A, Costa SD, Zenclussen AC: Endocrine factors modulating immune responses in pregnancy. *Front Immunol.* 2014;5:196. doi: 10.3389/fimmu.2014.00196.

- [30] Szekeres-Bartho J, Wilczynski JR, Basta P, Kalinka J: Role of progesterone and progestin therapy in threatened abortion and preterm labour. *Front Biosci.* 2008;13:1981-90.
- [31] Alok A, Mukhopadhyay D, Karande AA: Glycodelin A, an immunomodulatory protein in the endometrium, inhibits proliferation and induces apoptosis in monocytic cells. *Int J Biochem Cell Biol.* 2009;41:1138-47. doi: 10.1016/j.biocel.2008.10.009.
- [32] Tibbetts TA, Conneely OM, O'Malley BW: Progesterone via its receptor antagonizes the pro-inflammatory activity of estrogen in the mouse uterus. *Biol Reprod.* 1999;60:1158-65.
- [33] Gellersen B, Fernandes MS, Brosens JJ: Non-genomic progesterone actions in female reproduction. *Hum Reprod Update.* 2009;15:119-38. doi: 10.1093/humupd/dmn044.
- [34] Kowalik MK, Rekawiecki R, Kotwica J: The putative roles of nuclear and membrane-bound progesterone receptors in the female reproductive tract. *Reprod Biol.* 2013;13:279-89. doi: 10.1016/j.repbio.2013.09.001.
- [35] Sharma S: Natural killer cells and regulatory T cells in early pregnancy loss. *Int J Dev Biol.* 2014;58:219-29.
- [36] Henderson TA, Saunders PT, Moffett-King A, Groome NP, Critchley HO: Steroid receptor expression in uterine natural killer cells. *J Clin Endocrinol Metab.* 2003;88:440-9.
- [37] Inoue T, Kanzaki H, Imai K, Narukawa S, Katsuragawa H, Watanabe H, Hirano T, Mori T: Progesterone stimulates the induction of human endometrial CD56+ lymphocytes in an in vitro culture system. *J Clin Endocrinol Metab.* 1996;81:1502-7.
- [38] Carlino C, Stabile H, Morrone S, Bulla R, Soriani A, Agostinis C, Bossi F, Mocci C, Sarazani F, Tedesco F, Santoni A, Gismondi A: Recruitment of circulating NK cells through decidual tissues: a possible mechanism controlling NK cell accumulation in the uterus during early pregnancy. *Blood.* 2008;111:3108-15. doi: 10.1182/blood-2007-08-105965.
- [39] Czaja K, Kaleczyc J, Sienkiewicz W, Majewski M, Lakomy M: Peptidergic innervation of the porcine oviduct studied by double-labelling immunohistochemistry. *Folia Histochem Cytobiol.* 1996;34:141-50.
- [40] Czaja K, Kaleczyc J, Pidsudko Z, Franke-Radowiecka A, Łakomy M: Distribution of efferent neurones innervating the oviduct in the pig. *Folia Morphol (Warsz).* 2001;60:243-8.
- [41] Sjöberg NO, Johansson ED, Owman C, Rosengren E, Walles B: Cyclic fluctuation in noradrenaline transmitter of the monkey oviduct. *Acta Obstet Gynecol Scand.* 1977;56:139-53.

- [42] Marshall JM: Effects of ovarian steroids and pregnancy on adrenergic nerves of uterus and oviduct. *Am J Physiol.* 1981;240:C165-74.
- [43] Sporrang B, Helm G, Owman C, Sjöberg NO, Walles B: Electron microscopic and pharmacologic evidence for a functional adrenergic innervation of the smooth musculature in the human fallopian tube. *Brain Res Bull.* 1982;9:695-9.
- [44] Houdeau E, Rousseau A, Meusnier C, Prud'Homme MJ, Rousseau JP: Sympathetic innervation of the upper and lower regions of the uterus and cervix in the rat have different origins and routes. *J Comp Neurol.* 1998;399:403-12.
- [45] Zhu L, Xiao L, Che HS, Li YP, Liao JT: Uterine peristalsis exerts control over fluid migration after mock embryo transfer. *Hum Reprod.* 2014;29:279-85. doi: 10.1093/humrep/det429.
- [46] Zhu L, Chen J, Sun Y, Huang X, Xu H, Zhang X: Loss of nerve fibers in the oviduct isthmus in women with hydrosalpinx. *Acta Histochem.* 2013;115:609-15. doi: 10.1016/j.acthis.2013.01.008.
- [47] Zhang R, Feng XJ, Guan Q, Cui W, Zheng Y, Sun W, Han JS: Increase of success rate for women undergoing embryo transfer by transcutaneous electrical acupoint stimulation: a prospective randomized placebo-controlled study. *Fertil Steril.* 2011;96:912-6. doi: 10.1016/j.fertnstert.2011.07.1093.
- [48] Hashii K, Fujiwara H, Yoshioka S, Kataoka N, Yamada S, Hirano T, Mori T, Fujii S, Maeda M: Peripheral blood mononuclear cells stimulate progesterone production by luteal cells derived from pregnant and non-pregnant women: possible involvement of interleukin-4 and interleukin-10 in corpus luteum function and differentiation. *Hum Reprod.* 1998;13:2738-44.
- [49] Takabatake K, Fujiwara H, Goto Y, Nakayama T, Higuchi T, Maeda M, Mori T: Intravenous administration of splenocytes in early pregnancy changes the implantation window in mice. *Hum Reprod.* 1997;12:583-5.
- [50] Takabatake K, Fujiwara H, Goto Y, Nakayama T, Higuchi T, Fujita J, Maeda M, Mori T: Splenocytes in early pregnancy promote embryo implantation by regulating endometrial differentiation in mice. *Hum Reprod.* 1997;12:2102-7.
- [51] Egawa H, Fujiwara H, Hirano T, Nakayama T, Higuchi T, Tatsumi K, Mori T, Fujii S: Peripheral blood mononuclear cells in early pregnancy promote invasion of human choriocarcinoma cell line, BeWo cells. *Hum Reprod.* 2002;17:473-80.
- [52] Kosaka K, Fujiwara H, Tatsumi K, Yoshioka S, Sato Y, Egawa H, Higuchi T, Nakayama T, Ueda M, Maeda M, Fujii S: Human chorionic gonadotropin (HCG) activates monocytes to produce interleukin-8 via a different pathway from luteinizing hormone/HCG receptor system. *J Clin Endocrinol Metab.* 2002;87:5199-208.

- [53] Fujiwara H: Immune cells contribute to systemic cross-talk between the embryo and mother during early pregnancy in cooperation with the endocrine system. *Reprod Med Biol.* 2006;5:19-29.
- [54] Guerin LR, Moldenhauer LM, Prins JR, Bromfield JJ, Hayball JD, Robertson SA: Seminal fluid regulates accumulation of FOXP3+ regulatory T cells in the preimplantation mouse uterus through expanding the FOXP3+ cell pool and CCL19-mediated recruitment. *Biol Reprod.* 2011;85:397-408. doi: 10.1095/biolreprod.110.088591.
- [55] Chen T, Darrasse-Jèze G, Bergot AS, Courau T, Churlaud G, Valdivia K, Strominger JL, Ruocco MG, Chaouat G, Klatzmann D: Self-specific memory regulatory T cells protect embryos at implantation in mice. *J Immunol.* 2013;191:2273-81. doi: 10.4049/jimmunol.1202413.
- [56] Fujiwara H, Araki Y, Toshimori K: Is the zona pellucida an intrinsic source of signals activating maternal recognition of the developing mammalian embryo? *J Reprod Immunol.* 2009;81:1-8. doi: 10.1016/j.jri.2009.04.001.
- [57] Wakuda K, Takakura K, Nakanishi K, Kita N, Shi H, Hirose M, Noda Y: Embryo-dependent induction of embryo receptivity in the mouse endometrium. *J Reprod Fertil.* 1999;115:315-24.
- [58] Nance DM, Sanders VM: Autonomic innervation and regulation of the immune system (1987-2007). *Brain Behav Immun.* 2007;21:736-45.
- [59] Panuncio AL, De La Peña S, Gualco G, Reissenweber N: Adrenergic innervation in reactive human lymph nodes. *J Anat.* 1999;194:143-6.
- [60] Yuan XJ, Huang LB, Qiao HL, Deng ZP, Fa JJ: Uterine autonomic nerve innervation plays a crucial role in regulating rat uterine mast cell functions during embryo implantation. *Prostaglandins Other Lipid Mediat.* 2009;90:94-7.
- [61] Okitsu O, Kiyokawa M, Oda T, Miyake K, Sato Y, Fujiwara H: Intrauterine administration of autologous peripheral blood mononuclear cells increases clinical pregnancy rates in frozen/thawed embryo transfer cycles of patients with repeated implantation failure. *J Reprod Immunol.* 2011;92:82-7. doi: 10.1016/j.jri.2011.07.001.

