Review Article

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Use of biological and chemical molecules in regulating embryo implantation and endometrial receptivity

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Abstract

This review summarizes the drugs and chemicals that may modulate embryo implantation. Non-hormonal molecules, including aspirin, improved endometrial blood flow, while low molecular weight heparin, vitamin E, sildenafil, and atosiban modulated the expression of endometrial genes. Hormonal factors, including human chorionic gonadotropin and growth hormones, can regulate the expression of endometrial receptivity markers. Other immunomodulatory molecules, including granulocyte colony-stimulating factor, peripheral blood mononuclear cells, autologous platelet-rich plasma, and intralipid and intravenous immunoglobulins, may improve implantation rate by modulating endometrial immune functions. Medicinal extracts of the Chinese herbs *Paeonia lactiflora* and *Perilla frutescens* increased the expression of leukemia inhibitory factors in endometrial epithelial cells. Recently, the use of the commercially available Library of Pharmacologically Active Compounds with a high-throughput screening method has provided an approach to screen for compounds that may potentially enhance or suppress embryo implantation. Whether these biomedical findings translate into clinical effects that enhance or suppress embryo implantation requires further investigation.

Keywords: Embryo implantation, Endometrial receptivity, *In vitro* fertilization, Repeated implantation failure, Birth control, Emergency contraception

Introduction

Embryo implantation is a critical and complex biological process in the establishment of pregnancy[1]. Successful implantation requires synchronization of the receptive endometrium and a development-competent blastocyst[2]. Endometrial receptivity is the capacity of the endometrium to allow implantation of the embryo. The short period during which the uterine endometrium is receptive to blastocyst implantation is called the window of implantation (WOI). During the early secretory phase of the menstrual cycle, rising progesterone levels transform the estrogen-primed pre-receptive endometrium to a receptive state on days 20 to 24 of the 28-day menstrual cycle[3]. During the WOI, blastocysts enter the uterine cavity and appose to the endometrium. Embryo-maternal crosstalk further promotes adherence of the blastocyst to the endometrium during the attachment process[4]. The trophoblastic cells of the blastocyst attach to and penetrate the luminal epithelium and basal lamina before invading the decidualized stromal cells[5]. However, not all development-competent blastocysts can be implanted, and it is estimated that approximately 75% of early pregnancy loss is due to implantation failure[6,7]. Although high-quality embryos are transferred in most *in vitro* fertilization (IVF) cycles, certain patients still suffer from implantation failures, suggesting that endometrial receptivity is a critical parameter for successful implantation and pregnancy[8,9].

Repeated implantation failure

There are various definitions of repeated implantation failure (RIF). Some define it as the failure of a woman below 40 years of age to achieve a clinical pregnancy after transferring a minimum of four good-quality embryos at the cleavage stage, or two blastocysts in at least three fresh or frozen cycles[10,11], and there remains no universal consensus. RIF is a major challenge in IVF treatment because the underlying causes are unknown in the majority of cases. The etiologies of RIF are complex, and the risk factors include advanced maternal age, smoking status, high body mass index, and stress[12]. Changes in endometrial microRNA and mRNA expression have been demonstrated in women with RIF[13,14].

Endometrial receptivity markers

Markers of endometrial receptivity have long been investigated. Morphological changes in glandular and stromal endometrial cells are the gold standard for a receptive endometrium[15–17].

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Ultrasonography has been used to measure uterine blood flow and endometrial thickness, with some predictive value for pregnancy outcome[18–20]. An endometrial thickness of approximately 6 to 8 mm favors successful implantation[15,21,22], and a higher uterine blood flow resistance is associated with low pregnancy outcomes in women with RIF[23,24]. Resistance to blood flow in women with recurrent pregnancy loss is higher than that in women with normal pregnancies[25,26].

Leukemia inhibitory factor (LIF) is a member of the interleukin-6 family. It is expressed in the luminal and glandular epithelia of the endometrium[27–29]. LIF binds to the LIF receptor, which works with gp130 to activate the signaling pathway *via* signal transducer and activator of transcription 3. Embryos cannot be implanted into LIF knockout mice[30–32]. Furthermore, LIF from the uterine glands contributes to endometrium-embryo crosstalk during implantation and stromal cell decidualization[33]. Patients with higher LIF expression have a better chance of conceiving[34]. Similarly, fertile women express higher endometrial LIF levels than infertile women do in the WOI[29,35,36]. Therefore, LIF is believed to be a potential predictor of successful pregnancy[34,37].

Non-hormonal molecules

An increase in endometrial thickness and decrease in endometrial blood flow resistance are associated with improved pregnancy outcomes (Table 1)[38]. Aspirin and low-molecular weight heparin (LMWH) have been used to improve endometrial microcirculation. Low-dose aspirin can increase blood flow in the uterine arteries by dilating the blood vessels and inhibiting platelet aggregation[39,40]. Heparin regulates insulin-like growth factor-I (IGF-I) and IGF-binding protein-1 (IGFBP-1) to promote decidualization during implantation[41]. Moreover, in certain studies, the anticoagulant action of heparin prevented thrombosis and increased implantation rates in women with thrombophilia and repeated IVF failures[59–61]. Although a recent meta-analysis did not show a significant impact of LMWH administration on both clinical pregnancy and live birth rates in non-thrombophilic women with RIF[38], the beneficial effects of LMWH in women with thrombophilia undergoing IVF warrant further investigation[62].

Vitamin E may improve endometrial response because of its antioxidant and anticoagulant properties. Vitamin E appears to increase endometrial thickness and overcome the anti-estrogenic effect of clomiphene citrate in IVF treatment through its antioxidant effect[43,63,64]. Moreover, the anticoagulant effect of vitamin E may increase endometrial blood flow[42]. Supplementation with vitamin E improves the expression of low-density lipoprotein receptor, interleukin-1, and tumor necrosis factor alpha in patients with implantation failure[43]. A randomized controlled trial (RCT)[65] showed that the implantation rate was significantly higher in the treatment group of vitamin E and D3 in women with polycystic ovarian syndrome (PCOS) under intra-cytoplasmic sperm injection than in those PCOS infertile women without treatment; however, clinical support of vitamin E in the success of IVF is insufficient.

The use of vaginal sildenafil (Viagra) has gradually drawn attention as an adjuvant therapy for IVF. Sildenafil, an inhibitor of type 5-specific phosphodiesterase, can prevent cyclic guanosine monophosphate degradation and amplify the vasodilatory effects of nitric oxide[66]. Several studies have suggested the beneficial effects of vaginal sildenafil on endometrial receptivity through the improvement of uterine blood flow and an increase in endometrial thickness[44–47]. Atosiban, a mixed receptor antagonist of oxytocin and vasopressin V1a, can inhibit uterine contractions and increase endometrial perfusion[48]. In some studies, administration of atosiban improved implantation and pregnancy rates in patients with RIF[67,68], although other studies have indicated a limited role of atosiban in improving IVF success in unselected patients[69,70]. A previous study showed that atosiban treatment starting from the third embryo transfer (ET) cycle may be effective in improving embryo implantation and clinical pregnancy rates, because patients who have undergone three or more ET cycles were inclined to have higher uterine contractions and serum oxytocin levels[71]. Further investigation of the role of atosiban in embryo implantation is required.

Hormones

Steroid hormones regulate endometrial receptivity during the menstrual cycle. In IVF, hormone replacement therapy improves the implantation rate in patients with a thin endometrium (≤8 mm)[72]. It has been suggested that the administration of estrogen and progesterone helps patients with RIF to synchronize the uterine environment conducive to embryo implantation[73,74].

Table 1.

Summary of molecules that contribute to the blood flow or endometrial receptivity markers.

Groups	Molecules	Effects on	
		Blood flow	Receptivity markers
Non-hormone molecules	Aspirin	+[39,40]	–
	LMWH	+[39,40]	IGFBP-1, IGF-1[41]
	Vitamin E	+[42]	LDLR, IL-1, TNFα[43]
	Sildenafil	+[44–47]	–
	Atosiban	+[48]	–
Hormones and cytokines	hCG	–	IGFBP-1, VEGF, MMP-9, TIMP[49–51]
	GH	+[52]	IGF-I, VEGF, ITGB3[52]
Immunomodulatory molecules	G-CSF	–	–
	PBMC	–	LIF, VEGF[53]
	PRP	–	MMP-1, MMP-3, MMP-7[54]
	Intralipid	–	–
	IVIG	–	–
Traditional Chinese Medicine	*Paeonia lactiflora*	–	LIF[55]
	Perilla frutescens	–	LIF[56]
	Chinese Bushen Huoxue prescriptions	–	ITGB3, LIF[57]
	Wenshen Yangxue decoction	+	VEGF[58]

Presence or absence of characteristics represented as follows: present, plus (+) symbol; absent, minus (–) symbol.

G-CSF: granulocyte colony-stimulating factor; GH: growth hormone; hCG: intrauterine human chorionic gonadotrophin; IGF-I: insulin-like growth factor-I; IGFBP-1: insulin-like growth factor binding protein-1; IL-1: Interleukin-1; ITGB3: integrin subunit beta 3; IVIG: intravenous immunoglobulins; LDLR: low-density lipoprotein receptor; LMWH: Low-molecular weight heparin; MMP-1/3/7/9: matrix metalloproteinase 1/3/7/9; PBMC: peripheral blood mononuclear cell; PRP: platelet-rich plasma; TNFα: tumor necrosis factor alpha; VEGF: vascular endothelial growth factor; TIMP: matrix metalloproteinases.

Intrauterine human chorionic gonadotropin (hCG) infusion can improve pregnancy outcomes during IVF cycles[75,76]. hCG is produced by syncytiotrophoblasts[77] and induces progesterone secretion by promoting the development of the corpus luteum in the ovary[78]. hCG may prolong endometrial receptivity to facilitate implantation by downregulating intrauterine IGFBP-1 expression[49,79] in the late luteal phase. IGFBP-1 is a well-identified decidualization marker that regulates implantation by interacting with trophoblast-derived IGFs. hCG may enhance the endometrial response by increasing vascular endothelial growth factor (VEGF) expression for endometrial angiogenesis[50]. Moreover, hCG can induce LIF[51] expression, upregulate matrix metalloproteinase 9 expression, and inhibit tissue inhibitors of matrix metalloproteinases expression, thereby facilitating embryo invasion in the endometrial tissue[50]. A recent meta-analysis, which included 15 RCTs with a total of 2763 participants, concluded that infertile patients receiving intrauterine hCG injection before ET had a significantly higher implantation (31.6% *vs.* 22.5%), clinical pregnancy (47.8% *vs.* 32.8%), and live birth rates (44.9% *vs.* 29.8%) than those of the untreated or placebo controls. In addition, the miscarriage rate was significantly lower (12.5% *vs.* 18.6%) than that in the control group[80].

Growth hormone (GH) can also enhance endometrial receptivity and improve oocyte quality by activating IGF-I or promoting steroidogenesis of follicle-stimulating hormone[81]. Administration of GH stimulates endometrial blood flow and expression of cytokines such as endometrial VEGF, IGF-1, and integrin subunit beta 3, resulting in endometrial gland proliferation, blood vessel formation, and thickening of the endometrium[52]. In fact, administration of GH in RIF or non-RIF patients undergoing IVF has a positive effect on pregnancy outcomes[82–84].

Melatonin is a neuroendocrine hormone that is secreted by the pineal gland. Melatonin may be an efficient antioxidant agent for oocyte maturation and embryonic development[85]. Besides, recent research has shown that melatonin receptors 1A and 1B were expressed in human endometria[86]. Melatonin significantly increased the implantation sites (16.0±1.68 *vs.* 14.4±1.91; P <0.01) and litter sizes (13.9±1.23 *vs.* 12.6±1.39, P <0.05) of mice. The increased effect of melatonin on implantation sites and litter sizes, possibly through upregulation of PRA, HB-EGF, and p53 expression in the uterus, may contribute to endometrial development and uterine receptivity[87]. In another study, melatonin treatment in mice upregulated genes involved in pregnenolone synthesis in the ovary and Ihh expression in the uterine endometrium, which may mediate endometrial development and improve endometrial receptivity[88].

Immunomodulatory molecules

The immune system plays a pivotal role in embryo implantation[89]. Immunomodulatory therapies, such as subcutaneous or intrauterine administration of granulocyte colony-stimulating factor (G-CSF), intrauterine infusion of peripheral blood mononuclear cells (PBMCs), autologous platelet-rich plasma (PRP), and intralipid and intravenous immunoglobulins (IVIG), have been used to treat patients with RIF[90–94].

G-CSF, as a hematopoietic-specific cytokine, improves embryonic development, implantation, and trophoblast invasion in patients with RIF[95,96]. Human recombinant G-CSF plays a regulatory role in endometrial remodeling, local immune modulation, and cellular adhesion pathways[97]. G-CSF increases phagocytosis and oxidative processes, which are important for embryo implantation, by regulating endometrial vascular remodeling, local immune modulation, and cellular adhesion[98]. A recent meta-analysis suggested that G-CSF administration increases implantation and clinical pregnancy rates in patients with RIF[99]. Transvaginal perfusion of G-CSF significantly increased clinical pregnancy rates compared with that of the placebo (risk ratio [RR] = 1.563, 95% confidence interval [CI] = 1.122–2.176). Implantation rates were also significantly increased in patients with thin endometrium or repeated IVF failure under G-CSF treatment (RR = 1.887, 95% CI = 1.256–2.833)[99]. However, there was no significant increase in endometrial thickness[99].

Intrauterine PBMC infusion is another treatment option for patients with RIF. Human PBMCs obtained from pregnant women can promote the spread and invasion of murine blastocysts *in vitro*[100]. Moreover, PBMCs promote the invasion of trophoblastic BeWo cells *in vitro*[101]. Intrauterine administration of PBMCs enhances endometrial receptivity in mice by stimulating the endometrial expression of LIF and VEGF[53]. A meta-analysis of three RCTs that investigated the effect of intrauterine PBMC infusion on IVF outcomes in women with RIF showed a significant increase in the chances of clinical pregnancy (fixed effects model, RR = 2.18; 95% CI = 1.58–3.00; P <0.00001; I^2 = 0%) and live birth (RR = 2.41; 95% CI = 1.40–4.16; P = 0.002) in treated women[38]. In line with this, intrauterine administration of PBMCs increases clinical pregnancy rates in patients with RIF undergoing IVF-ET cycles[102–104], although a recently published meta-analysis study reported improvements in clinical pregnancy rates, but not live birth rates, in women with RIF after administration of PBMCs[91].

PRP infusion is a novel therapy in IVF[105]. PRP can facilitate embryo implantation by affecting endometrial thickness and vascularity[106]. Its therapeutic effect on endometrial growth and receptivity is due to the action of platelet-derived growth factors on cell proliferation and neo-endothelial cell generation for tissue growth, and also due to its anti-microbial and anti-inflammatory properties in uterine infections[107]. In two RCTs that investigated the effect of intrauterine PRP on IVF outcomes in women with RIF, clinical pregnancy rates were higher in the PRP group than in the control group (48.3% *vs.* 23.26%, P = 0.001; 44. 89% *vs.* 16.66%, P = 0.003, respectively)[55,56].

Intralipid, an intravenous fat emulsion, could be an effective and safe treatment for patients with RIF[108]. The benefits and efficacy of intravenous intralipid therapy in patients are controversial in clinical studies[109–111], however, a meta-analysis of 12 studies with 2676 participants in a recently published review[112] showed that treatment with intralipid improved implantation (odds ratio [OR]: 2.97, 2.05–4.29), pregnancy (OR: 1.64, 1.31–2.04), and live birth rates (OR: 2.36, 1.75–3.17), with a decrease in miscarriage rates (OR: 0.2, 0.14–0.30). This review highlights the benefits of intralipid in patients with poor reproductive history.

IVIG is considered a potential treatment for RIF and failed IVF. A previous study demonstrated that IVIG treatment increased the pregnancy rate of patients with RIF from 26% to 94% and the live birth rate from 18% to 80% compared with those not receiving IVIG[113]. A recent meta-analysis also showed that IVIG could improve implantation, pregnancy (OR = 1.82, 95% CI = 1.14–2.89; P = 0.01), and live birth rates (OR = 2.17, 95% CI = 1.30–3.61; P = 0.003) in patients with RIF to those of the control group[114]. Therefore, IVIG may be a good therapeutic agent for improving pregnancy outcomes in patients with RIF, particularly for those with immunological abnormalities[115].

Traditional Chinese medicine

Chinese herbs and acupuncture are adjuvant therapies used for patients undergoing IVF[116,117]. Accumulating data suggest that Chinese herbs increase endometrial thickness and improve embryo quality, which may benefit IVF treatment. *Paeonia lactiflora* (Chinese peony) and *Perilla frutescens* (Beefsteak plant) are Chinese herbs that increase LIF expression in endometrial epithelial cells and favor the adhesion of trophoblastic JAr cells *in vitro*[118,119]. Paeoniflorin, extracted from *P. lactiflora*, improves embryo implantation rates in a murine implantation failure model[120]. However, the clinical efficacy and safety of Chinese herbs require further investigation in clinical trials. In addition, several studies have shown that Chinese Bushen Huoxue prescriptions can upregulate the expression of endometrial receptors ER, PR, integrin β3, LIF, and other molecules, facilitating the development of pinopodes in the WOI and enhancing endometrial receptivity[57]. Another Traditional Chinese medicine (TCM), the Wenshen Yangxue decoction, could improve endometrial receptivity and promote endometrial angiogenesis by regulating the expression of PI3K, HIF-1α, and VEGF[58].

Emergency contraception

Birth control can be achieved through physical therapy and medication[121,122]. Emergency contraception (EC) is defined as the use of any drug or device after unprotected intercourse to prevent unwanted pregnancies[123]. In fact, >25% of all pregnancies are estimated to be unintended[124,125]; combined oral contraceptives (Yuzpe method), levonorgestrel, ulipristal acetate (UPA), and mifepristone (RU486) have commonly been used for medical abortion (Table 2).

RU486 is an orally active progesterone antagonist that acts through progesterone receptors. It was developed to terminate pregnancies and was also used for EC[146]. RU486 functions by blocking the effects of progesterone[140]. RU486 administration also impairs the ovulatory process. In the pre-ovulatory phase, RU486 blocks or delays ovulation in a dose-dependent manner. At a dose of 10 mg RU486, the development of the dominant follicle can be arrested or continued without rupture[141]. In women treated with high RU486 (3 mg/kg, orally) doses for three consecutive days, follicle development is disrupted or the dominant follicle is functionally destroyed with inhibition of ovulation[142,143]. Both UPA and RU486 can suppress the acrosome reaction of sperm[136,137]. The contraceptive effect of RU486 is dose- and time-dependent. A single dose of oral RU486 from 10 to 600 mg is sufficient to prevent pregnancies[144,145,147,148]. A single low dose of RU486 delays or inhibits ovulation, whereas high doses affect endometrial receptivity and prevent embryo implantation[144,145]. In an *in vitro* co-culture model, RU486 treatment (10^{-5} mol/L) for 5 days significantly suppressed the attachment of human blastocysts to reconstituted endometrial tissue consisting of human endometrial stromal and epithelial cells[145].

Screening of small molecules on endometrial receptivity

The Library of Pharmacologically Active Compounds (LOPAC) is a commercially available source for high-throughput screening of targets. It contains 1280 pharmacologically active compounds, with 58% related to neurotransmission, 9% to cell signaling, 6% to ion channels, and 3% to hormones. All major target classes, such as kinases, proteases, G protein-coupled receptors, and molecules involved in gene regulation and neurotransmission, are included in these 1280 pharmacologically active compounds. This library includes marketed drugs and pharmaceutically relevant compounds. More than 100 related publications were found in PubMed. LOPAC has been used for antiviral and antifungal drug screening[149,150], neurotransmitter screening[151], drug discovery against hepatitis C and human immunodeficiency viruses[152], and neurological diseases[153]. Importantly, LOPAC has been used to identify inhibitors of the canonical Wnt signaling pathway in colon cancer cells by evaluating β-catenin stability in a high-throughput model[154], and neurological[155] and angiogenic factors[156] in zebrafish embryo screening.

We modified a high-throughput attachment assay[157] in our laboratory, to screen the LOPAC for small molecules that enhance and/or suppress embryo implantation. The screening procedures are shown in Fig. 1. By using receptive (Ishikawa and RL95-2) and non-receptive (HEC1-B and AN3CA) endometrial epithelial cells cocultured with trophoblastic (BeWo) spheroids generated using Aggrewell and labeled with a fluorescent dye, this assay can be used to study the effect of LOPAC on the attachment rate of spheroids *in vitro*. Our results showed that Nemadipine-A[158] decreased spheroid attachment. In line with this, molecules that control the function of ion channels have been reported to modulate endometrial receptivity and embryo implantation[159–163]. Further analysis and confirmation with *in vitro* and *in vivo* models were used to explore their clinical implications.

Conclusion

Endometrial receptivity plays a vital role in successful implantation and pregnancy in women undergoing IVF. Ongoing research focuses on the use of non-hormonal molecules (aspirin, LMWH, vitamin E, sildenafil, and atosiban) to improve endometrial blood flow or vascularization. Hormonal factors, including hCG, GH, and melatonin, regulate the expression of receptivity

Table 2.

Summary of the effects of molecules in emergency contraception.

Molecules	Effects on		
	Human sperm function	Follicular development and ovulation	Endometrial receptivity and embryo implantation
Yuzpe	ND	+[126]	ND
Levonorgestrel	–[127–129]	+[130]	±[131–135]
Ulipristal acetate	+[136,137]	+[138,139]	±[131–135]
Mifepristone	+[136,137]	+[140–143]	+[144,145]

Presence or absence of effects represented as follows: present, plus (+) symbol; absent, minus (–) symbol; conflicting data, plus/minus (±) symbol.
ND: not determined.

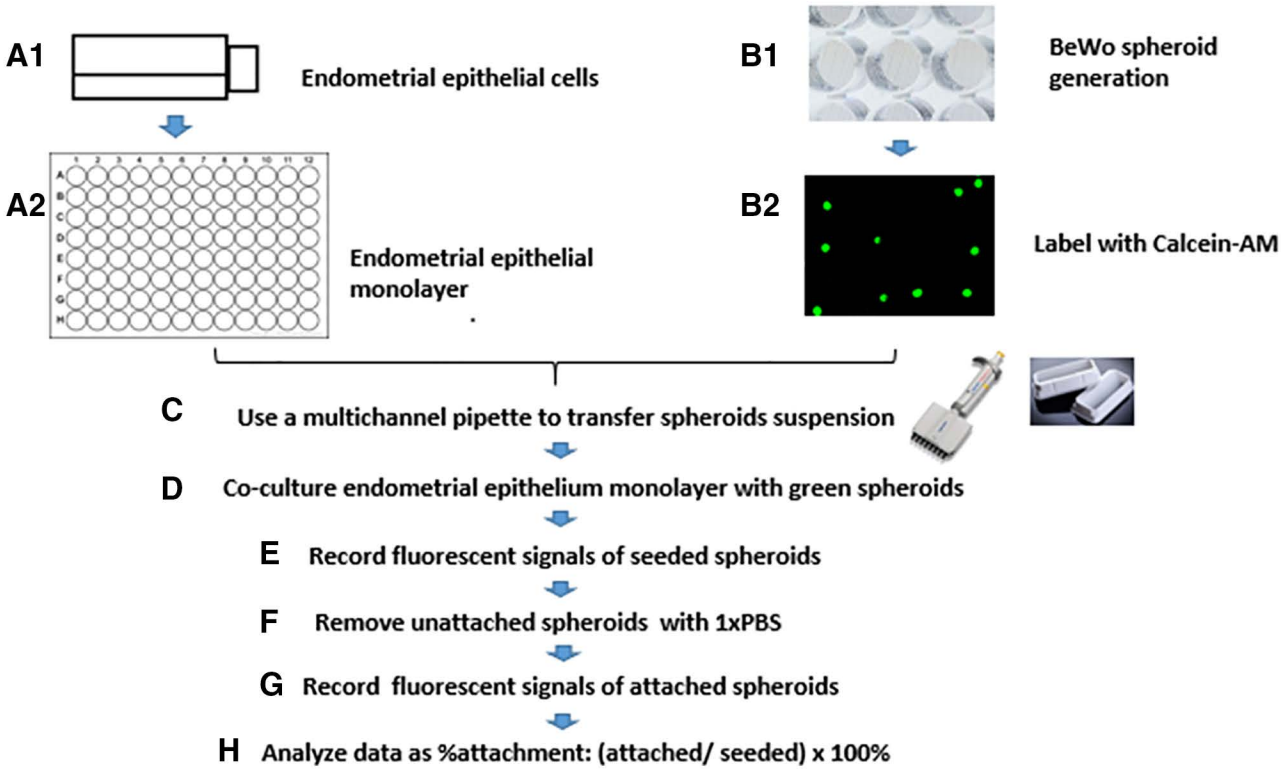

Fig. 1. Schematic diagram showing the screening strategy of the LOPAC. (A) The endometrial epithelial cells were cultured in a culture flask (A1), trypsinized and plated on 96-well plates to form the monolayers (A2) with or without LOPAC treatment for 24 h. (B) Concurrently, BeWo spheroids were generated using AggreWell (B1) and labeled with calcein-AM (green), a fluorescence dye. (C) BeWo spheroids with fluorescence labels were transferred onto the monolayers using a multichannel pipette. (D) The endometrial monolayer was cocultured with green spheroids for 1 h. (E) The fluorescence signals of seeded spheroids were recorded using a plate reader. (F) The unattached spheroids were removed by phosphate-buffered saline. (G) The fluorescence signals of attached spheroids were then recorded. (H) The attachment effect was expressed as the calculation of the percentage of the ratio of fluorescence signals (attached/seeded).

markers. The effects of immunosuppressive or immunomodulatory agents, including G-CSF, PBMC, PRP, and IVIG, have been studied for their effects in modulating implantation. Laboratory studies have shown that *P. lactiflora* and *P. frutescens* increase LIF expression in endometrial epithelial cells. Certain TCM prescriptions improve endometrial receptivity by regulating hormone receptors and growth factors. Finally, the use of libraries of small molecules (eg, LOPAC) combined with a high-throughput screening method may provide an approach to screen for compounds that may potentially enhance or suppress embryo implantation. However, whether these biomedical findings translate into clinical applications requires further investigation.

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None.

Author contribution

X.C., S.S., and K.L. conceived the concept of this study, designed and experimental approach. X.C. and S.S. performed the experiments. E.N., R.L., W.Y., and K.L. provided critical intellectual support, assisted with data analysis, and edited the manuscript. X.C., S.S., and K.L. wrote the manuscript. All authors contributed to the final editing and reviewed the manuscript.

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Conflicts of interest

All authors declare no conflict of interests.

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