—Review—

Embryonic modulation of endometrial receptivity

Sakae Goto*

Goto Ladies Clinic, Osaka 569-1116, Japan

Abstract: Implantation requires a receptive endometrium, a functionally normal embryo and communication between the developing embryo and maternal tissues (cross-talk). Two-step embryo transfer (two-step ET) and stimulation of endometrium embryo transfer (SEET) are procedures for ET based on concept of embryonic modulation of endometrial receptivity. In two-step ET, a cleaved embryo is transferred on day 2, and a blastocyst is transferred on day 5. The pregnancy and implantation rates with two-step ET were found to be higher than those with cleaved ET and double blastocyst transfer. We speculated that the cleaved embryos transferred on day 2 modulate endometrial receptivity and improve the implantation rate for blastocysts transferred on day 5. In SEET, embryo culture supernatant (ECS) is injected into the uterine cavity prior to blastocyst transfer to stimulate the endometrium and provide an optimum environment for implantation of forthcoming blastocysts. The pregnancy and implantation rates with SEET were found to be higher than those with blastocyst transfer. Higher implantation and pregnancy rates with SEET could be explained by embryonic factors produced in the culture medium that modulate endometrial receptivity. Lysophosphatidic acid was detected in ECS. Two-step ET and SEET are effective options for ET based on the concept of cross-talk between embryos and maternal tissues.

Key words: Embryo, Endometrium, Cross-talk, Two-step ET, SEET

Introduction

It has been proposed that the communication link between the developing embryo and maternal tissues (cross-talk) while the embryo travels through the fallopian tubes and becomes implanted in the endometrium plays an important role in successful embryo implantation [1–5].

Blastocyst transfer (BT) has been proposed as a means of improving the implantation rate compared with that using cleaved embryo transfer [6–8]. However, in BT, cross-talk between the developing embryo and the endometrium is absent until the blastocyst is transferred to the uterus, and the pregnancy rate using BT is limited to about 40% [9].

Although many studies have shown that embryos mediate their own environment in the maternal tract [1–5], only a few clinical approaches of embryo transfer using the cross-talk system have been reported. Accordingly, to improve the pregnancy outcome of BT, we designed two procedures for embryo transfer based on the concept of the embryonic modulation of endometrial receptivity: two-step embryo transfer [10, 11] and stimulation of endometrium embryo transfer (SEET) [12, 13].

In this review, we discuss the effectiveness of two-step embryo transfer and SEET in IVF-ET.

Two-step Embryo Transfer

In the two-step ET procedure, a cleaved embryo is transferred on day 2, and a blastocyst is transferred on day 5.

Two-step ET was adapted in patients with repeated ET failure, because single embryo transfer is recommended by Japan Society of Obstetrics and Gynecology for avoiding multiple pregnancy.

In two-step ET, a second best-quality cleaved embryo is selected for the first step of ET on day 2 based on the morphologic scores of the cleaved embryos. The remaining embryos, including the best embryo, are transferred to an environment with BlastAssist System 2 medium (MediCult, Jyllinge, Denmark) on day 3 and cultured until day 5. On day 5, the blastocyst with the best morphologic score according to the criteria of Gardner *et al.* [6] is selected for transfer in the second step of ET [10, 11].

Our study showed that two-step ET resulted in a pregnancy rate and implantation rate higher than those with

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*To whom correspondence should be addressed.
e-mail: gotoms@tcn.zaq.ne.jp

Table 1. Baseline characteristics and outcomes in cleaved embryo transfer and two-step embryo transfer groups

	Cleaved embryo group (n=60)	Two-step group (n=124)	Significance
Age of patient (year) (mean ± SD)	32.9 ± 3.5	33.5 ± 4.1	NS
Period of infertility (year) (mean ± SD)	5.3 ± 2.6	6.3 ± 2.7	NS
Basal FSH level (mIU/ml) (mean ± SD)	6.7 ± 2.2	7.9 ± 1.3	NS
No. of oocytes fertilized (mean ± SD)	6.4 ± 2.8	6.6 ± 2.8	NS
No. of embryos transferred	3	2.9 ± 0.3	NS
No. of clinical pregnancies[a]	18	74	
Clinical pregnancy rate per transfer (%)	30.0	59.7	$P<0.001$
No. of ongoing pregnancies[b]	16	64	
Ongoing pregnancy rate per transfer (%)	26.7	51.6	$P<0.01$
Implantation rate per embryo (%)[c]	11.1	27.8	$P<0.001$
No. of multiple pregnancies	2	19	
Multiple pregnancies rate (%)	11.1	25.7	NS

NS=not significantly different. [a]Clinical pregnancy was identified by development of a gestational sac. [b]Ongoing pregnancy was defined as the presence of cardiac activity. [c]Implantation rate was determined by dividing the number of gestational sacs by the number of embryos transferred. Note: modified from data in Goto *et al*., 2003 [11].

Table 2. Baseline characteristics and outcomes in day 2 ET or two-step ET in patients who have two embryos on day 2

	Day 2 ET (n=90)	Two-step ET (n=90)	Significance
Age of patient (year) (mean ± SD)	37.7 ± 4.8	37.4 ± 5.1	NS
Period of infertility (year) (mean ± SD)	8.1 ± 4.5	8.1 ± 5.3	NS
No.of previous ART cycles	3.3 ± 4.4	3.5 ± 4.1	NS
Basal FSH level (mIU/ml) (mean ± SD)	7.7 ± 2.9	8.4 ± 4.3	NS
No. of oocytes retrieved (mean ± SD)	3.8 ± 2.5	4.0 ± 2.2	NS
No. of oocytes fertilized (mean ± SD)	2.2 ± 0.5	2.2 ± 0.5	NS
Fertilization rate (%)	56.7	54.0	NS
No. of embryos transferred (mean ± SD)	2 ± 0	1.6 ± 0.5	$P<0.0001$
No. of high grade embryos on day 2[a]	144/180 (80.0%)	152/180 (84.4%)	NS
No. of clinical pregnancies	17	30	
Clinical pregnancy rate per transfer(%) [b]	18.9	33.3	$P<0.05$
Implantation rate per embryo (%)[c]	9.4	17.2	$P<0.05$
No. of twin pregnancies	0	1	
Twin pregnancies rate (%)	0.0	3.3	NS

NS=not significantly different. [a]High-grade embryos were defined as grade I or grade II 4-cell embryos and as grade I or grade II or grade III 5- to 8-cell embryos on day 2. [b]Clinical pregnancy was identified by development of a gestational sac. [c]Implantation rate was determined by dividing the number of gestational sacs by the number of embryos transferred. Note: modified from data in Goto *et al*., 2005 [10].

conventional day-2 ET [10, 11] (Table1 and 2). Several other studies also showed the advantages of the two-step ET strategy in human IVF/ICSI cycles [14–17]. The high pregnancy and implantation rates with two-step ET seem to be due in part to the transfer of a blastocyst on day 5 because the implantation rates of blastocysts have been reported to be higher than those of cleaved embryos [6]. However, our previous study showed that two-step ET resulted in pregnancy and implantation rates higher than those with double blastocyst transfer [18]. Thus, another possible explanation for the high pregnancy rate in two-step ET is modulation of the endometrium by embryos for implantation. It is possible that the cleaved embryos transferred on day 2 modulate endometrial receptivity and improve the implantation rate for blastocysts transferred on day 5.

Although single embryo transfer has recently been recommended for avoidance of multiple pregnancy, two-step ET is still an effective strategy for some patients suffering from repeated implantation failure.

Table 3. Outcomes of treatment with SEET and BT in patients with repeated ART failure

	SEET (n=23)	BT (control) (n=25)	*P*-value
No. of clinical pregnancies	20	12	0.006
single pregnancies	17	10	
twin pregnancies	3	2	
Clinical pregnancy rate per transfer(%)[a]	87	48	0.006
Implantation rate per embryo (%)[b]	71.9 (23/32)	37.8 (14/37)	0.007
Serum beta-hCG (IU/mL) on day 30	248 ± 184	138 ± 163	0.036
Estradiol (pg/ml) on day 23	370 ± 224	350.5 ± 195	0.764
Progesterone (pg/ml) on day 23	6.7 ± 3.6	7.1 ± 2.8	0.682

[a]Clinical pregnancy was identified by development of a gestational sac. [b]Implantation rate was determined by dividing the number of gestational sacs by the number of embryos transferred. Note: modified from data in Goto *et al.*, 2007 [12].

Stimulation of Endometrium Embryo Transfer (SEET)

The embryo produces several factors during its development to signal its presence to the maternal organism, and several embryonic factors that modulate endometrial receptivity have been detected in embryo culture supernatant (ECS) [19–25].

In SEET, ECS is injected into the uterine cavity prior to BT to stimulate the endometrium and provide an optimum environment for implantation of forthcoming blastocysts. Frozen-thawed blastocysts are used in the hormone replacement cycle in SEET. The blastocysts and ECS used in the SEET cycle are obtained as described next [12, 13].

In the oocyte retrieval cycle perior to the study cycle in our previous work [12, 13], patients were stimulated using standard GnRH agonist/FSH protocols or antagonist/FSH protocol. Ovulation was triggered when the second-leading follicle was >18 mm in diameter. The oocytes were retrieved transvaginally under ultrasonographic guidance 36 h after human chorionic gonadotropin (hCG) administration. Oocytes were fertilized conventionally or via intracytoplasmic sperm injection. The embryos were initially cultured in BlastAssist System 1 (MediCult, Jyllinge, Denmark). On day 2, depending on the number of cleaved embryos and the morphological grade of those embryos for any given patient, we cultured at least the top three grades of cleaved embryos until day 5 in BlastAssist System 2 (MediCult, Jyllinge, Denmark). Then embryos that had developed to the blastocyst stage were frozen. Two to four embryos were cultured in groups under mineral oil in 50-μl droplets of culture media (BlastAssist System 2). At most, 4 embryos were cultured in one droplet of the culture medium (BlastAssist System 2). Thus, when the patient had 5 embryos for extended culture, 5 embryos were divided into 2 and 3 embryos and cultured in separate droplets.

The ECS of BlastAssist System Medium 2 was preserved at −20°C. The blastocysts and the ECS were cryopreserved until the SEET cycle.

In the SEET cycle, transdermal oestradiol (Estraderm M, Kissei, Tokyo, Japan) was used in combination with a vaginal progesterone suppository (Utrogestan, Fuji Pharma Co., Ltd., Tokyo, Japan) for hormonal replacement (HRT). Preparation of the endometrium was started on day 2 of the HRT cycle and achieved in a step-up regime (2.16 mg to 4.32 mg). The progesterone suppository (600–1,200 mg/day) was started on day 15. ECS injection was performed transcervically into the uterus using a fai con IVF catheter (Fuji Systems, Tokyo, Japan) on day 17 or day 18 of the HRT cycle. The catheter was loaded with 20μl of ECS and inserted into the uterine cavity, and the ECS was released slowly when the tip of the catheter was about 1 cm from the fundus of the uterine cavity. Frozen-thawed blastocysts were transferred on day 20 of the HRT cycle. ET was performed transcervically using a fai con IVF catheter.

In our preliminary study, SEET was performed in patients with a relatively poor pregnancy prognosis who had already undergone established procedures unsuccessfully [12]. The clinical pregnancy rates in the SEET and BT groups were 87.0% and 48.0%, respectively (*P*=0.006), and the implantation rates were 71.9% and 37.8%, respectively (*P*=0.007). The clinical pregnancy and implantation rates in the SEET group were significantly higher than those in the BT group (Table 3).

Another SEET study was performed in patients who underwent IVF for the first time with a single blastocyst [13]. In that study, patients were randomly allocated to three groups: 48 women in the BT group, 48 women in the stimulation (ST) group who had been injected with

Table 4. Baseline characteristics and outcomes of patients in BT, ST and SEET

	low-grade blastocysts			high-grade blastocysts			*P*
	BT (n=23)	ST[c] (n=19)	SEET (n=23)	BT (n=25)	ST (n=29)	SEET (n=25)	
Age of patient (years)	32.3 ± 3.1	32.1 ± 3.3	33.7 ± 3.2	34.0 ± 3.6	33.6 ± 3.9	32.7 ± 4.0	0.58
Period of infertility (months)	61.3 ± 26.2	61.4 ± 30.5	71.7 ± 40.0	68.5 ± 32.8	59.1 ± 30.2	60.5 ± 34.9	0.61
Basal FSH level (mIU/ml)	5.8 ± 1.4	5.7 ± 2.5	6.3 ± 1.7	5.8 ± 1.7	5.9 ± 1.7	6.1 ± 2.0	0.48
No. of oocytes retrieved	14.0 ± 5.6	14.7 ± 4.1	15.7 ± 4.4	14.2 ± 4.9	14.7 ± 6.3	15.2 ± 5.9	0.50
No. of oocytes fertilized	10.5 ± 4.0	10.6 ± 4.1	11.7 ± 3.9	11.6 ± 4.2	10.9 ± 4.3	12.6 ± 5.3	0.38
No. of chemical pregnancies	15	9	14	16	22	23	0.024
Implantation rate per embryo (%)[a]	65.2	47.4	60.9	64	75.9	92	
No. of clinical pregnancies	12	8	9	14	20	20	0.032
Clinical pregnancy rate per transfer (%)[b]	52.2	42.1	39.1	56	69	80	

[a]Implantation rate was determined by detecting serum beta-hCG. [b]Clinical pregnancy was identified by development of a gestational sac. [c]ST group was injected culture medium into the uterus before blastocyst transfer. Note: modified from data in Goto *et al.*, 2009 [13].

culture media into the uterus before BT, and 48 women in the SEET group. Odds ratios of successful implantation rates for ST and SEET in patients with high-grade blastocysts, with BT as a reference, were 2.58 and 6.46 without adjustment (*P*=0.02 for trend) and 5.91 and 9.20 after adjusting for basal FSH levels and period of infertility. The odds ratios for clinical pregnancies were 2.47 and 4.32 without adjustment (*P*=0.03 for trend) and 4.46 and 5.10 with adjustment (*P*=0.05 for trend), respectively. In groups with low-grade blastocysts, such tendencies were not observed. The study showed that SEET was an effective method for increasing implantation rate and pregnancy rate for first-time ART patients who had a high-grade frozen-thawed blastocsyst in the HRT cycle (Table 4). On the other hand, SEET was not found to be effective for patients who were transferred a frozen-thawed blastocyst in the natural ovulatory cycle (data not shown).

Recently, results of several studies in which ECS was injected into the uterus before ET have been reported [26–29]. Some studies showed the advantage of SEET, but other studies showed no effectiveness of SEET. However, the methodology in those studies differed from that in our studies. We used high-grade frozen-thawed blastocysts in the HRT cycle, and we injected 20 μl of ECS at a point 1 cm from the fundus of the uterine cavity where embryos were transferred, because some embryonic factors might affect the endometrium locally. In studies with negative outcomes, ECS was injected into the lower part of the uterus [27], was injected only 30 min before ET [28] or was injected in fresh ET cycles [29]. SEET in those studies should be referred to be “modified SEET,” not SEET.

Although further studies are needed to evaluate SEET, SEET is an effective ET procedure based on the concept of embryonic modulation of endometrial receptivity for patients with a high-grade blastocyst in the HRT cycle without risk of multiple pregnancy.

Lysophosphatidic Acids (LPAs) in ECS

Lysophosphatidic acid is one of the simplest glycerophospholipids with one fatty acid chain and a phosphate group as a polar head. Although LPA had been viewed just as a metabolic intermediate in de novo lipid synthetic pathways, it has recently been paid much attention as a lipid mediator [30]. LPA acts on specific G protein-coupled receptors to elicit a wide range of cellular responses, ranging from cell proliferation and migration to neurite remodeling and blastocyst implantation (Fig. 1) [31].

Recent studies have shown that LPA signaling influences embryo spacing and uterine receptivity in mice [32, 33]. Deletion of LPA3 in mice led to uneven embryo spacing, possibly caused by a defect in uterine contraction, and delayed implantation caused by a defect in uterine receptivity. The lipid signaling system is also an integral part of the establishment and maintenance of pregnancy in pigs [34]. Liszewska *et al.* [35] investigated the LPA pathway during early pregnancy in sheep. Quantitative RT-PCR showed that the LPA-generating enzyme autotaxin was expressed in the endometrium and conceptus. More recently, Achache *et al.* [36] reported that LPA3 levels were comparatively lower in patients with recurrent implantation failure. Thus, we investigated whether LPAs are present in ECS as a potential cross-talk signal from the embryo to the endometrium [37]. LPAs in ECS were

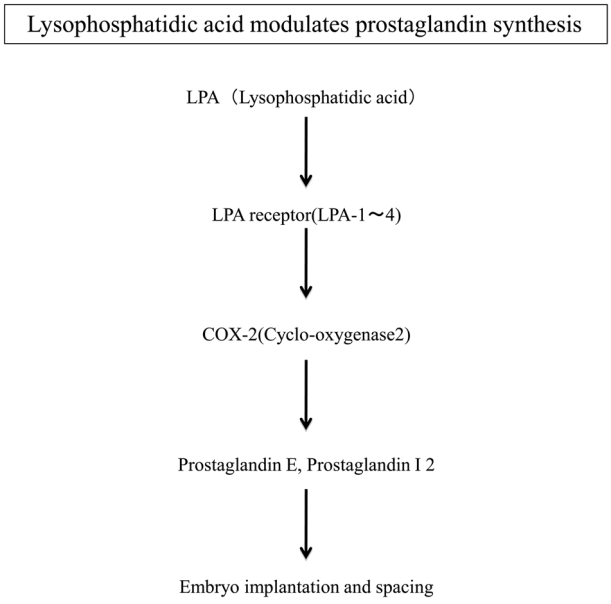

Fig. 1. Lysophosphatidic acid (LPA) modulates prostaglandin synthesis via LPA receptor in the endometrium. Note: Van Meeteren LA,Molecular WH 2007 [31].

extracted by the Bligh-Dyer method. Extracted LPAs were converted to trimethylsilyl (TMS) derivatives. Gas chromatography (GC)/selected ion monitoring (SIM), and GC/mass spectrometry (MS) were used to analyze LPAs derivatized with TMS. LPA-C16:0, 16:1, 18:0, 18:1, and 18:2 were detected in ECS. The results of the study indicated that the five LPA species including three unsaturated LPAs produced by embryos are candidate embryonic signals to the endometrium for implantation.

Conclusion

Two-step embryo transfer and SEET are ET procedures based on the concept of embryonic modulation of endometrial receptivity.

Although two-step embryo transfer has a risk of multiple pregnancy, it is an effective ET procedure for repeated ART failure. In SEET, ECS is obtained from a medium in which the patient's own embryos are cultured. Thus, ECS for SEET is easy to obtain and is safe, with no risk of unknown viral infection. SEET is an effective ET procedure for patients who have high-grade blastocysts in the HRT cycle. LPAs are candidate embryonic signals to the endometrium for implantation.

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