

# Improvement of Live Birth Rate Following Frozen-Thawed Blastocyst Transfer by Combination of Prednisolone Administration and Stimulation of Endometrium Embryo Transfer

Taketo Inoue<sup>1,2\*</sup>, Yoshiyuki Ono<sup>1</sup>, Yukiko Yonezawa<sup>1</sup>, Junji Kishi<sup>1</sup>, Nobuyuki Emi<sup>1</sup>

<sup>1</sup>Ono Ladies Clinic, Ono, Japan

<sup>2</sup>Department of Biophysics, Kobe University Graduate School of Health Sciences, Kobe, Japan

Email: [taketoein@yahoo.co.jp](mailto:taketoein@yahoo.co.jp)

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## Abstract

The endometrial condition is a significant factor for successful pregnancy. To regulate endometrial function in fertility treatment, prednisolone (PSL) is administered for suppression of increased natural killer cells and stimulation of endometrium embryo transfer (SEET) to enhance communication between embryo and maternal tissues. We attempted to improve the endometrial condition by PSL administration and SEET during frozen-thawed blastocyst transfer (FBT). Patients took PSL (5 mg) 3 times daily for 3 days after ovulation during the FBT cycle. To analyse effects of PSL combined with SEET, we determined rates of chemical pregnancy, clinical pregnancy, foetal heart movement (FHM) and live birth. Rates of chemical pregnancy, clinical pregnancy and FHM were significantly higher in the PSL(+)/SEET(+) (57.7%, 50.0% and 46.2%, respectively) and PSL(+)/SEET(-) (53.3%, 46.7% and 46.7%, respectively) groups than in the PSL(-)/SEET(+) (30.3%, 18.2% and 18.2%, respectively) and PSL(-)/SEET(-) (22.4%, 22.4% and 18.4%;  $P = 0.0043$ ,  $0.0081$  and  $0.0055$ , respectively) groups. The live birth rate was significantly higher in the PSL(+)/SEET(+) group than in the PSL(+)/SEET(-), PSL(-)/SEET(+) and PSL(-)/SEET(-) groups (42.3%, 26.7%, 18.2% and 12.2%, respectively;  $P = 0.0237$ ). PSL combined with SEET may be a useful adjunct to assisted reproductive technology in women who repeatedly fail to conceive by infertility treatment.

## Keywords

Frozen-Thawed Blastocyst Transfer, Infertility, Live Birth, Prednisolone, Stimulation of

\*Corresponding author.

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## Endometrium Embryo Transfer (SEET)

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### 1. Introduction

In 2010, an estimated 48.5 million couples have been reported to be infertile worldwide [1] and >5 million children have been born after assisted reproductive technology [2]. For *in vitro* fertilisation (IVF) and intracytoplasmic sperm injection (ICSI), clinical pregnancy rates per transfer have been reported to be 32.9% and 32.0%, respectively, and for frozen-thawed embryo transfer, pregnancy rates per thawing have been reported to be 20.9% [3].

For successful pregnancy, the endometrial condition is important. Accordingly, to regulate endometrial function, infertility patients who have had recurrent spontaneous abortions have been treated with drugs such as prednisolone (PSL) [4]-[6] and aspirin [7]-[9] combined with luteal support. In the endometrium of patients who have had recurrent spontaneous abortions, natural killer (NK) cells are increased [10]. A previous study showed that PSL suppressed NK cell cytotoxicity [11]. Accordingly, several studies have attempted to improve pregnancy rates by administration of PSL in patients after embryo transfer [4]-[6]. However, PSL treatment of fresh embryo derived from ICSI transfer did not improve pregnancy rates [5]. On the other hand, pregnancy rates were successfully improved after fresh embryo transfer by PSL treatment [4] [6]. Moreover, for successful embryo implantation, communication between the developing embryo and maternal tissues (crosstalk) may be important [12]. Previous studies have attempted to induce crosstalk by inserting embryo culture supernatant, which may contain several factors, such as interleukin-1, hCG and vascular endothelial growth factors from pre-implantation embryos, into a patient's uterus before blastocyst transfer [13]-[15]. As a result, pregnancy rates were successfully increased.

We focused on the effect of PSL administration during frozen-thawed blastocyst transfer (FBT) cycle combined with stimulation of endometrium embryo transfer (SEET). However, effects of PSL are still unclear during the FBT cycle. Accordingly, we investigated the effect of PSL combined with SEET on improvement of pregnancy rates during luteal support for FBT.

### 2. Materials and Methods

#### 2.1. Patients

A retrospective study was performed on data obtained from 138 FBT cycles between October 2010 and December 2011 [115 ovum pick-up (OPU) cycles]. Informed consent was obtained regarding the dosage of PSL and SEET. All the procedures were performed in accordance with the Declaration of Helsinki.

#### 2.2. Ovarian Stimulation/*in Vitro* Fertilisation/*Intracytoplasmic*sperm Injection

For ovarian stimulation, the long, short or gonadotropin-releasing hormone antagonist protocols were used depending on the patient. In poor responders, clomifene citrate (50 mg Spacromin tablet; Pola Pharma Inc., Tokyo, Japan) and human menopausal gonadotropin (hMG; ASKA Pharmaceutical Co., Ltd., Tokyo, Japan) were used for ovarian stimulation. GnRH analogue acetate (Fuji Pharma Co., Ltd., Tokyo, Japan), human menopausal gonadotropin and GnRH antagonist (Cetrotide®; Shionogi & Co., Ltd., Osaka, Japan) were used in all protocols. When at least 2 follicles reached 18 - 20 mm in diameter (determined by transvaginal ultrasonography), 5000 IU of human chorionic gonadotropin (hCG; Fuji Pharma) was administered. OPU was performed 35 h after hCG injection. Oocytes were inseminated by IVF or ICSI. ICSI was performed as described previously [16]. In brief, spermatozoa were selected by hyaluronic acid (HA). HA-bound spermatozoa were injected into oocytes using a piezo micro manipulator (Prime Tech Ltd., Ibaraki, Japan). Fertilised eggs were cultured in Single Step Medium™ (Irvine Scientific, Santa Ana, CA, USA) with 10% Serum Substitute Supplement (Irvine Scientific) until Day 5. Two morphological blastocyst categories were defined according to the criteria of Gardner *et al.* [17]. Grades 3AA, 4AA, 4AB, 4BA, 5AA, 5AB, 5BA, 6AA, 6AB and 6BA were categorised as high-grade blastocysts, and the others were categorised as low-grade blastocysts [15].

### 2.3. Cryopreservation/Thawing/Blastocyst Transfer

Blastocysts were cryopreserved and thawed using the Cryotop technique, as described previously [18], with the modification that thawing was performed at room temperature. A frozen-thawed blastocyst was transferred to a uterus. During luteal support for FBT cycles, patients were administered 2 mg of chlormadinone acetate (Lutorial; Shionogi & Co., Ltd.) 3 times daily after ovulation, and 0.72 mg transdermal estradiol patches (Estrana<sup>®</sup> Tape; Hisamitsu Pharmaceutical Co., Inc., Tokyo, Japan) were applied on the abdomen for 10 weeks after the initiation of menstruation. On the day of blastocyst transfer, if serum progesterone concentration was <15 ng/ml, the women were injected with 125 mg of 17 $\alpha$ -hydroxyprogesterone caproate once every 5 days [19]. Patients took 5 mg of PSL (prednisolone tablet; Towa Pharmaceutical Co., Ltd., Osaka, Japan), 3 times daily for 3 days after ovulation. The dosage of PSL was set at 15 mg/day based on previous references [6]. SEET [13] [14] was performed 2 or 3 days before FBT.

### 2.4. Statistical Analysis

The average patient age, OPU frequency and number of motile spermatozoa were evaluated using one-factor analysis of variance. These data are presented as means  $\pm$  standard deviations (SDs). Ratios of blastocysts derived from IVF/ICSI and blastocyst grade and rates of chemical pregnancy, clinical pregnancy, foetal heart movement (FHM) and live birth were evaluated using chi-square test and residual analysis. A probability level of  $P < 0.05$  was considered to indicate statistical significance.

## 3. Results

To investigate the effect of PSL combined with SEET during luteal support for FBT, we confirmed rates of chemical pregnancy, clinical pregnancy, FHM and live birth. No significant differences were observed in backgrounds of patients (average patient age, OPU frequency and number of motile spermatozoa) and characteristics of blastocysts (rates of fertilisation and development, ratio of blastocysts derived from IVF/ICSI and ratio of high to low-grade blastocysts) among all groups (Table 1). Rates of chemical pregnancy, clinical pregnancy and FHM were significantly higher in the PSL(+)/SEET(+) and PSL(+)/SEET(-) groups than in the PSL(-)/SEET(+) and PSL(-)/SEET(-) groups ( $P = 0.0043$ ,  $0.0081$  and  $0.0055$ , respectively; Table 1). Furthermore, the live birth rate was significantly higher in the PSL(+)/SEET(+) group than in the PSL(+)/SEET(-), PSL(-)/SEET(+) and PSL(-)/SEET(-) groups ( $P = 0.0237$ ; Table 1).

**Table 1.** Effect of prednisolone combined with SEET during frozen-thawed blastocyst transfer cycle.

	PSL(+)/SEET(+)		PSL(+)/SEET(-)		PSL(-)/SEET(+)		PSL(-)/SEET(-)		P value
Age of women	33.0 $\pm$ 5.0		35.4 $\pm$ 4.5		35.3 $\pm$ 4.4		32.8 $\pm$ 5.4		0.0676
No. of ovum pick-up cycles	23		25		30		37		
Ovum pick-up frequency	2.5 $\pm$ 2.5		2.3 $\pm$ 1.5		2.8 $\pm$ 1.6		2.5 $\pm$ 2.7		0.9090
No. of motile spermatozoa ( $\times 10^6$ /ml)	53.1 $\pm$ 45.7		62.5 $\pm$ 35.4		46.4 $\pm$ 36.0		40.9 $\pm$ 34.6		0.1837
No. of recovered oocytes	171		216		186		295		
No. of 2PN2PB oocytes	110	(64.3%)	154	(71.3%)	133	(71.5%)	214	(72.5%)	0.2761
No. of blastocysts	68	(61.8%)	95	(61.7%)	84	(63.2%)	127	(59.3%)	0.9060
No. of blastocyst transfers	26		30		33		49		
Blastocysts derived from IVF/ICSI	12/14		20/10		19/14		26/23		0.4560
Ratio of high- to low-grade blastocysts	14/12		15/15		18/15		21/28		0.7060
No. of chemical pregnancies	15	(57.7%)*	16	(53.3%)*	10	(30.3%)	11	(22.4%)**	0.0043
No. of clinical pregnancies	13	(50.0%)*	14	(46.7%)*	6	(18.2%)	11	(22.4%)	0.0081
No. of foetal heart movement	12	(46.2%)*	14	(46.7%)*	6	(18.2%)	9	(18.4%)	0.0055
No. of live births	11	(42.3%)**	8	(26.7%)	6	(18.2%)	6	(12.2%)*	0.0237

PN, pronucleus; PB, polar body. Significant difference between each group at  $P < 0.05$  using chi-square test and residual analysis. \*:  $P < 0.05$ , \*\*:  $P < 0.01$ .

No adverse effects of PSL were observed. In all successful pregnancies, all mothers and babies were healthy.

#### 4. Discussion

The present study showed that pregnancy rates were successfully improved by PSL administration, and the live birth rate was successfully and significantly increased by PSL combined with SEET during the FBT cycle. Several studies have attempted to administer PSL to patients during fresh embryo transfer cycles [4]-[6]. PSL treatment of fresh embryos derived from ICSI transfer did not improve pregnancy and implantation rates (PSL vs. control; 49.0% vs. 50.0% and 23.6% vs. 23.3%, respectively [5]). On the other hand, pregnancy rates were successfully improved by PSL treatment after fresh embryo transfer in patients with auto-antibodies [4] [6]. Hasegawa *et al.* [4] reported that administration of PSL and low-dose aspirin therapy to women with positive anti-nuclear antibodies but without anti-phospholipid antibodies significantly improved pregnancy and implantation rates (40.6% vs. 14.8% and 20.3% vs. 6.8%, respectively). Taniguchi [6] showed that implantation (12.2% vs. 0%) and clinical pregnancy (29.3% vs. 0%) rates were improved by PSL administration; however, the live birth rate was not (14.6% vs. 0%). In the present study, rates of clinical pregnancy and FHM were improved by administration of PSL during FBT cycles. In addition, our results showed that the live birth rate did not improve during FBT cycles by PSL alone.

Tehranejad *et al.* [15] reported that term deliveries increased and abortions decreased by stimulation of the endometrium with high-grade blastocyst culture supernatant perfusion before fresh blastocyst transfer. Considering these results, we attempted to improve the live birth rate during FBT cycles by administering the combination of PSL and SEET to enhance crosstalk. Our study showed that the live birth rate was successfully and significantly increased by the combination of PSL and SEET during the FBT cycle ( $P = 0.0237$ ). A comparison between PSL(-)/SEET(+) and PSL(-)/SEET(-) groups revealed no obvious differences in rates of clinical pregnancy, in contrast to the results of a previous study [14]. Goto *et al.* [14] reported that SEET may be an effective method for increasing implantation and pregnancy rates (SEET, 80.0%; blastocyst transfer, 56.0%) in patients receiving assisted reproductive technology treatment for the first time who had high-grade blastocysts (pregnancy rates of low-grade blastocysts; SEET, 39.1%; blastocyst transfer, 52.2%). Moreover, in contrast to the results of the previous study by Tehranejad *et al.* [15], the live birth rate did not improve by SEET. In this study, ratios of low-grade to high-grade blastocysts were approximately half the number of blastocysts derived from IVF/ICSI in each group. Accordingly, our results suggested that SEET alone was not efficacious during FBT involving low-grade blastocysts.

In the present study, although ratios of low-grade blastocysts were approximately half the number in each group, the live birth rate was successfully improved by the combination of PSL and SEET during the FBT cycle. For early normal pregnancy, uterine CD56<sup>bright</sup> NK cells are necessary, probably because they secrete both pro- and anti-inflammatory cytokines in relative proportions that determine the degree of inflammation at the fetomaternal interface [20]. On the other hand, in women with a history of recurrent miscarriage [20] and recurrent failed implantation [21] [22], uterine NK (uNK) cells were found to be increased in their endometrium. Elevated uNK cell density has been shown to be associated with lower endometrial expression of 11 $\beta$ -hydroxysteroid dehydrogenase type 1 and mineralocorticoid receptor [23]. Moreover, expression of mineralocorticoid receptor-dependent genes, such as dehydrogenases/reductase superfamily 3 and retinol saturase that are involved in lipid metabolism and retinoid acid biosynthesis and storage, decreased [23]. Accordingly, we speculated that by supplementing the decreased corticoids via PSL treatment, lipid metabolism [24] and retinoid acid biosynthesis/storage [25] [26], which are essential for embryogenesis and maintenance of pregnancy, could be improved. Consequently, the live birth rate was successfully increased in the present study.

Moreover, PSL treatment modified the endometrial environment for embryogenesis and maintenance of pregnancy through reduction of the numbers of endometrial macrophages and uNKs [27] [28], which suppressed the cytolytic activity of NK cells [11] and associated highly muscularised vessels [28]. The present study showed that the combination of PSL and SEET may be a useful adjunct to assisted reproductive technology in women who repeatedly fail to conceive by infertility treatment.

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