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Lack of an increase in endometrial thickness from the day of oocyte retrieval to the day of embryo transfer results in optimal pregnancy outcome after fresh *in vitro* fertilization cycles

체외수정시술시 자궁내막두께 변화가
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서울대학교 대학원
의학과 산부인과학 전공
정 미 나

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지도 교수 김 석 현

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의학과 산부인과학 전공
정 미 나

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위 원 장 _____

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위 원 _____

Lack of an increase in endometrial thickness from the day of oocyte retrieval to the day of embryo transfer results in optimal pregnancy outcome after fresh *in vitro* fertilization cycles

Mina Jeong

Department of Medicine, Obstetrics and Gynecology

The Graduate School

Seoul National University

Abstract

Objective: This study aimed to evaluate the effect of changes in endometrial thickness (EMT) on pregnancy outcome during *in vitro* fertilization and embryo transfer (IVF–ET) cycles.

Methods: In total, 370 fresh IVF cycles performed at a single fertility center from January 2017 to November 2018 were retrospectively analyzed. EMT on the day of human chorionic gonadotropin injection, ovum pick–up (OPU), and embryo transfer (ET) were examined and analyzed according to the presence and absence of clinical and ongoing pregnancy. Subsequently, pregnancy outcomes (i.e., clinical and ongoing pregnancy) were compared according to the change in EMT. The ‘non–increase’ group was

defined when EMT was the same or decreased from the OPU day to the ET day, whereas the 'increase' group was defined when cycles had thicker EMT on the ET day than on the OPU day.

Results: EMT on the OPU day was thicker in cycles with ongoing pregnancy than in cycles without ongoing pregnancy (9.4 ± 1.6 mm versus 9.0 ± 1.9 mm, p = 0.016). There was no other association between each EMT and pregnancy outcomes. EMT increased more from the OPU day to the ET day in cases with non-pregnant cycles than in those with clinical and ongoing pregnancy. Clinical and ongoing pregnancy rates were both significantly higher in the non-increase group than in the increase group. After adjusting for female age, the serum anti-Müllerian hormone level, transferred number of good-quality embryos, and EMT on the OPU day, clinical pregnancy (adjusted odds ratio [aOR] = 1.649, 95% confidence interval [CI]: 1.033-2.633) and ongoing pregnancy rates (aOR = 1.796, 95% CI: 1.108-2.911) were still significantly higher in the non-increase group than in the increase group.

Conclusion: Clinical and ongoing pregnancy rates were higher in the non-increase group than in the increase group of EMT from the OPU day to the ET day after fresh IVF cycles.

Keywords : infertility, endometrium, in vitro fertilization, pregnancy

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Introduction

Although *in vitro* fertilization (IVF) has become a widely accepted therapy for infertility, the pregnancy rate by IVF has not increased significantly in recent years (1). A healthy embryo needs to be implanted in a receptive endometrium during the window of implantation, which is about the day 20 to 24 of an idealized 28-day cycle for a successful pregnancy (2). Endometrial receptivity has been regarded as one of the most important and challenging steps for success in assisted reproductive technology. Although there are recent methods for histologically or genetically evaluating endometrial receptivity such as endometrial receptivity array test, thickness and pattern of the endometrium measured by ultrasonography have been mainly investigated as surrogate markers for endometrial receptivity (3, 4).

Many studies have reported the importance of the endometrial thickness (EMT) on pregnancy outcome during the IVF procedure and most of them discovered that a thin endometrium adversely affects pregnancy rates (5, 6). Most of the existing studies measured EMT on the day of human chorionic gonadotropin (hCG) injection in fresh IVF cycles and EMT at the end of the estrogen phase in frozen-thawed embryo transfer (FET) cycles, and proved their effect on pregnancy rates. The optimal cutoff value of EMT for IVF success has been suggested to be 7 mm, and values below this cutoff are considered to be associated with a lower chance of pregnancy (7), leading to the cancellation of an embryo transfer (ET).

The endometrial condition can change according to the menstrual

period, not only in natural menstrual cycles but also in IVF cycles (8, 9). In a study of 30 apparently fertile women with regular menstrual cycles that used three-dimensional ultrasonography, EMT and endometrial volume increased significantly during the follicular phase, reaching a plateau around the time of ovulation, and remained relatively constant during the luteal phase (10). It has been reported that the distribution of EMT and endometrial pattern were similar in spontaneous and stimulated cycles (11). In patients undergoing IVF treatment, the day of ET is usually considered to be in the middle of the implantation window. Thus, it is reasonable to regard the endometrial condition on the ET day as more representative of endometrial receptivity (12). However, there is a paucity of data about the effect of EMT in the luteal phase or its change within IVF cycles on successful pregnancy outcome.

Baker et al. (13) demonstrated that EMT and the echogenic pattern in the follicular and luteal phases were not predictive of pregnancy outcomes in oocyte donation cycles. Haas et al. (14) examined hormonally prepared FET cycles and introduced the concept of "endometrial compaction". They demonstrated that FET cycles in which EMT decreased (compaction) after applying progesterone showed higher ongoing pregnancy rates, and that the greater the degree of compaction, the better the pregnancy outcome. Afterwards, they further analyzed FET cases with only elective single ETs of a euploid embryo after a pre-implantation genetic test (PGT) for aneuploidy (PGT-A) to exclude the impact of embryo quality (15). In this study, ongoing pregnancy rates were significantly higher in cycles whose endometrial lining compacted on the ET day than in those without endometrial compaction, which supported the result of their first publication.

Since Haas et al. first highlighted the impact of endometrial compaction, research on the predictive value of EMT change has been continued, and the results are controversial. Contrary to Haas et al.' s study, Bu et al. (12) reported that increased EMT after progesterone administration resulted in higher clinical pregnancy rates during the first FET cycles using a single blastocyst. Meanwhile, other three studies could not find any significant association between EMT change in response to progesterone and live birth outcomes in FET cycles (16-18).

So far, no study has evaluated the association between EMT changes and pregnancy rates in fresh IVF cycles. Therefore, the present study aimed to investigate the effect of EMT changes from the day of hCG triggering through the ovum pick-up (OPU) day to the ET day on pregnancy rates in fresh IVF cycles.

Methods

1.1. Study Design and Population

This study included 370 IVF and ET (IVF-ET) cycles conducted at a single private fertility center from January 2017 to November 2018. This study was approved by the Institutional Review Board (IRB) of Seoul Rachel Fertility Center (IRB No. RTR-2020-01). This was an observation-only study using medical chart review and the need of informed consent was waived.

Medical records of the subjects were reviewed retrospectively by a clinician. Fresh IVF cycles using fresh autologous oocytes and sperm were included in the analysis. Only cycles in which all EMT on the days of hCG triggering, OPU, and ET was measured were included. When EMT on the hCG day was <7 mm, it was excluded from the analysis. PGT cycles and cases whose pregnancy outcome could not be traced were also excluded.

All female patients underwent an interview to obtain a detailed medical history, physical examination, and transvaginal ultrasonography. Basal serum hormonal assays including anti-Müllerian hormone (AMH) and hysterosalpingo contrast sonography were also performed in all female subjects. Male partners underwent a medical interview, basal hormone tests, and semen analysis.

We recorded general patient information such as patient age, gravidity, and body mass index. Cycle-specific information such as the number of oocytes retrieved or EMT during the IVF cycles were also examined.

1.2. IVF procedure

Controlled ovarian stimulation (COS) started on menstrual days 2–3 with gonadotropins of recombinant follicle–stimulating hormones (Gonal–F, Merck–Serono, Darmstadt, Germany; Follitrope, LG Life Sciences, Seoul, Korea; and Puregon, NV Organon, Oss, the Netherlands), with or without highly purified human menopausal gonadotropin (IVF–M HP, LG Life Sciences). The initial gonadotropin dosage ranged from 150 to 300 IU/day, and was determined for each patient according to the ovarian reserve markers and female age. During the COS cycle, all patients were monitored using serial transvaginal ultrasonography, and the gonadotropin dose was adjusted if necessary. When the leading follicle reached 14 mm in diameter, a daily gonadotropin–releasing hormone (GnRH) antagonist injection (Cetrotide, Merck–Serono) was added. When at least one of the follicles reached 18 mm or more in diameter, 250 ㎍ of recombinant hCG (Ovidrel, Merck–Serono) and/or 0.2 mg of a GnRH agonist (Decapeptyl, Ipsen Pharma, Paris, France) was administered for final oocyte maturation.

Oocyte retrieval was performed with transvaginal ultrasonography at 34–36 hours after hCG triggering. Oocyte maturity was evaluated by microscopic examination, and oocytes with a single polar body and expanded cumulus–corona were regarded as mature oocytes. Fertilization was done by conventional IVF or intracytoplasmic sperm injection using sperm collected from the male partner on the day of OPU. Fertilization was confirmed on the next day by observing two pronuclei, and all normally fertilized embryos were cultured in the culture medium (G–1™/G–2™ PLUS, Vitrolife, Gothenburg, Sweden) in an incubator with a temperature

of 37 ℃ and 6% carbon dioxide/5% oxygen. Three to five days after oocyte retrieval, ET was conducted under guidance of transabdominal ultrasonography. One or two embryos were transferred in all patients. Embryos were transferred in cleavage or blastocyst status depending on the quality and number of embryos, and the patient's condition.

Embryo quality was evaluated by experienced embryologists before transfer. Cleavage stage embryos were graded using the following morphological grading system: grade 1, equal-sized blastomeres without cytoplasmic fragmentation; grade 2, equal-sized blastomeres and <10% of cytoplasmic fragmentation; grade 3, blastomeres of unequal size and variable fragmentation without apparent morphologic abnormalities; grade 4, blastomeres of equal or unequal size and significant fragmentation (>10%); and grade 5, few blastomeres of any size and severe fragmentation (50%) (19). Cleavage-stage embryos of grade 1 or 2 were regarded as good quality embryos.

Blastocysts were scored according to the grading system proposed by Gardner (20): B1, early blastocyst with a blastocoel less than one-half the volume of that of the embryo; B2, with a blastocoel one-half the volume of that of the embryo or more; B3, full blastocyst with a blastocoel completely filling the embryo; B4, expanded blastocyst with a blastocoel volume larger than that of the blastocyst, and a thinning zona; B5, hatching blastocyst with a trophectoderm starting to herniate through the zona; and B6, hatched blastocyst, having completely escaped the zona. For blastocysts graded as B3 to B6, development of the inner cell mass and trophectoderm was assessed. The inner cell mass was graded as A, tightly packed, many cells; B, loosely grouped, several cells;

or C, very few cells. The trophectoderm was graded as follows: A, many cells forming a cohesive epithelium; B, few cells forming a loose epithelium; or C, very few large cells. Good quality blastocysts were defined as follows: blastocele B3 or higher, inner cell mass A/B, or trophectoderm A/B.

The luteal phase was supported using micronized vaginal progesterone (Lutinus, Ferring, Saint-Prex, Switzerland) or vaginal progesterone gel (Crinone, Merck-Serono) and an injection of intramuscular progesterone depot as needed, beginning one day after oocyte retrieval. After confirmation of pregnancy, luteal phase support was continued until the week 8 of gestational age.

1.3. Outcome measures and statistical analysis

Main outcome measures were EMT and its changes, and pregnancy outcomes. During the IVF cycle, EMT was measured with transvaginal ultrasonography by the experienced attending physician at every visit, except on the ET day when EMT was measured using transabdominal ultrasonography. When performing ultrasonography, the maximal echogenic distance between the junction of the endometrium and myometrium was measured in the mid-sagittal plane, including the cervical canal.

Pregnancy outcome was assessed 14 days after oocyte retrieval by measuring serial the serum hCG level. If the serum hCG level exceeded 5, it was considered as implantation. The implantation rate was calculated as the ratio of the number of implanted embryos to the number of transferred embryos. Confirmation of the intrauterine gestational sac on transvaginal ultrasonography was regarded as clinical pregnancy. Ongoing pregnancy was defined

when pregnancy was sustained until after week 20 of gestational age.

EMT values on the hCG triggering day, OPU day, and ET day were examined, and the impact of EMT on pregnancy outcomes (i.e., clinical and ongoing pregnancy) was analyzed. Subsequently, the EMT changing pattern such as an increase or decrease between the days and its relationship to pregnancy outcomes was investigated. Then cycles were divided into two groups according to the EMT changes from the OPU day to the ET day: 'increase' group, EMT became thicker on the ET day than on the OPU day, and 'non-increase' group, EMT did not change or decreased from the OPU day to the ET day. Then, several characteristics and pregnancy outcomes were compared between these two groups.

Numerical data are presented as mean ± standard deviation, and categorical variables are expressed as number with percentage. Pearson's chi-square test was used to compare proportions between the two groups. Continuous variables were analyzed by the Student's *t*-test or Mann-Whitney *U* test. EMT increase or non-increase from the OPU day to ET day was regarded as a categorial variable and was used in subsequent regression analyses. Logistic regression analysis was performed to evaluate the independent association of prognostic factors and ongoing pregnancy. Multivariable logistic regression analysis was also performed to adjust for confounding factors that can affect pregnancy outcome during IVF-ET cycles. All statistical analyses were performed with IBM SPSS ver. 21.0 (IBM Corp., Armonk, NY, USA). A p-value $<.05$ was considered statistically significant.

Results

The overall clinical pregnancy and ongoing pregnancy rates were 40.5% (150/370) and 34.9% (129/370) per ET, respectively. EMT and changes in EMT according to the pregnancy outcomes are presented in Tables 1 and 2, respectively. Each EMT value on the hCG triggering day, OPU day, and ET day was not significantly different according to the presence and absence of a clinical pregnancy. EMT on the OPU day was thicker in cycles with ongoing pregnancy than in cycles without ongoing pregnancy (9.4 ± 1.6 mm versus [vs.] 9.0 ± 1.9 mm, p = 0.016), but EMT on the hCG day or ET day were similar according to the ongoing pregnancy outcome. The values of change, in other words, the delta, of EMT from the OPU day to the ET day were significantly different according to the pregnancy outcomes. EMT increased more from the OPU day to the ET day in non-pregnant groups than in both clinically and ongoing pregnant groups. The other changes of EMT from the hCG day to the ET day were comparable according to the pregnancy outcomes.

Table 1. EMT according to the presence and absence of a clinical pregnancy

	Clinical pregnancy		*p−value*
	Yes (n = 150)	No (n = 220)	
EMT (mm) on the hCG day	9.6 ± 1.5	9.5 ± 1.8	0.490
EMT (mm) on the OPU day	9.3 ± 1.6	9.1 ± 1.9	0.061
EMT (mm)on the ET day	9.8 ± 1.7	10.1 ± 2.1	0.527
Δ EMT (mm) from the hCG day to the OPU day	−0.2 (−4.1, 3.0)	−0.3 (−5.3, 10.0)	0.278
Δ EMT (mm) from the hCG day to the ET day	0 (−4.9, 4.2)	0.4 (−4.0, 8.0)	0.122
Δ EMT (mm) from the OPU day to the ET day	0.3 (−2.4, 4.5)	1.0 (−4.3, 7.0)	0.009

Values are presented as mean ± standard deviation or median (range).

EMT, endometrial thickness; hCG, human chorionic gonadotropin; OPU, ovum pick−up; ET, embryo transfer.

Table 2. EMT according to the presence and absence of an ongoing pregnancy

	Ongoing pregnancy		*p−value*
	Yes (n = 129)	No (n = 241)	
EMT (mm) on the hCG day	9.6 ± 1.5	9.5 ± 1.8	0.495
EMT (mm) on the OPU day	9.4 ± 1.6	9.0 ± 1.9	0.016
EMT (mm) on the ET day	9.8 ± 1.7	10.0 ± 2.1	0.631
Δ EMT (mm) from the hCG day to the OPU day	0 (−4.1, 3.0)	−0.4 (−5.3, 10.0)	0.074
Δ EMT (mm) from the hCG day to the ET day	0 (−4.9, 4.2)	0.4 (−4.0, 8.0)	0.169
Δ EMT (mm) from the OPU day to the ET day	0.2 (−2.4, 3.9)	0.9 (−4.3, 7.0)	0.003

Values are presented as mean ± standard deviation or median (range).

EMT, endometrial thickness; hCG, human chorionic gonadotropin; OPU, ovum pick−up; ET, embryo transfer.

When comparing the increase group and the non-increase group, the basal clinical and cycle-specific characteristics were similar between the groups (Table 3). Clinical outcomes such as the number of retrieved oocytes or fertilization rates did not significantly differ between the two groups according to the increment in EMT from the OPU day to the ET day (Table 4).

Pregnancy outcomes were compared between the increase group and the non-increase group (Table 5). Both clinical and ongoing pregnancy rates per ET were significantly higher in the non-increase group than in the increase group. According to multivariable logistic regression analysis, pregnancy rates were still significantly higher in the non-increase group than in the increase group after adjusting for female age, the basal serum AMH level, number of good-quality embryos transferred, and EMT on the OPU day (adjusted odds ratio [aOR] = 1.649, 95% confidence interval [CI]: 1.033-2.633 for clinical pregnancy; aOR = 1.796, 95% CI: 1.108-2.911 for ongoing pregnancy).

Table 3. Basal characteristics according to the presence and absence of an increment in EMT from the OPU day to the ET day

	EMT increase (n = 239)	EMT non-increase (n = 131)	*p*-value
Age (yrs)	36.3 ± 3.7	36.6 ± 3.4	0.343
Age of the male partner (yrs)	38.2 ± 4.1	38.7 ± 4.5	0.499
Body mass index (kg/m^2)	20.5 ± 3.5	20.8 ± 2.4	0.940
Duration of infertility (yrs)	4.0 ± 2.5	4.1 ± 2.8	0.641
Type of infertility			0.624
Primary infertility	145/232 (62.5%)	73/122 (59.8%)	
Secondary infertility	87/232 (37.5%)	49/122 (40.2%)	
Cause of infertility			0.501
Unexplained	25 (10.5%)	18 (13.7%)	
Male factor	54 (22.6%)	27 (20.6%)	
Tubal factor	3 (1.3%)	4 (3.1%)	
Ovulatory factor	5 (2.1%)	4 (3.1%)	
Combined and others	152 (63.6%)	78 (59.5%)	
Serum AMH level (ng/mL)	3.3 ± 3.1	3.2 ± 2.7	0.922
Basal serum FSH level (mIU/mL)	9.0 ± 4.9	8.6 ± 5.1	0.103

Values are presented as mean ± standard deviation or number (percent).

EMT, endometrial thickness; OPU, ovum pick-up; ET, embryo transfer; yrs, year; AMH, anti-Müllerian hormone; FSH, follicle-stimulating hormone.

Table 4. Clinical outcomes according to the presence and absence of an increment in EMT from the OPU day to the ET day

	EMT increase (n=239)	EMT non-increase (n= 131)	*p*-value
Serum E2 level on the hCG day (pg/mL)	1666.4 ± 1297.3	1690.9 ± 1275.8	0.699
Serum P4 level on the hCG day (ng/mL)	0.6 ± 0.4	0.7 ± 0.4	0.053
No. of follicles≥11mm on the hCG day	8.8 ± 4.7	8.9 ± 4.8	0.912
No. of follicles≥14mm on the hCG day	7.0 ± 4.3	7.3 ± 4.6	0.623
No. of oocytes retrieved	7.4 ± 5.0	8.1 ± 5.9	0.671
Oocyte retrieval rate, % (n)	84.4 (1777/2105)	90.4 (1054/1166)	0.292
No. of mature oocyte retrieved	4.3 ± 3.7	4.7 ± 4.2	0.621
Oocyte maturation rate, % (n)	58.3 (1036/1777)	58.6 (618/1054)	0.914
Fertilization rate, % (n)	72.2 (1213/1681)	77.5 (768/991)	0.064
No. of embryos transferred	1.6 ± 0.5	1.7 ± 0.5	0.173
No. of GQEs transferred	1.1 ± 0.8	1.2 ± 0.8	0.118

Values are presented as mean ± standard deviation or percent (number).

EMT, endometrial thickness; OPU, ovum pick-up; ET, embryo transfer; E2, estradiol; P4, progesterone; hCG, human chorionic gonadotropin; no., number; GQE, good-quality embryo.

Table 5. Pregnancy outcomes according to the presence and absence of an increment in EMT from the OPU day to the ET day

	EMT increase	EMT non-increase	*p*-value	aOR* (95% CI)	*p*-value
Implantation rate, % (n)	47.2 (199/422)	57.4 (135/235)	0.137	1.387 (0.881-2.815)	0.158
CPR per ET, % (n)	36.4 (87/239)	48.1 (63/131)	0.029	1.649 (1.033-2.633)	0.036
OPR per ET, % (n)	30.5 (73/239)	42.7 (56/131)	0.018	1.796 (1.108-2.911)	0.018

Values are presented as number (percent).

OPU, ovum pick-up; ET, embryo transfer; EMT, endometrial thickness; aOR, adjusted odds ratio; CI, confidence interval; CPR, clinical pregnancy rate; OPR, ongoing pregnancy rate.

*Multivariable logistic regression after adjusting for confounding factors (female age, the basal anti-Müllerian hormone level, EMT on the OPU day, and number of good-quality embryos transferred)

Regarding prognostic factors for ongoing pregnancy (Table 6), EMT on the OPU day showed no significant relationship with the likelihood of an ongoing pregnancy, whereas EMT non-increase from the OPU day to the ET day (p = 0.03) independently predicted higher ongoing pregnancy rates.

Table 6. Predictive factors for ongoing pregnancy

	p-value	OR	95% CI	
			Lower	Upper
Age	<0.001	0.848	0.785	0.915
AMH level	0.718	1.015	0.937	1.098
EMT on the OPU day	0.808	1.017	0.887	1.166
No. of GQEs transferred	<0.001	2.159	1.570	2.968
EMT non-increase from the OPU day to the ET day	0.030	1.761	1.056	2.938

OR, odds ratio; CI, confidence interval; AMH, anti-Müllerian hormone; EMT, endometrial thickness; OPU, ovum pick-up; no., number; GQE, good-quality embryo; ET, embryo transfer.

Discussion

This study revealed that change in EMT was predictive of the pregnancy outcome in fresh IVF-ET cycles. Ongoing pregnancy rates were higher for cycles whose EMT did not increase from the OPU day to the ET day, than for those whose EMT increased on the ET day rather than on the OPU day. This association remained significant after controlling for possible confounders that could affect pregnancy outcome in IVF cycles.

EMT on the day of the hCG injection is a well-known prognostic factor for IVF success (5, 6), with a widely accepted threshold of ≥7 mm (7). However, EMT on the hCG day was comparable between the pregnant and non-pregnant groups in our study. In the present study, cycles with EMT <7 mm on the hCG day were excluded from the analysis, and this probably caused different results from what we generally know. Indeed, clinical pregnancy rates of the excluded cases in which EMT on the hCG triggering day was <7 mm (n = 28) were significantly lower than those of the analyzed cases whose EMT was ≥7 mm on the hCG day (14.3% vs. 40.5%, $p < 0.01$), and the same trend was observed for ongoing pregnancy rates (data not shown).

A few studies noted the value of the endometrial condition in the luteal phase around the ET day, and the results are controversial. Among them, three studies reported a significant correlation between increased endometrial volume on the ET day and IVF success (21-23). Hou et al. (24) reported that each endometrial echo pattern on 1, 2, and 3 days after OPU, especially that on OPU + 2 days, showed high predictive efficiency for clinical pregnancy rates in fresh IVF cycles. In our study, EMT on the ET day was not

related to pregnancy rates after IVF cycles. This difference may be due to the different measurement variables and method. Previous studies measured the endometrial volume or echogenic pattern using transvaginal ultrasonography, whereas our study measured EMT on the ET day using transabdominal ultrasonography.

The endometrial condition can similarly change during the menstrual cycle according to the ovarian-derived steroid hormones, both in spontaneous and stimulated cycles (25). In the follicular or proliferative phase, the endometrium is exposed to estrogen, causing an increase in EMT and linear growth of the endometrial glands. Endometrial proliferation ceases 2–3 days after ovulation. However, there is a continuing increase in gland tortuosity and secretion, and accretion of blood vessels within the constrained endometrium under the influence of progesterone, resulting in increased endometrial density (8). During this luteal or secretary phase, progesterone also regulates expression of numerous biomarkers such as cell adhesion molecules and growth factors via progesterone receptor (PR)s, triggering decidualization of the endometrial stromal fibroblasts (26). Estrogen receptor (ER)s are increased during the proliferative phase in response to estrogen and then downregulated in response to progesterone in the secretary phase. This decline in ERs at the time of implantation, observed in most mammals, is known to be critical for endometrial receptivity (27). Continued growth in the endometrium during the luteal phase around the ET day may be due to an inadequate effect of progesterone, specifically PR deficiency or resistance (14).

Several causes for PR resistance have been proposed including chronic endometrial inflammation and genetic causes such as PR gene polymorphisms or altered microRNA expression (28). Chronic

inflammatory condition, typically endometriosis, is a prime candidate for inducing progesterone resistance. Accretion of cyclooxygenase associated with endometriosis has been shown to augment endometrial aromatase expression with increased estrogen activity and increased expression of SIRT1 and BCL6, both of which are molecular mediators of progesterone resistance (29, 30). Some studies compared the endometrial transcriptome during the secretary phase from women with and without endometriosis, and reported dysregulation of several progesterone-related genes in ectopic endometrium of endometriosis patients, suggesting a poor response to progesterone (31, 32). Moreover, ERs in the endometrium are often not down-regulated in women with endometriosis. One biomarker of endometrial receptivity, beta 3 integrin subunit, was reported to be reduced or absent in some women with endometriosis and such defects were accompanied by inappropriate overexpression of ER-alpha during the mid-secretory phase (27). Our subjects with increased endometrial thickness on the ET day probably had a similar mechanism of progesterone resistance.

Progesterone resistance is likely not related to the actual circulating concentration of progesterone. Histologic endometrial development in the luteal phase has been reported to be similar whether serum progesterone levels are normal or abnormally low (33). Alternatively, one could speculate that the estrogen-progesterone ratio is important, as too much estrogen causes the endometrium to continuously grow, causing it to no longer be compact. In a fresh IVF cycle, ovarian stimulation may have a detrimental effect on endometrial receptivity (34, 35). Supraphysiologic estrogen levels after COS can negatively affect

the normal estrogen–progesterone ratio in the endometrium and can lead to the failure of endometrial compaction (14). Indeed, although the main results of our study on EMT changes and pregnancy rates are in line with Haas et al.'s findings, the ratio of cycles with endometrial compaction >5% was 21.4% (79/370) in our study, which was far less than the 42.44% (115/271) in Haas et al.'s study. Moreover, in our study, the pregnancy rate was higher when EMT did not increase on the ET day compared to the OPU day, but the delta values of EMT from the OPU day to the ET day were positive in both the pregnant and non–pregnant groups. Since our center only measured serum estrogen and progesterone levels on the hCG injection day, these hormone levels and their ratios could not be evaluated or compared on the OPU and ET days. Further studies are needed including ones that measure the estrogen–progesterone ratios and concentrations of ERs and PRs from the hCG day to the ET day.

The present study has severe limitations. First, an inherent limitation is its retrospective design. Second, the study had a relatively small and heterogeneous study population. Patients with endometriosis or a decreased ovarian reserve that can affect the endometrial receptivity were included in the present study, and it was difficult to conduct subgroup analysis because many patients had complex and multiple etiologies. Further study using strict inclusion criteria is required. Third, two important factors for implantation are embryo and endometrial receptivity, but embryo quality and numbers were not unified in this study. Although we found a significant association between the EMT change and ongoing pregnancy rates after controlling for embryo quality, further studies that accurately exclude the impact of embryo quality

are necessary. Fourth, even though highly experienced physicians tried to measure EMT in the same manner, there might have be inter-observer variability. Lastly, EMT on the ET day was measured with transabdominal ultrasonography, so the accuracy of this value may have been low.

Nevertheless, the current study also has several strengths. First, this study was performed in one center, so the overall IVF procedure, specifically the methods of COS, embryo culture, and luteal phase support, were relatively unified. Second, we conducted multiple regression analysis to adjust for various confounders, thus improving the validity of our results. Finally, it is meaningful in that this is the first study to reveal the association of the change in EMT from the hCG injection day to the ET day with pregnancy outcome after fresh IVF cycles. To the best of our knowledge, this is the first study to explore the effect of EMT change after progesterone administration in fresh IVF cycles.

In conclusion, fresh IVF cycles that showed no increase in EMT on the ET day in response to progesterone administration after OPU resulted in better ongoing pregnancy outcomes than those that showed an increase in EMT on the ET day rather than on the OPU day. EMT on the day of hCG triggering have been mainly measured and considered to be a crucial factor for IVF success. This study's results suggest that EMT change from the OPU day to the ET day may be a more important clue for predicting pregnancy outcome, and this may be a novel perspective for counseling and managing patients during fresh IVF cycles. Further well-designed prospective studies should be conducted to confirm these findings.

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요약(국문초록)

목표: 본 연구는 체외수정시술에서 자궁내막두께의 변화양상이 임신결과에 미치는 영향에 대해 분석하고자 하였다.

방법: 2017년 1월부터 2018년 11월까지 한 난임시술기관에서 시행된 총 370개의 체외수정시술 주기를 후향적으로 분석하였다. 체외수정시술 기간 중 난포의 최종 성숙을 위해 인간 융모성 생식선 자극호르몬을 투여하는 날, 난자채취일, 배아이식일에 모두 자궁내막 두께를 측정하여 임상적 임신율 및 20주 이후까지 지속되는 임신율과의 관계를 분석하였다. 또한 난자채취일로부터 배아이식일로의 자궁내막두께 변화에 따라 난자채취일보다 배아이식일에 자궁내막두께가 증가하지 않은 '비증가군'과 배아이식일에 자궁내막두께가 더 두꺼워진 '증가군' 으로 나누어 두 군의 임신 결과를 비교하였다.

결과: 20주 이후까지 임신이 지속되는 군에서 그렇지 않은 군보다 난자채취일의 자궁내막두께가 더 두꺼웠다 (9.4 ± 1.6 mm vs. 9.0 ± 1.9 mm, p = 0.016). 이외에 각 자궁내막두께와 임신결과의 연관성은 확인되지 않았다. 자궁내막두께의 변화와 관련해서는, 임신군과 비교하여 비임신군에서 난자채취일에 비해 배아이식일에 자궁내막두께가 더 많이 증가하였다. 이에 따라 난자채취일로부터 배아이식일로의 자궁내막두께 증가군과 비증가군을 비교하였을 때 비증가군에서 임상적 임신율과 20주 이후 지속되는 임신율이 모두 유의하게 높았다. 임신 결과에 영향을 줄 수 있는 여성환자의 나이, 항뮬러관호르몬 농도, 양질의 배아 이식 수, 난자채취일의 자궁내막두께를 모두 보정하고 분석하였을 때도 이 자궁내막두께 변화와 임신율에 대한 연관성은 여전히 유의하게 확인되었다 (임상적 임신율에 대한 보정된 오즈비 (adjusted odds ratio (aOR) = 1.649, 95% 신뢰구간 (confidence interval (CI)): 1.033−2.633, 20주 이후 지속되는 임신율에 대한 aOR = 1.796, 95% CI: 1.108−2.911).

결론: 체외수정 시술에서 난자채취일과 비교하여 배아이식일에 자궁내막

두께가 증가하지 않았을 때 증가한 군과 비교하여 임상적 임신율 및 20주 이후까지 지속되는 임신율이 더 높았다.

주요어 : 난임, 자궁내막, 체외수정, 임신
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