전 용 필 교수지도
석사학위 청구논문

Efficiency of Freeze-All Embryo Transfer on Pregnancy in the Patients with Poor Ovarian Reserve

2020년 5월

성신여자대학교 대학원

생 물 학 과

이 지 현

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이 논문을 석사학위논문으로 제출함

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Adviser: Yong-Pil Cheon, Ph.D.

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Sungshin Women's University

Graduate School

Department of Biology

Jee-Hyun Lee

Certificate of Committee Approval

Be accepted partial fulfillment of the

requirements for the degree of:

Master of Science

Signatures

Chairperson	Park, Hyun-Tae Ph.D
Committee member	Kang, Chang-Su, Ph.D
Committee member	Cheon, Yong-Pil, Ph.D

Sungshin Women's University

Graduate School

ABSTRACT

Efficiency of Freeze-All Embryo Transfer on Pregnancy in the Patients with Poor Ovarian Reserve.

Jee-Hyun Lee
Department of Biology
Graduate School
Sungshin Women`s University

Serum levels of anti-Müllerian hormone (AMH) have been demonstrated to positively correlate with the size of the primordial follicle pool and considered to marker for ovarian reserve. As a woman ages, there is a decrease in blood level of AMH by the decrease the number of growing follicles. According to the known evidences, due to the development of methods for freezing and thawing of embryos during in vitro fertilization (IVF), fresh embryos can be frozen and withheld for embryo transfer (ET) in fresh cycles depending on the patient's physiological and histological status, and thawed at the appropriate time for patients. And many studies have shown that this method is similar to the fresh cycle pregnancy rate. So far, it is not well uncovered about the positive effects of freeze-all embryo transfer (FAET) in poor ovarian revere groups. To improve the IVF-ET in poor responder or poor ovarian-responder (POR), a study is needed to determine whether FAET has the same results in the ovarian low-response group compared with normal-

response group. The normal and poor responder groups can be distinguished according to internationally defined ovarian response deterioration standards. Based on the criteria the IVF-ET patients were distinguished and compared by the clinical criteria with retrospective study method (from March 2018 to November 2019 at Suzy Maria Hospital). The patients were classified into fresh cycle and FAET cycle. The characteristics of the patients were compared and analyzed by dividing the normal ovary response group and the low ovary response group. Analyze the results of FAET in the normal ovarian response group and the POR group. In the POR group, the results of fresh cycle embryo transfer and thawing embryo transfer were analyzed. As a result of the analysis, there was no statistically significant difference between the fresh cycle and thawed after FAET cycle . Although there was no statistical significance, the tendency of the pregnancy rate to decrease as the ovarian function decreased overall was clear. Although needed more number of case, FAET had tendency to more positive in successful pregnancy in normal responder after 42 years old but not showed similar patterns in poor responder. The characters which mostly affect on pregnancy in 38≦~<40 was the numbr of collected oocytes and tranfered embryos, in 40≦~<42 was the blood P4 level, in 42≦~<44 was no significant criteria, and in ≧44 was the number of transfered embryos in poor responder fresh ET groups. On the other hand, in the poor responder with FAET, the characters which mostly affect on pregnancy was no significant criteria in 38≦~<40, the blood P4 level in 40≦~<42, the blood levels of FSH and AMH in 40≦~<42, and the blood levels of

FSH and E2, BMI, and the number of transfered embryos in ≧44 group. Therefore, it seems to be necessary to make more careful decisions about the timing of transplantation of the poor-response group.

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INTRODUCTION

Although, in vitro fertilization procedures have made great strides as a way to overcome infertility and over a decade, the number of pregnancies and childbirths in Korea has been reduced to less than 1.0 a year. It is time to increase the pregnancy rate even if you overcome infertility to solve the expected social problems. Particularly, patients suffering from infertility are getting older (Sartorius et al, 2010), or declined ovarian function by unknown reasons regardless of age are met a hard hurdle. So in these patients, age and decreased ovarian function should be overcome by direct or indirect approaches to increase the success rate of in vitro fertilization. Oocyte or embryo cryopreservation protocols are one of the indirect approaches and prevalent method in critiques (Chen et al., 2016).

This study was started to consider which is more effective embryo transfer time for patients with low ovarian reserve. Repeated clinical studies have demonstrated that blood anti-Müllerain hormone (AMH) levels correlate strongly to antral follicle count and are more accurate than age and other conventional serum markers (follicle-stimulating hormone, 17β-estradiol (E2), inhibin B) in predicting preovulatory oocyte supply in response to ovulation induction (Broekmans et al.,2009; Vet et al., 2002). Relative to these conventional ovarian serum makers, AMH appears to vary significantly less throughout the menstrual cycle or with perturbations of the endocrine system (Hansen et al., 2011; Iliodromiti et al., 2014).

As a major cause of infertility, ovarian dysfunction may be suggested along with an increase in patient age (Busnelli et al., 2015; Ferraretti et al., 2011; Iliodromiti et al., 2015). The degree of ovarian function can be determined by AMH in blood and total antral follicle count (AFC) (Dewailly et al., 2014; Fleming et al., 2012; Hansen et al., 2011; Visser et al., 2006). AMH is a glycoprotein hormone secreted by the granulosa cells of preantral and small antral follicles (Dewailly et al., 2014). Blood levels of AMH have been demonstrated to positively correlate with the size of the primordial follicle pool and the number of antral follicles (Andersen et al., 2015; Hansen et al., 2011). According to the Bologna Criteria, a consensus was reached on the minimal criteria needed to define poor ovarian response (POR) (Ferraretti et al., 2011). The group of patients with low ovarian function is defined as; i) Advanced maternal age (≥40 years) or any other risk factor for POR, ii) A previous POR (≤3 oocytes with a conventional controlled ovarian hyperstimulation (COH) protocol), iii) An abnormal ovarian reserve test (i.e. AFC < 5–7 follicles or AMH < 0.5–1.1 ng/ml).

In recent years, including techniques for measuring physiological hormone levels for endometrial receptors in the fresh COH cycle (Roque et al., 2015; Shapiro et al., 2015) and Due to the development of embryo freezing technology, embryo cryopreservation has become a routine application technique for IVF, increasing the chance to perform which freezes all embryos (Cobo at al., 2012; Roque et al., 2015). Freeze-all embryo transfer (FAET) improve outcomes (Weinerman and Mainigi, 2014). During COH, excessive follicular development and

supraphysiologic serum concentrations of E2 can lead to a premature rise of progesterone (P4) in the late follicular phase (Bosch et al., 2010 ; Venetis et al., 2007, 2008), resulting in asynchrony associated with implantation failure (Younis, 2011). Because of these conflicting findings, further larger studies are required in the future to determine whether extended culture or embryo freezing is the preferred route for managing patients with elevated P4. POR patients who receive IVF treatment have an average pregnancy rate of 15% or less, which is very low compared to those who do not (La Marca et al., 2015; Polyzos et al., 2012; Tarlatzis et al., 2003). When considering the effects of hormones and stimulation, comparing various criteria effecting pregnancy and directions will help poor responders.

So far, the patient's age is the best available validate the ovarian reserve and responsibility, and blood AMH levels project well the number of follicles. FAET or fresh ET have been decided by a few criteria including the number of pick-upped oocyte or P4 level (Ferraretti et al., 2011; Mizrachi et al., 2020). Although FAET become prevalent in poor responder or old age groups, it is necessary to evaluate the effect of a better method among fresh ET and FAET on IVF results of POR patients in establishing the direction of clinical trials to improve the pregnancy rate of the POR target group

Materials and Methods

Institutional Review Board Approval

This study was based on the basic database of Suzi Maria center, which at the time of this presented analysis included 1,300 consecutive autologous fresh IVF cycles. This study is a retrospective study in which no patient's personal information is entered and was done without the consent of the patient. The study was reviewed and approved by the Center's Institutional Review Board

Study design

Retrospectively compared fresh 1091 fresh embryo transfer and 237 vitrified/warmed embryo transfer between March 2018 and November 2019 of cleavage embryos or blastocysts originating from the Controlled Ovarian Stimulation cycles. We also analyzed embryo recovery after vitrification/warming or maternal age had an impact on clinical outcomes. Most of the culture medias were used Maria Medical Foundation.

Hormonal assay

Blood was collected and AFC determined on day 2–4 of the menstrual cycle. Blood samples were analyzed for circulating E2 and P levels on the day of human chorionic gonadotropin(hCG) administration. Serum and plasma specimens were tested for AMH, follicle-stimulating hormone (FSH), and E2 with the use of the automated Beckman Coulter Access immunoassay analyzer . Serum AMH levels were assessed in serum by

the enzyme linked immunosorbent assay (ELISA) method (Beckman Coulter, USA). All assays were commissioned by Green Cross Medical corporation.

Study Population

During the study period, 1,091 fresh ET cycles and 237 freeze all/ thawed ET cycles of 867 patients were examined. In the normal responder fresh ET group, 383 patients in the 577 cycles are control group. In the experimental group, the poor responder group included 388 patients in 612 ET cycles. Which under the Bologna criteria qualified as ‘poor responder’ cycles because they produced three or fewer oocytes and involved women above age 40 and/or with AMH <0.5~1.1 ng/mL. In this study, the ‘poor responder’ group was classified as AMH <1.1 ng/mL and the study was conducted. Based on standard age groups used by the Society for Assisted Reproductive Technology (SART; <35; 35–37; 38–40; 41–42; and 43–44 years)(Lauren A et al, 2017), In this paper, the age group is classified as follows (38≤~<40, 40≤~<42, 42≤~<44, 44≤).

Table 1. Characteristics of patients defined as normal ovarian responders (AMH ≥ 1.1 ng/ml)

Variable	Fresh ET cycles (n=577),Mean ± SD	Freeze-all ET cycles (n=139), Mean ± SD	P value
Age (years)	40.80 ± 2.15	40.47 ± 2.20	0.110
Body mass index (kg/m²)	22.17 ± 4.82	22.23 ± 3.44	0.861
AMH level (ng/mL)	2.59 ± 1.65	3.77 ± 2.76	<.001 [a]
FSH level on day 2 or 3 of menses (mIU/mL)	8.01 ± 3.13	7.07 ± 2.67	<.001 [a]
E2 level on trigger day (pg/mL)	2428.16 ± 1369.12	3411.92 ± 2243.49	<.001 [a]
Progesterone level on trigger day (ng/mL)	0.68 ± 0.55	1.87 ± 1.80	<.001 [a]
HCG day Endometrium thickness (mm)	9.58 ± 1.48	9.00 ± 0.95	<.001 [a]
Retrieved oocytes (*n*)	9.94 ± 5.48	13.78 ± 9.00	<.001 [a]
2PN zygote (*n*)	7.43 ± 4.20	9.94 ± 6.21	<.001 [a]
3 days good embryos (*n*)	2.86 ± 2.54	3.10 ± 2.79	0.349
Transferred embryos (*n*)	2.62 ± 0.62	2.64 ± 0.64	0.689

(✳ [a] Significance was reached at P<0.05)

Table 2. Characteristics of patients defined as poor ovarian responders (AMH < 1.1 ng/ml)

Variable	Fresh ET cycles (n=514),Mean ± SD	Freeze-all ET cycles (n=98),Mean ± SD	P value
Age (years)	42.06 ± 2.56	41.69 ± 2.63	0.205
Body mass index (kg/m²)	22.12 ± 3.18	21.83 ± 2.55	0.327
AMH level(ng/mL)	0.51 ± 0.29	0.52 ± 0.29	0.868
FSH level on day 2 or 3 of menses (mIU/mL)	11.75 ± 5.75	12.34 ± 8.56	0.523
E2 level on trigger day (pg/mL)	1153.55 ± 751.78	1170.74 ± 994.78	0.878
Progesterone level on trigger day (ng/mL)	0.60 ± 0.54	1.27 ± 2.72	0.006[a]
HCG day Endometrium thickness(mm)	8.96 ± 1.38	8.93 ± 1.19	0.857
Retrieved oocytes (*n*)	3.74 ± 2.57	3.27 ± 3.04	0.152
2PN zygote (*n*)	2.96 ± 1.97	2.76 ± 2.42	0.441
3 days good embryos (*n*)	1.34 ± 1.33	1.19 ± 1.31	0.325
Transferred embryos (*n*)	2.13 ± 0.83	2.17 ± 0.81	0.648

(✳ [a] Significance was reached at P<0.05)

Ovarian stimulation and luteal phase support for fresh embryo transfer

Gonadotropin-releasing hormone (GnRH) agonist long protocol or GnRH antagonist protocol was used for follicle growth. Most patients used an antagonist protocol. Patients underwent COS with recombinant follicle stimulating hormone (FSH) (Gonal-f, MerckSerono, Darmstadt, Germany) starting on day 2 or 3 of menses, with doses ranging from 150 to 450 international units per day, according to the patient's age, in a step-down protocol. A GnRH antagonist (Orgalutran, MSD, New Jersey, United States) was used to prevent a premature luteinizing hormone (LH) surge. It was used for pituitary suppression when a leading follicle achieved 14 mm. Final oocyte maturation was induced with recombinant human chorionic gonadotropin (hCG) (Ovidrel, MerckSerono, Darmstadt, Germany) when follicles reached a diameter of 18 mm. The patients underwent Transvaginal ultrasound-guided oocyte retrieval 36 hours after the trigger. Luteal phase Support started on the day of oocyte retrieval. All patients received vaginal micronized progesterone in gel form (Crinone8%, MerckSerono, Darmstadt, Germany) in a single daily administration. Progesterone was used for R13 days, when a pregnancy test was performed, and until 9 weeks if pregnancy was confirmed.

Ovarian stimulation and luteal phase supporting for vitrified/warmed embryo transfer

In the normal ovulation patients, the endometrium preparation protocol was based on detection of ovulation during a natural cycle. E2, P4 and

LH were monitored. Some of the normal ovulation patients were administered hCG for accurate synchronization of the endometrium. In patients with irregular cycles, 2.5 to 5 mg letrozole (Femara, Novartis, Basel, Switzerland) was used for endometrium preparation on cycle day 3. Then, follicle growth was monitored by ultrasound. When the follicles reached the criteria of maturation, hCG (10,000 IU) was injected to trigger ovulation..

Oocyte collection and insemination

Oocytes were retrieved 36 hours after hCG administration by means of transvaginal ultrasound–guided aspiration. Cumulus oocyte complex (COC) aspirated using 19G aspiration needle with flushing media (FF, Maria Medical Foundation, Seoul, Korea). COC were picked under a stereomicroscope. After collection, COCs were washed and transferred to a incubated by placing them in a insemination media (MRC#D01, Maria Medical Foundation, Seoul, Korea) prepared in overnight. Sperm was treated by a two-step swim-up method (MRC#SC, MRC#D01, Maria Medical Foundation, Seoul, Korea). Oocytes were denuded from cumulus cells immediately after oocyte retrieval for ICSI. Conventional IVF and ICSI procedures were performed 3–4 hours after oocyte retrieval.

Embryo culture and evaluation of embryo morphology

Fertilization was assessed 16–18 hours post-insemination by the presence of two pronuclei. The embryos were cultured sequentially in cleavage and blastocyst medium (MRC#D13 and MRC#D46, Maria

Medical Foundation, Seoul, Korea) 40μL covered with Hypure Heavy oil (93621, Kitazato, Tokyo, Japan) 1ml in 5 well culture dish (16004, Vtrolife) prepared in overnight. In a humidified or dried atmosphere containing 6% CO_2, 5% O_2, and 89% N_2 and maintained at 37ºC for 105 hours. Our laboratory distinguishes embryos in five stages, with the second stages selected as good embryos. A “good” quality day 3 embryo was defined as having more than 7-blastomeres of equal size and less than 20% fragmentation. The quality of blastocyst-stage embryos was assessed in the morning of day 5, which was categorized according to the criteria of Gardner and Schoolcraft (Gardner, Schoolcraft, 1999 ; Kang et al., 2011)

Embryo vitrification procedure

Vitrification was performed by dividing it into a cleavage stage and blastocyst stage. Briefly, cleavage embryos were transferred into Vitrification Solution 1 (VS1) consisting of 1.5 M ethylene glycol (EG, Sigma, St. Louis, United States) dissolved in Dulbecco’s phosphate buffered saline (DPBS, Maria Medical Foundation, Seoul, Korea). supplemented with 10% serum protein supplement (SPS, Origio-SAGE, CooperSurgical, Trumbull, Connecticut, United States) for 2 min 30 sec in cleavage embryos. embryos were transferred into Vitrification Solution 2 (VS2) consisting of 5.5 M ethylene glycol, 1 M sucrose (Sigma, St. Louis, United States) dissolved in DPBS supplemented with 10% SPS for 20 seconds. All the steps were performed at 37°C. After the exposure to VS2, embryos were quickly loaded into an electron microscopy (EM) grid (Cooper Surgical, Trumbull, Connecticut, United States) or thin plastic

strip (TPS, SPL Life Science, Seoul, Korea), and plunged into LN_2. Blastocyst embryos were transferred into Vitrification Solution 1 (VS1) consisting of 7.5% ethylene glycol and 7.5% dimethyl sulphoxide (DMSO, Sigma, St. Louis, United States) dissolved in DPBS supplemented with 20% SPS for 2 min 30sec in blastocyst embryos after an initial shrinkage. Embryos were transferred into Vitrification Solution 2 (VS2) consisting of 15% EG, 15% DMSO, 0.5 M sucrose dissolved in DPBS supplemented with 20% SPS for 20 sec. All the steps were performed at 37°C. After the exposure to VS2, embryos were quickly loaded into an EM grid or thin plastic strip, and plunged into LN_2. In the case of the expanded embryos, artificial shrinkage was performed using laser (RI, Cooper Surgical, Trumbull, Connecticut, United States)

Embryo warming procedure

Cleavage embryos loaded on EM grid or TPS were transferred for 2 min 30 sec to a warming solution containing 1 M sucrose dissolved in DPBS supplemented with 20% SPS followed by transfer to a second warming solution containing 0.5 M sucrose dissolved in DPBS supplemented with 20% SPS for 2 min 30 sec. Transfer to third warming solution containing 0.25 M sucrose dissolved in DPBS supplemented with 20% SPS for 2min 30sec, transfer to forth warming solution containing 0.125 M sucrose dissolved in DPBS supplemented with 20% SPS for 2 min 30 sec, and last transfer to warming solution containing only DPBS supplemented with 20% SPS for 2 min 30 sec The embryos were then washed three times in MRC#D46 and cultured in an inner-well dish for further culture.

Blastocyst embryos loaded on EM grid or TPS were transferred for 2 min 30 sec to a warming solution containing 0.5 M sucrose dissolved in DPBS supplemented with 20% SPS followed by transfer to a second warming solution containing 0.25 M sucrose dissolved in DPBS supplemented with 20% SPS for 2 min 30 sec. Third warming solution containing 0.125 M sucrose dissolved in DPBS supplemented with 20% SPS for 2 min 30 sec, and last transfer to warming solution containing only DPBS supplemented with 20% SPS for 2 min 30 sec The embryos were then washed three times in MRC#D46 and cultured in an inner-well dish for further culture at 37°C in an atmosphere of 6% CO_2, 5% O_2 and 89% N_2. The post-thawing survival of the embryos was observed under an inverted microscope 16 to 20 hours after warming, and assisted hatching by laser.

Embryo transfer

Embryo transfers were performed on day 3 or in the blastocyst stage with the use of abdominal ultrasound guidance. After the embryo was immersed in Embryo Glue (Vitrolife, Sweden) 0.5mL for 2 to 5 min, 0.2mL was inserted into the uterus using a catheter (COOK medical, Bloomington, Indiana, United States). On the cleavage stage, two embryos were transferred under the age of 35, and three were transferred over the age of 35. On the blastocyst stage, embryos were transferred under the age of 35 and two over the age of 35, and the principles recommended by the Ministry of Health and Welfare were followed.

Clinical outcomes

All data were obtained from medical records and follow-up lasted until the 12th week of pregnancy, The main outcome measure was ongoing pregnancy rate. Implantation rate (IR), positive pregnancy test rate and clinical pregnancy rate were secondary outcome measures. Positive pregnancy test was defined by elevated (>20 pg/mL) ß-hCG levels measured 11 days after ET. Clinical pregnancy ratio was defined by the presence of at least one gestational sac on ultrasound examination vs the number of transferred cycles.

Statistical analysis

Baseline characteristics were presented as number, mean+SD where appropriate. The data were examined using ANOVA or chi-square analysis to determine whether the differences in implantation and pregnancy rates were significant for each group (IBM SPSS statistics, version 25). The results were considered statistically significant at p-values of <0.05.

Result

Baseline characteristics

In normal ovarian response group, the physiological characteristics were statistically different between fresh ET and FAET groups except the age, body mass index (BMI) (Table 1). 3 days good embryos and transferred embryos were also not statistically significant. On the other hand, in poor ovarian response group, physiological characteristics were not statistically different between fresh ET and FAET groups except blood P4 levels (Table 2). This is because, in many literatures, patients with high P4 levels freeze all because of the fact that early rise of p4 level does not help implantation in the uterine (Huang et al., 2012).

Table 3. Age difference classification of basic characteristics according to the fresh ET and FAET of two AMH groups. (a) age 38≤~<40 (b) age 40≤~<42 (c) age 42≤~<44 (d) age 44≤

(A)

Variable	age 38≤~<40 (AMH≥1.1ng/ml)		P value	age 38≤~<40(AMH<1.1ng/ml)		P value
	Fresh ET (n=255)	Freeze-all ET (n=63)		Fresh ET (n=132)	Freeze-all ET (n=18)	
Age (years)	38.93±0.60	38.52±0.53	<0.05[a]	39.04±0.70	38.56±0.51	<0.05 [a]
ody mass index (kg/m²)	22.48±6.43	21.84±3.20	0.27	21.89±3.69	21.77±2.84	0.87
AMH level(ng/mL)	2.69±1.69	4.34±2.85	<0.05 [a]	0.50±0.28	0.52±0.34	0.80
FSH level (mIU/mL)	7.82±2.87	7.19±2.47	0.09	11.27±5.57	13.50±6.20	0.17
Ξ2 level on trigger day (pg/mL)	2516.93±1051.28	3852.80±2286.30	<0.05 [a]	1169.35±666.14	1585.11±1041.43	0.13
ᵖ4 level on trigger day (ng/mL)	0.68±0.54	1.93±1.70	<0.05 [a]	0.39±0.31	1.99±3.78	0.12
HCG day Endometrium thickness(mm)	9.60±1.49	9.00±0.95	<0.05 [a]	9.10±1.54	8.74±1.43	0.40
Retrieved oocytes (*n*)	10.95±5.17	17.70±10.19	<0.05 [a]	4.12±2.58	5.22±4.41	0.31
2PN zygote (*n*)	8.21±3.96	12.68±7.04	<0.05 [a]	3.32±2.09	4.17±3.33	0.31
3 days good embryos (*n*)	3.30±2.50	3.97±3.01	0.11	1.48±1.46	1.39±1.20	0.76
Transferred embryos (*n*)	2.58±0.58	2.62±0.58	0.64	2.26±0.81	2.50±0.62	0.15

(✳ [a] Significance was reached at P<0.05)

(B)

Variable	age 40≤~<42 (AMH≥1.1ng/ml)		P value	age 40≤~<42(AMH<1.1ng/ml)		P value
	Fresh ET (n=158)	Freeze-all ET (n=37)		Fresh ET (n=125)	Freeze-all ET (n=33)	
Age (years)	40.90±0.68	40.68±0.47	<0.05 [a]	40.81±0.74	40.32±0.47	<0.05 [a]
ody mass index (kg/m²)	21.67±2.81	22.96±3.52	<0.05[a]	21.83±2.99	21.56±2.39	0.57
AMH level(ng/mL)	2.73±1.73	3.36±2.97	0.22	0.53±0.28	0.58±0.30	0.36
FSH level (mIU/mL)	8.08±3.02	6.76±2.00	<0.05 [a]	11.12±5.80	10.48±7.25	0.63
E2 level on trigger day (pg/mL)	2424.61±1444.71	3061.96±2344.33	0.14	1272.17±749.87	1424.80±1187.39	0.50
P4 level on trigger day (ng/mL)	0.63±0.36	1.22±0.79	<0.05 [a]	0.45±0.31	0.79±0.82	<0.05 [a]
HCG day Endometrium thickness(mm)	9.50±1.30	9.14±0.86	0.07	9.02±1.28	9.22±1.17	0.43
Retrieved oocytes (*n*)	9.66±5.60	11.70±7.55	0.13	4.02±2.59	3.59±2.95	0.44
2PN zygote (*n*)	7.20±4.32	8.38±5.03	0.20	3.27±2.05	3.03±2.48	0.59
3 days good embryos (*n*)	2.64±2.53	2.81±3.02	0.75	1.48±1.37	1.27±1.39	0.42
Transferred embryos (*n*)	2.64±0.64	2.46±0.80	0.21	2.25±0.77	2.22±0.92	0.85

(✳ [a] Significance was reached at P<0.05)

(C)

Variable	age 42≤~<44 (AMH≥1.1ng/ml)		P value	age 42≤~<44(AMH<1.1ng/ml)		P value
	Fresh ET (n=105)	Freeze-all ET (n=23)		Fresh ET (n=110)	Freeze-all ET (n=13)	
Age (years)	42.77±0.60	42.52±0.51	<0.05 [a]	42.86±0.75	42.57±0.51	0.08
ody mass index (kg/m²)	21.77±2.88	22.00±3.71	0.79	22.74±2.80	21.84±1.98	0.14
AMH level(ng/mL)	2.36±1.46	3.08±1.99	0.11	0.51±0.31	0.48±0.26	0.74
FSH level (mIU/mL)	8.04±3.21	7.13±2.78	0.18	11.59±5.02	12.59±10.77	0.74
Ξ2 level on trigger day (pg/mL)	2699.68±1860.81	3659.23±2257.50	0.07	1206.81±711.23	1086.94±614.37	0.51
ᵖ4 level on trigger day (ng/mL)	0.80±0.68	1.57±1.55	<0.05 [a]	0.54±1.04	1.25±1.85	0.18
HCG day Endometrium thickness(mm)	10.14±1.85	8.88±1.03	<0.05 [a]	9.07±1.46	8.64±0.74	0.11
Retrieved oocytes (*n*)	9.98±5.42	9.70±4.48	0.79	4.02±2.80	2.86±2.25	0.09
2PN zygote (*n*)	7.28±4.06	7.30±3.42	0.97	2.99±1.94	2.50±1.79	0.35
3 days good embryos (*n*)	2.67±2.34	1.91±1.65	0.07	1.17±1.29	1.71±1.54	0.23
Transferred embryos (*n*)	2.70±0.57	2.91±0.29	<0.05 [a]	2.16±0.82	1.93±0.73	0.28

(✳ [a] Significance was reached at P<0.05)

(D)

Variable	age 44≤ (AMH≥1.1ng/ml)		P value	age 44≤ (AMH<1.1ng/ml)		P value
	Fresh ET (n=58)	Freeze-all ET (n=16)		Fresh ET (n=147)	Freeze-all ET (n=24)	
Age (years)	45.16±0.87	44.69±1.01	0.10	45.25±1.18	45.10±1.09	0.49
ody mass index (kg/m²)	22.84±3.39	22.38±3.81	0.66	22.11±3.06	22.68±3.23	0.37
AMH level(ng/mL)	2.16±1.48	3.45±2.59	0.07	0.51±0.29	0.48±0.27	0.52
FSH level (mIU/mL)	8.65±4.19	7.27±4.35	0.27	12.85±6.28	13.45±9.92	0.76
Ξ2 level on trigger day (pg/mL)	1567.06±896.19	2062.39±960.51	0.09	1004.12±833.47	688.13±621.27	<0.05 [a]
ᵖ4 level on trigger day (ng/mL)	0.58±0.66	2.71±2.69	<0.05 [a]	0.27±0.21	0.45±0.70	0.25
HCG day Endometrium thickness(mm)	8.94±1.15	8.84±1.11	0.79	8.69±1.22	8.76±1.22	0.81
Retrieved oocytes (*n*)	6.22±5.04	9.06±5.04	0.06	2.95±2.20	1.83±1.32	<0.05 [a]
2PN zygote (*n*)	4.95±4.17	6.56±3.44	0.12	2.33±1.66	1.70±1.18	<0.05 [a]
3 days good embryos (*n*)	1.84±2.70	2.06±1.34	0.66	1.20±1.19	0.73±1.05	<0.05 [a]
Transferred embryos (*n*)	2.55±0.78	2.75±0.68	0.33	1.90±0.87	2.00±0.79	0.53

(✳ [a] Significance was reached at P<0.05)

Clinical outcomes after fresh embryo transfer or vitrified/warmed embryo transferred in Normal character (AMH ≥ 1.1 ng/mL) vs Poor character (AMH < 1.1 ng/mL)

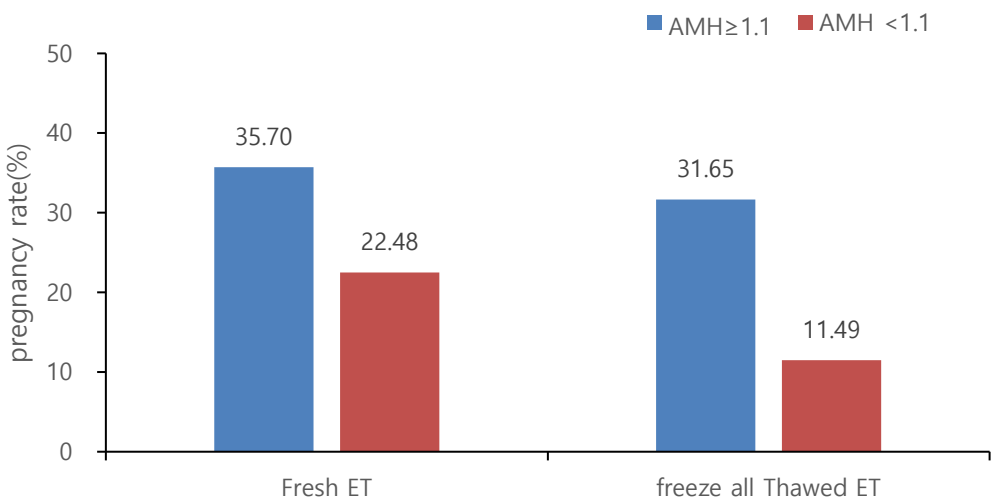


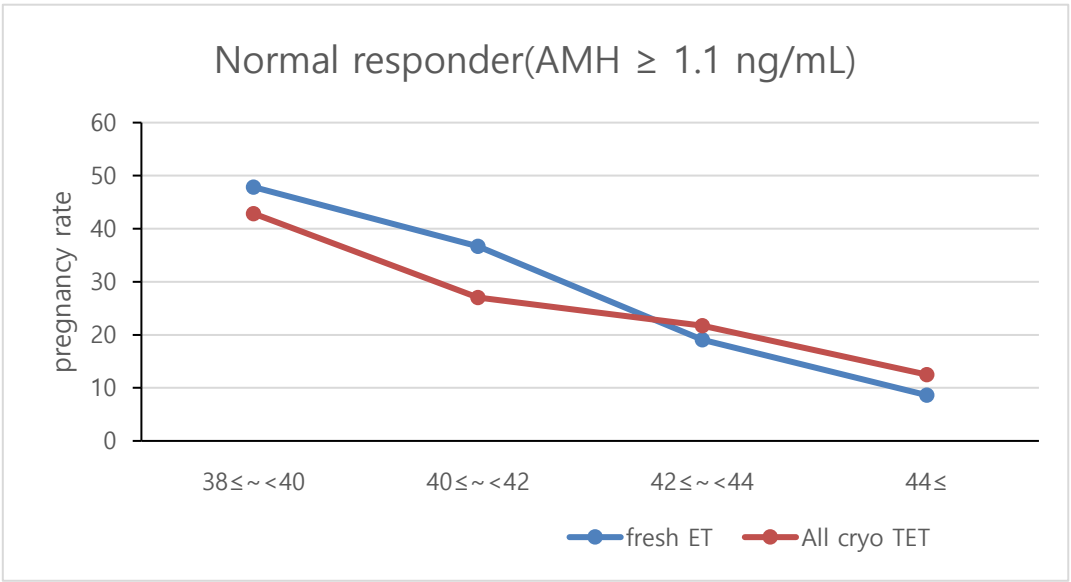
Figure 1. Comparison of pregnancy rates between normal responder(AMH ≥ 1.1 ng/mL) and poor responder(AMH < 1.1 ng/mL).

The clinical pregnancy rate was no statistically difference between normal response fresh ET and FAET (37.50% and 31.65%, respectively). However, the clinical pregnancy rate was statistically significant (P<0.05) between fresh ET and FAET of the poor response group (22.48% and 11.49%, P<0.024, χ^2 test).).

Trends in clinical outcomes in both normal response (AMH ≥ 1.1 ng/mL) and poor response (AMH < 1.1 ng/mL) groups according to changes in age difference

Although, there was no statistical significance, in the normal response group (Figure 2(A)) from 38 to 42 years, fresh ET showed a slightly higher pregnancy rate than FAET (fresh ET 47.84% vs FAET 42.86%). However, in FAET group, the pregnancy rate showed a slightly better than the fresh ET starting from the age of 42 as the age increased (fresh ET 19.00% vs FAET 21.74%). The poor response group overall indicated that fresh ET were higher in pregnancy rates than FAET (Figure 2(B)).

(A)



(B)

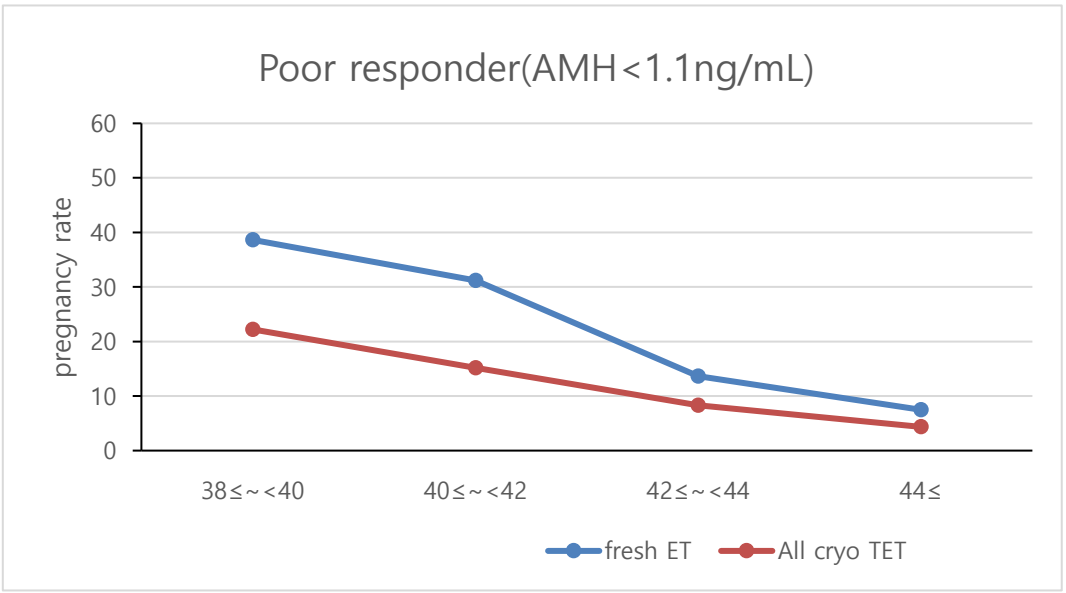


Figure 2. Age difference on pregnancy rate. (A) normal responder group, (B) poor response group.

Differences in AMH and factors affecting pregnancy rates in fresh ET or freeze-all ET

In the fresh ET of the normal response group, as the age increased, the pregnancy rate decreased significantly (OR=0.736, P＜0.05). In addition, the pregnancy rate decreased as the P4 level increased (OR=0.392, P<0.05), and the pregnancy rate decreased statistically as the retrieved oocytes were increased (OR=0.87, P<0.05). On the other hand, the number of embryos (OR=1.202, P<0.05), the number of embryos of good quality on the 3 days (OR=1.187, P<0.05), and the number of transferred embryos (OR=1.935, P<0.05) showed statistically significant increase in pregnancy rate. In the FAET, the only increase in the total number of embryos showed a statistically significant increase in pregnancy rate (OR=1.323, P<0.05).

In the fresh ET of the poor response group, the pregnancy rate decreased statistically as the P4 level increased (OR=0.131, P<0.05). On the other hand, on the 3days more embryos of good quality (OR=1.427, P<0.05), the more the number of transferred embryos (OR=2.255, P<0.05), and on the hCG triggering day, the thicker the endometrium (OR=1.402, P<0.05), the higher the pregnancy rate. In the FAET, the pregnancy rate decreased statistically with age (OR=0.62, P<0.05).

Table 4. Factors affecting pregnancy rates with ET method according to AMH difference.

	Effect factor	Mean difference (95% CI)	P-value
fresh ET (AMH≥1.1)	Age	OR=0.736 (CI 0.664~0.816)	<0.000
	Progesterone	OR=0.392 (CI 0.208~0.737)	<0.004
	Retrieved oocytes	OR=0.87 (CI 0.78~0.97)	<0.012
	2PN zygotes	OR=1.202 (CI 1.043~1.386)	<0.011
	3day good embryos	OR=1.187 (CI 1.078~1.307)	<0.000
	Transferred embryos	OR=1.935 (CI 1.342~2.791)	<0.000
Freeze-all ET (AMH≥1.1)	2PN zygotes	OR=1.323 (CI 1.048~1.669)	<0.018
fresh ET (AMH<1.1)	Progesterone	OR=0.131 (CI 0.042~0.409)	<0.000
	3day good embryos	OR=1.427 (CI 1.166~1.747)	<0.001
	Transferred embryos	OR=2.255 (CI 1.479~3.437)	<0.000
	HCG day Endometrium thickness	OR=1.402 (CI 1.116~1.762)	<0.004
Freeze-all ET (AMH<1.1)	Age	OR=0.62 (CI 0.419~0.917)	<0.017

(✳ OR : Odd ratio, CI : Confidence intervals)

Table 5. Factors affecting pregnancy rates with age.

	38≤~<40	**40≤~<42**	**42≤~<44**	**44≤**
fresh ET (AMH≥1.1)	3day good Ems, Transferred Ems	progesterone Transferred Ems		FSH, AMH
Freeze-all ET (AMH≥1.1)	AMH, Retrieved Oos, 2PN zygotes, 3day good Ems,		E2 level, Endometrium thickness	Age, E2 level, 3day good Ems
fresh ET (AMH<1.1)	Retrieved Oos, 2PN zygotes, 3day good Ems, Transferred Ems	Progesterone 3day good Ems, Transferred Ems		Age, Transferred Ems
Freeze-all ET (AMH<1.1)		progesterone	Age, AMH, BMI	

Effects of age difference on pregnancy rate

Factors influencing the difference in fresh ET or FAET method according to the difference in AMH were classified according to age. It was performed student's t-test, and all showed statistical significance of P value less than 0.05 (Table 5).

At age 38≤~<40, the number of retrieved oocytes, the number of good embryos at 3 days, and the number of transferred embryos were classified as factors affecting pregnancy. On the other hand, at age 40≤~<42, progesterone appeared to be a factor influencing AMH difference and fresh ET or FAET. At age 42≤~<44, In fresh ET, no specific factors were found to affect AMH difference, but in FAET, E2 level and endometrium thickness affected pregnancy rate in the normal response group, but age, AMH, and BMI affected the pregnancy in the poor response group. And at the age 44≤, The physiological factors of FSH and AMH influenced the pregnancy rate in fresh ET in the normal response group. In the fresh ET of the poor response group, age and the number of transferred embryos were found to affect the pregnancy rate.

Discussion

One of the main reasons for cryopreservation or ‘freeze-all’ of embryos has been attributed to a method of decreasing the risk of ovarian hyperstimulation syndrome (Fatemi et al., 2014). Some clinics are now advocating for a policy for elective cryopreservation of all cycles based on the endometrial peri-implantation environment (Weinerman et al., 2014), reduced pregnancy rates associated with fresh embryo transfers (Shapiro et al., 2011). SART CORS data to demonstrate that infants conceived in fresh embryo transfer cycles have a higher risk of low birth weight than infants conceived after frozen embryo transfer cycles, potentially owing to the supraphysiologic E2 levels resulting from ovarian stimulation (Kalra el al., 2011). Another 'freeze all' is due to an increase premature progesterone rise negatively correlated with live birth rate in IVF cycles (Huang et al., 2012). Actually, in most studies, the methods to determine the cutoff value have been main focus. The cutoff value for the progesterone level on hCG triggering day in this study was 1.5ng/mL (Bosch et al, 2003).

In the Roque group, the results of the study on the 'freeze all' policy of the normal response and poor response groups (Roque et al., 2015, 2018). The normal response group were all measured IVF outcomes (implantation, clinical pregnancy rate, and ongoing pregnancy rate) were higher in the freeze-all group. But poor response group were no significant difference in the clinical pregnancy rate (14.1% vs 13.7%,

respectively). The results of Roque groups are different from this study. In this study, there was no difference between fresh ET and FAET pregnancy rates (37.50% and 31.65%, respectively) in the normal response group, and in the poor response group, fresh ET was statistically significantly better than FAET (22.48% and 11.49%, $P<0.024$).

Biomarkers of ovarian reserve, FSH and AMH are not useful to assess the reproductive potential among late-reproductive age women (Steiner et al., 2017). More recent studies have contradicted these studies, finding similar pregnancy rates in patients with normal and elevated basal FSH levels (Esposito et al., 2002 ; Haadsma et al., 2009). FSH levels may be of less value in predicting IVF outcomes than previously thought. Whereas decline in oocyte quantity corresponds with elevated FSH and advancing age, a correlation between basal FSH and oocyte quality is less clear. In 30-44 older reproductive-age women, low AMH and high FSH levels are not associated with reduced fecund ability, or a lower cumulative probability of conceiving by 6 or 12 cycles of pregnancy attempt (Steiner et al., 2017). Nevertheless, physiological factors have been shown to affect pregnancy rates after age 40. Mean serum FSH levels consistently increased with increasing age in infertile women and increased levels of FSH and decreased AMH can be considered as a marker for reduced fertility potential (Raeissi et al., 2015). Interestingly, the blood FSH level had meaning in the older age group (≧44 years). In other groups, there were no significant meaning in pregnancy rate.

Repeated clinical studies have demonstrated that serum AMH levels correlate strongly to antral follicle count and are more accurate than age and other conventional serum markers in predicting preovulatory oocyte supply in response to ovulation induction. In the case of the blood AMH level, pregnancy rate was effected only ≧44 in fresh ET group of normal response group. On the other hand, in poor response group, it was affected only 38≦~<40 group at FAET. E2 and P4 are critical in uterine responsibility and decidualization. The effects of them on pregnancy were various by the blood AMH levels and age. It means the blood AMH level is not critical factor for uterine receptibility. It means that it should be considered the age and other factors such as the blood levels of E2 or FSH when decide clinical decision.

In summary, there was no statistically significant difference between the fresh cycle and thawed after FAET cycle . Although there was no statistical significance, the tendency of the pregnancy rate to decrease as the ovarian function decreased overall was clear. Although needed more number of case, FAET had tendency to more positive in successful pregnancy in normal responder after 42 years old but not showed similar patterns in poor responder. The characters which mostly affect on pregnancy in 38≦~<40 was the numbr of collected oocytes and tranfered embryos, in 40≦~<42 was the blood P4 level and number of transfered embryos, in 42≦~<44 and ≧44 were the number of transfered embryos in poor responder fresh ET groups. On the other hand, in the poor responder with FAET, the characters which mostly affect on pregnancy

was no significant criteria in 38≦~<40, the blood P4 level in 40≦~<42, the blood levels of FSH and AMH in 40≦~<42, and the blood levels of FSH and E2, BMI, and the number of transfered embryos in ≧44 group. Therefore, it seems to be necessary to make more careful decisions about the timing of transplantation of the poor-response group.

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논 문 개 요

항뮬레리안 호르몬(AMH)의 혈청 수준은 원시 모낭 풀의 크기와 양에 긍정적 상관 관계가 있는 것으로 입증되었으며, 여성이 나이가 들어감에 따라 AMH 의 혈청 농도가 감소한다고 알려져 있다. 여러 논문에 따르면 체외수정 시술 시 배아의 동결과 해동에 관한 방법의 발전으로 인해 환자의 호르몬 상태나 자궁내막 상태에 따라 신선주기에서의 이식을 보류하고 모든 배아를 냉동하여 환자에게 적정한 시기에 해동시켜 이식하는 방법이 많이 사용되고 있으며 이 방법은 신선주기의 임신률과 비슷하다는 많은 연구 결과를 보여왔다. 이러한 결과들이 난소 저반응군의 환자에게도 동일하게 적용될 수 있는가에 대한 연구가 필요하였다. 국제적으로 규정지은 난소 기능 저하 기준에 따라 난소 기능이 정상인 군과 정상 이하의 군으로 나누었을 때, 난소 저반응군에 해당하는 환자들에게도 신선 배아 이식 대신 모든 배아를 동결하여 두었다가 치료나 보강 후에 이식하는 것이 효용성이 있는 것인지에 알아보는 것이 이 연구의 목적이다. 이 연구는 수지 마리아 병원에서 2018 년 3 월부터 2019 년 11 월까지 시행된 난자채취 후 이식과 해동 이식을 시행한 환자에 대해 이루어진 후향적 연구이다. 신선주기와 모든 배아 냉동 후 이식을 한 환자를 나누고, 정상 난소 반응군과 난소 저반응군을 나누어 환자의 특징을 비교 분석하였다. 정상 난소 반응군과 난소 저반응 군의 모든 배아 냉동 이식의 결과를 분석하고, 난소 저반응군에서 신선주기 배아이식과 모든 배아 냉동 후 해동이식의 결과를 분석하였다. 분석 결과 난소 정상반응군과 난소 저하반응군

모두 기신선주기와 모든 배아의 해동 이식에서의 임신율은 통계적으로 유의한 차이점이 보이지 않았으나, 난소 저반응 군에서는 신선주기의 배아이식이 더 높은 임신율의 경향을 보였다. 하지만, 나이에 따른 임신율의 저하 경향을 보면, 전체적 임신율을 모든 나이대 별로 신선 배아이식이 높게 나타나는 경향을 보였다. 그러나, 모두 냉동 후 해동 이식을 한 그룹이 신선 배아 이식을 한 그룹보다 더 완만한 임신율 저하율을 보임에 따라 그 방법이 꼭 잘못되었다고 할 수 없다. 따라서 저반응군의 이식 방법에 대해서 환자의 특성을 고려한 신중한 결정을 내려야 할 것으로 보인다.