P1-01 Comparison between Combined Fresh with Frozen Embryo Transfer and Accumulated Frozen Embryo Transfer in Natural or Mild Stimulation IVF/ICSI Cycle for Extremely Poor Responders

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Introduction
To investigate the outcomes between combined fresh with frozen embryo transfer and accumulated frozen embryo transfer in extremely poor responders.

Materials and Methods
We analyzed 116 patients fulfilling Bologna criteria were included in this study. This retrospective cohort study was conducted at our ART clinic from November 2016 to November 2017. A total of 116 patients with 269 natural or mild stimulation IVF/ICSI cycles were analyzed. The patient was divided into a combined fresh with frozen embryo transfer group (co-ET group; 56 patients) and an accumulated frozen embryo transfer group (a-ET group; 60 patients). If there were two or more AFC counts observed on the 3rd day of the menstrual cycle period, the patient received mild stimulation IVF using letrozole, HMG, and GnRH antagonist. Otherwise patient received natural IVF. Retrieved oocytes were inseminated and 2 pronucleate zygotes were cryopreserved and collected. In the co-ET group, fresh oocytes were retrieved and fertilized in the next cycle, and embryo transfer was performed simultaneously with frozen embryos if an appropriate number of embryos was collected according to the patient's age.

Results
There were no statistically significant differences in baseline characteristics between the co-ET group (56 patients with 128 cycles) vs. the a-ET group (60 patients with 141 cycles) with regards to age (38.96±3.81 vs. 39.0±3.76, p=0.76); baseline AMH (0.42±0.27 vs. 0.40±0.25, p=0.61); day 3 AFC count (1.74±1.06 vs. 1.64±0.92, p=0.76); basal FSH (13.3±5.80 vs. 15.1±8.01, p=0.295) and endometrial thickness (8.69±1.43 vs. 8.62±1.12, p=0.76).
No significant differences were found in IVF/ICSI outcomes between the two groups with regard to the total number of retrieved oocytes (211 vs. 239); the mean number of initiated cycles required for ET (3.12±1.26 vs. 2.78±1.22, p=0.14); the total number of 2PN rate [79.6%(156/196) vs.81.4%(175/215), NS]; total grade I, II embryo rate [45.1%(69/153) vs. 48.5%(78/163), NS] and the mean number of the transferred embryo (2.69±0.53 vs. 2.73±0.44, p=0.687). Also clinical pregnancy rate [23.2%(13/56) vs. 23.3%(14/60)], ongoing pregnancy rate [17.8%(10/56) vs. 16.6%(10/60), NS], miscarriage rate was [23.0%(3/13) vs. 28.5%(4/14), NS] and multiple pregnancy rate was [30%(3/10) vs. 40%(4/10), NS] not significantly differ from each other.

Conclusions
There were no significant differences in clinical results between combined fresh with frozen embryos (co-ET) and accumulated frozen embryo transfer (a-ET) in extremely poor responders. Therefore, co-ET could be taken into consideration due to reducing additional cryopreserving costs and it could be more patient-friendly.

P1-02 Effect of Progestin-primed Ovarian Stimulation Using Dydrogesterone or Medroxyprogesterone Acetate on IVF Outcomes Compared to GnRH Antagonist Protocol in Infertile Women of Advanced Age over 40 years

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Inrtroduction

This study was performed to investigate the effect of progestin-primed ovarian stimulation (PPOS) using dydrogesterone (DDG) or medroxyprogesterone acetate (MPA) on IVF outcomes in elderly women over the age of 40 compared to GnRH antagonist protocol.

Material and methods

A total of 220 infertile patients who underwent PPOS using 20mg of DDG per day (PPOS/DDG group, n=23) or 10mg of MPA per day (PPOS/MPA group, n=30) or GnRH antagonist protocol (control group n=162) for freeze-all practice after stimulated IVF between January 2019 and Dec 2022 was included in this study. Controlled ovarian stimulation (COS) results including the incidence of premature LH surge and IVF outcomes were compared among the three groups. Premature LH surge was defined as a serum LH level of ≥ 10 mIU/mL, and a serum progesterone level of ≥ 1.0 ng/mL occurring before the criteria for hCG administration are met. If patients underwent two or more cycles of IVF/ICSI during the study period, charts corresponding to the 1st IVF/ICSI cycle were reviewed and data of other IVF/ICSI cycles except 1st cycle were excluded from this analysis. Analysis of variance (ANOVA) test with Bonferroni's post hoc correction was used to compare mean values among three groups. Chi-square test and Fisher's exact test were used for the comparisons of fraction. Statistical significance was defined as $P<.05$. All analyses were performed by using SPSS statistical package for Windows, version 11.0 (SPSS Inc, Chicago, IL).

Results

Patients' characteristics were comparable among the three groups. The incidence of premature LH surge was significantly higher in PPOS/DDG group of 26.1% (6/23) compared with 3.3% (1/30) in PPOS/MPA group or 1.2% (2/162) in control group ($P = .034$, $P < .001$, respectively). The total dose of gonadotropin used was significantly higher in PPOS/MPA group than in PPOS/DDG or control groups ($P < 0.05$). There were no differences in the three groups with respect to the duration of COS and number of oocytes retrieved. However, the numbers of mature oocytes retrieved and grade I or II embryos were significantly higher in control group than in PPOS/DDG or PPOS/MPA groups ($P = .006$, $P < .001$, respectively).

Conclusions

PPOS using DDG was not effective in preventing premature LH surge in infertile women over 40 years of age. Also, the quality of oocytes and embryos obtained by PPOS using DDG or MPA was poor compared to those in the GnRH antagonist protocol in women with advanced age over 40 years.

P1-03 Follitropin Delta can Play a Good Role in Both Patients with High Serum AMH(Anti-Mullerian Hormone) Level and Patients with Low Serum AMH Level Who undergo in Vitro Fertilization (IVF) Procedure

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Introduction
To evaluation the clinical efficacy of follitropin delta in IVF procedure for the patients with high serum AMH level and patients with low serum AMH level.

Method
This is a prospective study performed by single physician at single primary fertility center. From December 2020 to November 2021, a total of 82 cycles of IVF procedures using partners' sperm were performed from the couples who were diagnosed as infertility. In 70% of total cycles, controlled ovarian hyperstimulation was performed using gonadotrophic releasing hormone(GnRH) antagonist protocol, and in another 30% of cycles, gonadotrophin releasing hormone agonist(GnRHa) protocol were applied. We measured the basal characteristics of the patients and the outcome of IVF procedure.

Results
Women's age were 37.0±4.0 years, and among them, 19(23%) were under 35years old, 24(29%) were 35 to 37 years old, 27 (33%) were 38 to 40 years old, and 12 (15%) were over 40years old. Their average AMH levels were 2.4±2.4ng/ml, 29% of patients had AMH level under 1.0ng/ml, 32% from 1.0 to 2.0 ng/ml, 11% from 2.0 to 3.0 ng/ml, 9% from 3.0 to 4.0 ng/ml, 7% from 4.0 to 5.0 ng/ml, and 12% had over 5.0ng/ml in serum AMH level. Total 34% of patients showed 1.2ng/ml or less on serum AMH level, 46% showed 1.2 to 4.0 ng/ml, and 20% showed 4.0ng/ml or higher level. The number of obtained oocytes were 8.9±5.0 and among them, 39% of total patients obtained 8 to 14 oocytes, 20% obtained 15 or more oocytes, only 7% obtained 20 or more oocytes, and 10% obtained less than 4 oocytes. Patients with 2.1ng/ml or higher serum AMH level retrieved 11.7±5.3 oocytes, meanwhile patients with less than 2.1ng/ml in serum AMH level retrieved 7.2±3.9 oocytes. Mean daily dose of follitropin delta were 10.3±2.5mcg, and total dosage were 121.8±25.5mcg. Duration of stimulation were 9.9±2.0 days. Pregnancy rate was diagnosed if serum beta human chorionic gonadotrophin(hCG) level was above 5ng/ml on 14th day after oocyte retrieval, and the result was 38%. Clinical pregnancy rate which was defined if gestational sac was visualized by transvaginal ultrasonography after at least 21st days after oocyte retrieval, and the result was 27%. For optimally retrieved group (8-14 oocytes were obtained), pregnancy rate were 47% and clinical pregnancy rate were 34%. For the patients with 1.0 ng/ml or less serum AMH level, pregnancy rate were 37% and clinical pregnancy rate were 24%. For the patients with 2.0 ng/ml or less serum AMH level, pregnancy rate were 36%, clinical pregnancy rate were 24%. For the patients with 4.0 ng/ml or more serum AMH level, pregnancy rate were 62.5% and clinical pregnancy rate were 43.7%. No cases of severe ovarian hyperstimulation syndrome developed.

Conclusion
In IVF procedure, not only for the patients with good ovarian reserve, but also for the patients with poor ovarian reserve, follitropin delta can play a good role. With its discriminative fixed dosing protocol based on patients' serum AMH level, body weight and previous response, follitropin delta gives more convenient way for both physicians and patients for ovarian stimulation with minimal risk of OHSS, without having detrimental effect on pregnancy rate.

P1-04 Serum Progesterone Level of Embryo Transfer Day has Different Threshold Value on Pregnancy Rate, Either among Fresh ET and Frozen-thawing ET Cycle, or between the Route of Luteal Phase Support

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Introduction
This study was designed to check to threshold level of serum P4 on ET day and evaluate the characteristics and difference of the threshold level between fresh and frozen ET cycles.

Method
This is a retrospective study performed by single physician at single primary fertility center. From January 2020 to January 2023, a total of 1870 cycles of ET procedures using partners' sperm were performed from the couples who were diagnosed as infertility. They were composed of 688 fresh ET cycles, 830 programmed FET and 352 ovulatory FET cycles. Each groups were separated and analyzed by the method of luteal phase support(vaginal vs. subcutaneous progesterone). We measured the basal characteristics of the patients and the outcome of fresh/frozen ET cycles.

Results
For fresh ET group, women's age was 36.56±3.61 years, and the number of their previously failed history was 3.41±2.95 times. Serum estradiol(E2) and P4 level on trigger day was 1550.50±1439.51, 0.53±0.34, separately. Mean number of retrieved eggs were 9.65±6.59 and mean value of P4 on retrieval day was 7.33±5.74. On ET day P4 was 41.92±30.00 and mean number of transferred embryo were 2.36±0.72. And the pregnancy rate was statistically different between ET day P4 level <60.0ng/ml group (25.19%) versus >60.0ng/ml group (43.64%). ($p<0.01$)
For programmed FET group, women's age was 35.80±4.32 years, and the number of their previously failed history was 2.81±2.71 times. Serum E2 and P4 level on LPS start day was 281.12±200.02, 0.23±0.81, separately. Mean value of P4 on ET day was 13.84±9.20 and mean number of transferred embryo were 1.93±0.74. And the pregnancy rate was not statistically different among ET day P4 levels. But for the patients with vaginal suppository, there was statistical difference between <8.0ng/ml group (47.47%) versus >8.0ng/ml group (56.90%). ($p<0.01$) And for the patients with subcutaneous progesterone, there was statistical difference between <10.0ng/ml group (21.05%) versus >10.0ng/ml group (45.70%). ($p<0.01$)
For ovulatory FET group, women's age was 34.57±4.09 years, and the number of their previously failed history was 3.00±1.84 times. Serum E2 and P4 level on LPS start day was 238.09±117.88, 0.81±1.249, separately. Mean value of P4 on ET day was 31.46±15.87 and mean number of transferred embryo were 1.64±0.74. And the pregnancy rate was statistically different between ET day P4 level <20ng/ml group (34.57%) versus >20ng/ml group (55.26%). ($p<0.01$). For the patients with vaginal suppository, there was statistical difference between <20.0ng/ml group (33.33%) versus >20.0ng/ml group (57.26%). ($p<0.01$). For the subcutaneous progesterone users, there were no statistical difference among ET day P4 levels.

Conclusion
Different threshold value of ET day P4 level should be applied among fresh and frozen ET, programmed and ovulatory FET, vaginal suppository users and subcutaneous injecting users. For fresh ET, 60ng/ml can be considered as a threshold. Meanwhile, for programmed FET, in vaginal suppository users, it should be 8ng/ml, or 10ng/ml for subcutaneous injecting patients. And for ovulatory FET cases, the threshold would be 20ng/ml.

Key Words: ET day serum progesterone, fresh embryo transfer, programmed frozen embryo transfer, ovulatory cycle frozen embryo transfer, pregnancy rate

P1-05 Effect of Blood Zinc Level on in Vitro Fertilization and Embryo Culture Performance

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Background and purpose
A transient release of zinc ions (Zn^{2+}) from the egg during fertilization (zinc spark) has been reported. This suggests that in addition to Ca^{2+} signal, Zn^{2+} signal also plays an important role in fertilization. In this study, we examined the effect of blood zinc levels on in vitro fertilization and embryo culture outcomes.

Subjects and methods
We enrolled patients aged <40 years old who underwent egg collection from September 2022 to May 2023. The participants were divided into the following two groups based on the blood test results prior to egg collection: low blood zinc level group (<80 μg/dl) and normal blood zinc level group (≥80 μg/dl). Thereafter, we retrospectively investigated the effects of blood zinc level on in vitro fertilization and embryo culture outcomes.

Results
There were 64 and 40 participants in the low and normal blood zinc level groups, respectively. There were no significant differences in the average age, BMI, numbers of eggs collected, fertilization rate, 2PN rate, multinucleation rate, and embryo utilization rate between the two groups. However, the blastocyst arrival rate was 38.2 ± 34.8% and 47.6 ± 33.8% in the low and normal blood zinc level groups, respectively, indicating a significantly lower rate in the low blood zinc level group (P=0.007).
We also performed analysis according to the insemination method. With conventional in-vitro fertilization (cIVF), no significant difference was observed in the number of eggs collected, fertilization rate, 2PN rate, multinucleation rate, blastocyst arrival rate, and embryo utilization rate between the two groups. With intracytoplasmic sperm injection (ICSI), there was no significant difference in the number of eggs collected, fertilization rate, 2PN rate, multinucleation rate, and embryo utilization rate between the two groups. However, the blastocyst arrival rate was 36.0 ± 35.7% and 49.1 ± 39.3% in the low and normal blood zinc level groups, respectively, indicating a significantly lower rate in the low blood zinc level group (P=0.035).

Conclusions
The low blood zinc level group had lower blastocyst arrival rate, suggesting that serum zinc levels may reflect the quality of fertilized eggs by assessing the zinc spark.

P1-06 More Reliable Ovarian Reserve Marker between AMH and AFC Discordance in Patients Undergoing IVF

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Background and Aims
The current study evaluates which marker could be more reliable in Korean patients with anti-Müllerian hormone (AMH) and antral follicle count (AFC) discordance, particularly accompanied with poor ovarian response (POR), and which patient-related factors contribute to such discordance of the markers.

Method
A total of 377 controlled ovarian stimulation (COS) cycles undergoing and in vitro fertilization (IVF) / Intracytoplasmic sperm injection (ICSI) and consequent embryo transfers were included. COS cycles were first divided into three groups using 50% confidence interval (CI) from the linear regression equation according to AMH levels expected from the given AFCs. In the second analysis, COS cycles were divided into four groups, using cut-offs adopted from Bologna criteria. Patient characteristics including obstetric and gynecological histories, serum hormone levels, outcomes of COS and clinical pregnancy rates are analyzed.

Results
Patients with higher AMH levels compared to their AFCs were more likely to display polycystic ovary syndrome (PCOS), and those with lower AMHs to AFCs tended to have underlying endometriosis. COS outcomes were more favorable in groups with higher AMH levels to AFCs, including oocyte yield, good-quality embryo rate and clinical pregnancies, than in groups with lower AMH levels.

Conclusion
Discordance between AMH levels and AFCs was observed in 16% of cases in the study population, and endometriosis, PCOS and age were regarded as discordant factors. AMH levels seemed to more accurately predict ovarian reserve in presence of discordance, having positive correlation with COS outcomes regardless of AFCs.

P1-07 Analysis of 836 ERA Cases Conducted in Korea

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Introduction
The Endometrial Receptivity Analysis (ERA) has been conducted worldwide to detect implantation windows.
In Korea, ERA was also introduced after 2019, and the tests are being actively conducted mainly for women who have experienced repeated implantation failures.

Material & Methods
We retrospectively analyzed the results of 836 ERA conducted in Korea from January 2019 until June 2023.

Results
Of the 836 ERA cases, the result were as follows; 7 cases (0.8%) were 2 days-prereceptive, 154 cases (18.4%) were 1 day-prereceptive, 84 cases (10.0%) were early rereceptive, 459 cases (54.9%) were receptive, 93 cases (11.1%) were late receptive, and 39 cases (4.7%) were 1 day postreceptive.
There was no difference in the distribution of ERA results according to age.
Among them, 495 cases were able to obtain data on the number of previous implantation failures. The distribution according to the number of implantation failures was as follows; 79 cases of #1-3 failures, 242 cases of #4-6 failures, 130 cases of #7-10 failures, 44 cases of >#11 failures.
Women who experienced more than 7 implantation failures had a lower rate of “Receptive" than those who experienced less than 6 implantation failures (76/174=43.7% vs 182/321=56.7%, p=0.005).

Conclusions
We found that the higher the number of implantation failures, the higher the probability that implantation windows were displaced.

P1-08 Effects of Short-Time Oxygen Concentration Changes on Human Embryonic Development: A Case Report

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Introduction
Several studies reported that low oxygen concentrations of 2 – 8% had a good effect on embryonic development in human embryo cultures. This study was conducted to investigate the effect of changes in oxygen concentrations following short-term oxygen supply interruption on embryonic development.

Material & Methods
This study analyzed patients undergoing treatment (treatment group, n =193) and the normal oxygen concentration group (control group, n =116), in which oxygen concentrations changed due to oxygen supply interruption, from December 2022 to February 2023.
The baseline characteristics were the patient's age, body mass index, number of previous cycles, duration of infertility, and anti-mullerian hormone.
Good morphological quality was investigated between the two groups at each of Days 2, 3, 4, and 5. On Days 2, 3, and Day 4, good quality embryos were defined as ≥ 4 cell, ≥ 8 cell and ≥ morulae regular blastomeres, no fragmentation, and no multinucleation. On Day 5, good quality blastocysts were defined as ≥ 3BB, following Gardner and Schoolcraft.

Results
There was no significant difference between the basic patient characteristics of the two groups, one in which the oxygen concentration was maintained normally (control group) and the treatment group in which the oxygen concentration was changed due to an interruption in oxygen supply (treatment group).
There was a significant difference found in the mean number of ≥ 4 cell good quality embryos on Day 2 between the control group and the treatment group (3.1 ± 2.7 vs. 2.2 ± 2.3, p=0.002). There was also a significant difference found between the two groups in the mean number of good quality embryos ≥ 8 cell on Day 3 (2.1 ± 2.4 vs. 1.4 ± 1.7, p=0.005).
There was no significant difference between the two groups in Morulae grades greater than or equal to good on Day 4. There was also no significant difference between the two groups in ≥3BB; that is, an expanding blastocoele, and no excluded blastomeres or fragments from the formation of the blastocyst on Day 5.

Conclusions
The investigation of how changes in oxygen concentration due to oxygen supply interruption affects embryonic development found that a short supply delay affected embryonic development in the early stage but did not affect embryonic development in the later stage.

P1-09 P-ICSI as a Strategic ICSI Method for Successful Overcoming Infertility

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Introdutcion

The in vitro selection of sperm for intracytoplasmic sperm injection (ICSI) is critical and directly influences the paternal contribution to preimplantation embryogenesis. Pysiological intracytoplasmic sperm injection involves identifying sperm that bind well to hyaluronic acid (HA) as a way to select for sperm with high genomic integrity and thereby decreases aneuploidy and miscarriage rates. We aimed to investigate the efficacy of the Pysiological intracytoplasmic sperm injection (P-ICSI) versus the Conventional intracytoplasmic sperm injection (C-ICSI) for improving fertility in couples undergoing infertility treatment.

Materials and Methods

This is the result of a retrospective analysis of the cases conducted at the Ilsan CHA Fertility Center from April 2022 to February 2023. This study targeted patients aged 38 years or older, who had undergone 3 or more cycles of IVF, had an AMH of 2.0 or more, and collected 15 or more oocytes. Sibling oocytes obtained from patient were divided into two groups according to the same criteria, and C-ICSI and P-ICSI were performed respectively. In both groups, embryos were cultured in a time-lapse incubator (Embryoscope, Vitrolife). Embryos were cultured as expanded blastocysts and trophectoderm biopsies were performed on day 5 or 6.

The fertilization rate, embryo score , blastocyst formation rates and euploidy rates of C-ICSI and P-ICSI were compared. Statistical significance was tested using the SPSS.

Results

As a result of conducting the study by performing P-ICSI and C-ICSI, respectively, in sibling eggs, significantly higher results were shown in the case of P-ICSI: fertilization rate (54.09% vs 74.05%; $p<0.001$), Embryo score (2.53 vs 3.84; $p=0.015$), blastocyst formation rate (32.37% vs 55.18%; $p<0.001$). Although there was no significant difference (25.29 vs 26.67; $p>0.05$), the percentage of polyploidy between the two groups was higher in the PICSI group.

Conclusions

According to the results of a study that performed different methods of fertilization in sibling eggs, we were able to demonstrate that performing P-ICSI contributed to higher quality embryos than performing C-ICSI. Although no significant difference could be identified in the PGT-A results, the P-ICSI embryos showed a high euploid ratio. This confirms once again the need to strategically select a modification method according to the patient's condition in performing ICSI.

P1-10 Assessing the Importance of Vitrification Day or Blastocyst Quality for Embryo Selection in Vitrified-warmed Cycles

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Introduction

Recently, many IVF centers have conducted extended culture, making blastocyst transfer a common practice. However, there is limited reporting on integrated thinking regarding vitrification day and blastocyst quality for embryo selection. This study aimed to determine whether adjusting the blastocyst quality to the same grade before vitrification could have similar clinical outcomes regardless of the vitrified day.

Material & Methods

This is a retrospective study of 736 FET cycles, in which only one blastocyst was warmed and transferred, excluding unqualified cases from 2134 FET cycles by one team in a single center at the CHA Fertility Center, Gangnam, between January 2019 to December 2021. We analyzed the changes in blastocyst quality before and after vitrification. And clinical outcomes according to quality and vitrified day were compared. The quality was analyzed using the blastocyst quality score (BQS), which can comprehensively reflect a degree of expansion and cell number of ICM and TE. BQS: score of (Expansion)×(ICM)×(TE). The score by grading: Early=1, Mid=2, Expanded=3, Hatching=4, Hatched=5, A=3, B=2, C=1. Classify: Top (45~24), Good (23~16), Average (15~7), Poor (6~1).

Results

The blastocysts with top or good grades before vitrification maintained top or good grades after warming, regardless of the day of vitrification (top: 84.9% and 100%, p=0.305, good: 68.0% and 91.7%, p=0.116). On the other hand, in average grade before vitrification, day 6 blastocysts had a significantly lower potential to have top or good grades after warming than day 5 (39.0% vs. 69.2%, p<0.001). Similarly, day 6 vitrified blastocysts with average grade had a significantly higher possibility to transfer poor grade blastocysts than day 5 (25.4% vs. 9.9%, p<0.001). As already widely known, our center also has a significantly better clinical pregnancy rate of day 5 vitrified blastocyst transfer than day 6 (48.7% vs. 39.1%, p=0.047). However, compared with vitrified blastocysts, which had top or good grades before vitrification, there was no difference in the clinical pregnancy rate according to the day of vitrification (49.2% vs. 55.6%, p=0.558).

Conclusions

Our findings suggest that top or good morphological grade blastocysts exhibit comparable clinical outcomes regardless of the vitrified day. It indicates that vitrification of sufficiently expanded blastocysts with good quality on day 6 could be a favorable option for infertility patients with slow blastulation who have blastocysts of poor quality or insufficient expansion to be vitrified on day 5.

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P1-11 Multiple Pregnancy after Single Blastocys Transfer

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Introduction
To establish the incidence of each chorionicity and risk factors for multiple pregnancy after single blastocyst transfer.

Materials and Methods
A retrospective chart review was performed for women who achieved clinical pregnancies after single blastocyst transfer from January 2020 to August 2022 in Bundang CHA hospital fertility center in South Korea. Outcome measures included the number of multiple pregnancies, possible risk factors for multiple pregnancies after single blastocyst transfer; comprising insemination method (conventional in vitro fertilization (IVF) and intra-cytoplasmic sperm injection (ICSI)), embryo biopsy for pre-implantation genetic study, assisted hatching (AH), hyaluronan-containing transfer medium, type of frozen-thawed embryo transfer (FET) cycle (ovulation or programed cycle) and female age.

Results
A total of 2,352 cycles of single blastocyst transfers were performed, of which 139 in the fresh cycle and 2,213 in the frozen-thawed cycle. Total of 1,230 clinical pregnancies (52.3%) were achieved including 38 multiple pregnancies, of which 17 MCDA twin, 19 DCDA twin, 1 DCTA and 1 TCTA triplet. Among 38 multiple pregnancy cases, 1 was performed in fresh cycle and 37 were performed in FET cycle; 27 were in ovulation cycle, and 10 were in programmed cycle.
The mean age of the singleton pregnancy group and the multiple pregnancy group were 34.67±3.697 and 33.89±2.957, respectively. Though not statistically significant, ICSI (OR: 2.021, p=0.365), embryo biopsy (OR: 2.253; p=0.282), hyaluronan-containing transfer medium (OR: 2.027; p=0.077), and fresh cycle (OR: 2.256; p=0.437) were associated with multiple pregnancy. AH (OR: 1.070; p=0.909) and female age (OR: 1.018, p=0.719) were not correlated with multiple pregnancies.

Conclusion
The incidence of multiple pregnancies after single blastocyst transfer was estimated to be 3.1%, of which MCDA and DCDA were 1.4% and 1.5%, respectively, and the others were 0.2%. In single blastocyst transfer, ICSI, embryo biopsy, fresh cycle, hyaluronan-containing transfer medium were associated with higher rates of multiple pregnancies although the statistics were not significant. Other clinical parameters, including AH, were not associated with changes in the number of twin pregnancies.

P1-12 Is There an Optimal Ovarian Stimulation Protocol to Obtain Euploid Embryos?

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Introduction
This study aimed to reveal whether type of ovarian stimulation protocols, type of gonadotropins, add-ons, or triggering method in ovarian stimulation affect obtaining euploid embryos.

Materials and methods
This is a retrospectively designed study which included total of 476 PGT-A (preimplantation genetic testing for aneuploidy) cycles, conducted in a single center between December 2021 and March 2023. Patients aged between 35 to 39 who had at least 1 blastocyst biopsied for PGT-A were included. The exclusion criteria were patients less than 35 years or older than 40 years of age, and patients with AMH (anti-Müllerian hormone) less than 1.0ng/mL. As the result of PGT-A, the cycles obtaining at least one euploid embryo were defined as group A, and the cycles with all aneuploidy as group B. The factors expected to affect the results were compared. Age, AMH, BMI(body mass index), the number of biopsied embryos, type of ovarian stimulation protocols including GnRH antagonist, GnRH agonist-long, GnRH agonist-short, PPOS(Progestin-primed ovarian stimulation), type of gonadotropins, duration of stimulation, total dose of gonadotropin, ovulation trigger regimen(hCG, GnRH agonist, dual trigger), additional oral formulations used(clomiphene citrate, aromatase inhibitor, or none), and cycle type(fresh or thawed) were analyzed by independent t-test and chi-square test. A subgroup analysis was performed to control the age factor, dividing the patients according to the age of 37.

Results
Group A and B included 275 and 201 PGT-A cycles, respectively. The mean age were 37.0±1.5 and 37.7±1.3 ($p<0.001$), AMH were 3.3±3.2ng/mL and 2.7±3.0ng/mL ($p=0.032$), BMI were 23.7±4.1 and 23.0±3.8 ($p=0.037$) and the number of biopsied embryos were 3.8±1.9 and 2.2±1.5($p<0.001$) showed statistically significant differences between group A and B. However, type of ovarian stimulation protocol and duration of stimulation, type of gonadotropin, additional oral formulations, total dose of gonadotropins, add-ons did not show statistical significance). In the subgroup analysis, patients age between 35 to 36 years showed statistically different mean number of biopsied embryos between groups ($p<0.001$). However, none of the other factors showed statistical significance.

Conclusions
From this study, the significant factor for obtaining euploid embryo was the mean number of biopsied embryos.

P1-13 Investigation of the Impact of Cryopreservation-Thawing Procedures on Mitochondrial Dynamics in Unfertilized Oocytes

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Objective
Cryopreservation of unfertilized oocytes has been established as a common technique for fertility preservation in adolescent and young adult (AYA) female cancer patients. However, the success rate of live births from cryopreserved unfertilized oocytes is only around 15%, which is not satisfactory. Previous research has shown a decrease in mitochondrial density and swelling within frozen oocytes through transmission electron microscopy (TEM), indicating potential mitochondrial damage caused by cryopreservation stress. In order to improve the techniques of oocyte cryopreservation and treatment outcomes, we aimed to examine the disturbances occurring during the cryopreservation-thawing process of MII oocytes and their impact on mitochondrial dynamics in unfertilized oocytes.

Methods
MII oocytes were obtained from ICR mice aged 6-12 weeks and divided into two groups: fresh oocytes and cryopreserved-thawed oocytes. Mitochondria were observed using scanning electron microscopy. To assess mitochondrial distribution and membrane potential, Mito-tracker (MT) and TMRE fluorescent staining were performed, and cluster distribution and area were analyzed in 2D and 3D using confocal laser microscopy. Mitochondrial distribution was also confirmed by TEM. Mitochondrial homeostasis evaluation included analysis of mitochondrial DNA copy numbers and mitochondrial homeostasis-related genes (MFN1, MFN2, Dnml1, Opa1, Fis1, SIRT) using RT-qPCR and digital PCR.

Results
Scanning electron microscopy revealed decreased mitochondrial density and swelling in the cryopreserved-thawed oocyte group. Confocal laser microscopy analysis of mitochondrial cluster distribution and 2D and 3D area showed a significant increase in area and decrease in cluster count in the cryopreserved-thawed group compared to fresh oocytes. TEM analysis also indicated a significant decrease in mitochondrial count. DNA copy number evaluation showed no significant difference between the two groups. However, significant changes were observed in mitochondrial-related genes (MFN2, Dnml1, SIRT3).

Conclusion
While the cryopreservation-thawing process induced disturbances in mitochondria, the damage was not substantial enough to lead to apoptosis judged by mitochondrial DNA copy numbers. Changes within the mitochondria might be responsible for inducing certain disruptions, potentially contributing to the reduced pregnancy success rates observed in oocyte cryopreservation.

P1-14 Adding the iDAScore Scoring to Standard Evaluation Blastocysts of Gardner Criteria can Predict Higher Pregnancy Rate

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Introduction
Gardner criteria based on morphological evaluation of ICM and TE has been mainly used for evaluation of blastocysts. In recent years, embryo evaluation methods based on deep learning models based on using time-lapse sequential images. With the aim of establishing a new evaluation that combines the Gardner criteria with the iDAScore (Vitrolife, Sweden) evaluation, we conducted a study on pregnancy prediction in single vitrified blastocyst transfer at single facility.

Material & Methods
From October 2022 to June 2023, oocytes were retrieved by minimal ovarian stimulation at our clinic, and after ICSI, normal fertilization was confirmed, and blastocyst culture was performed by time-lapse incubator (EmbryoScope Flex; Vitrolife, Sweden). Expanded blastocysts were frozen by the vitrification method and subjected to 588 cycles of single embryo transfer. The evaluation of transferred embryos was classified into AA, AB+BA, and BB according to the Gardner criteria. In addition, the iDAscore is divided into Excellent (9.9-9.0), Good (8.9-8.0), Fair (7.9-7.0), and Poor (6.9 or less) based on quartiles, and iDAScore scoring is transferred to morphological evaluation by Gardner criteria. We investigated the usefulness of Gardner criteria plus iDAScore evaluation for embryo selection to predict clinical pregnancy rate.

Results
The clinical pregnancy rates in AA, AB+BA, and BB evaluations according to the Gardner criteria were 84.6% (33/39), 70.9% (122/172), and 54.9% (207/377), respectively. The rate tended to be high ($p<0.0001$), and the AUC value by ROC curve analysis was 0.595 ($p<0.0001$). On the other hand, the clinical pregnancy rates for Excellent, Good, Fair, and Poor by iDAscore were 72.5% (129/178), 67.7% (84/124), 57.7% (82/142) %, and 46.5% (67/144) %, respectively. The higher the iDAScore, the higher the pregnancy rate ($p<0.0001$), and the AUC value by ROC curve analysis was 0.620 ($p<0.0001$). In addition, as a result of adding the iDAScore quartile evaluation to the evaluation for each Gardner criteria. Although there was no significant difference in the pregnancy rate by iDAScore evaluation in the AA and AB+BA (AA; $p=0.609$, AB+BA; $p=0.703$), there was a significant difference was observed only in the BB.

Conclusions
The results of this study indicated that both the Gardner criteria and the iDAScore are useful prediction model of pregnancy outcome. However, evaluation by Gardner criteria plus evaluation by iDAScore suggested that only for BB, the higher the iDAScore, the higher the pregnancy rate. It is possible that iDAScore is an indicator of embryo quality that cannot be evaluated by human visual observation alone for embryos with standard blastocyst morphology. Therefore, it was suggested that pregnancy rate prediction using iDAScore as a secondary indicator would lead to more accurate selection of favorable embryos when the embryos were the standard evaluation embryos in the Gardner criteria.

P1-15 Exposure to IGFBP-rP1 during the Oocyte Maturation Process Inhibits IGF-1R and AKT Activity in Murine MII Oocytes and Embryos

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Introduction

Deterioration of oocyte quality with maternal aging is one of the problems that need to be overcome in reproductive medicine. No senescence-related factors that are directly involved in oocyte deterioration in aged ovaries have been identified. Recently, the senescence-associated secretory phenotype (SASP), in which various proteins with physiological activity are over-secreted from senescent cells, has been reported, and we believe that the SASP is the essential cause of oocyte aging. We focused on insulin-like growth factor binding protein-related protein 1 (IGFBP-rP1), which blocks the activity of IGF-1 receptor (IGF-1R). Our previous studies revealed that IGFBP-rP1 was significantly upregulated in aged compared to young ovaries. In addition, exposure of immature oocytes to exogenous IGFBP-rP1 increased apoptosis in blastocysts. However, the mechanisms of increased apoptosis in blastocysts remain unclear. In this study, we investigated the effects of IGFBP-rP1 exposure on the maturation process of immature oocytes and the activity of IGF-1R and its downstream factors during early development.

Materials and methods

Immature oocytes were collected by puncturing the ovaries of mice and cultured for 15 h IVM. Oocytes cultured with (rP1 group) and without (control group) 30 ng/ml of IGFBP-rP1 were used. Expression levels and localization of IGF-1R and its downstream factors were analyzed by immunoblotting and immunofluorescent staining, respectively.

Results

A significant decrease in the developmental rate to blastocyst was found following exposure of oocytes to IGFBP-rP1 during the maturation process compared to the control group despite no differences in the rates of maturation and fertilization. At the MII oocyte, 2-cell, and blastocyst stages in the rP1 group, the level of IGF-1R activity was decreased compared to that in the control group. Furthermore, the level of expression and activity of AKT, one of the apoptosis-regulated factors, were also decreased in the rP1 group. Activated IGF-1R (detected on the cell membrane) and AKT (detected in the cytoplasm) levels were lower in the rP1 group than in the control group in all analyzed embryonic stages.

Conclusion

Our findings suggest that increased expression of IGFBP-rP1 in the ovaries causes over-expression of apoptosis in the blastocyst, and this may be due to a decrease in activated IGF-1R and AKT during early development.

P1-16 Effects of Proteoglycans on the Expression of Apoptosis-related Factors in Murine Embryos

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Introduction

In vitro aging of oocytes advances upon exposure of ovulated oocytes to in vitro environments and degrades the embryo quality due to high expression of apoptosis post-fertilization. We previously reported that proteoglycans (PGs) derived from salmon nasal cartilage suppressed apoptosis in embryos derived from in vitro aged oocytes by activating the epidermal growth factor receptor and its downstream PI3K/AKT pathways. In vitro aging reduces the mitochondrial membrane potential (MP) of oocytes, suggesting that mitochondria are involved in the apoptosis pathway, which is highly expressed in embryos. This study aimed to examine the effects of PGs on mitochondrial MP and expression of apoptosis regulators in murine embryos.

Materials and Methods

Murine ovulated oocytes were incubated for 6 h in vitro before IVF (aged group). Some aged oocytes were cultured for 96 h with 0.1 mg/ml PGs after IVF (aged+PGs group). Oocytes examined immediately after collection were used as controls. The mitochondria of embryos obtained after IVF were visualized with JC-1, a fluorescent probe with transmembrane permeability. Brightness of the fluorescent images were analyzed to calculate MP. The expression levels and localization of p53, an apoptosis-promoting factor, and Bcl-2-associated X (BAX), which is regulated by p53 and causes loss of mitochondrial MP and cytochrome c release, were analyzed by immunoblotting and immunofluorescence staining, respectively.

Results

In the 2-cell and blastocyst stages, the mitochondrial MP of embryos in the aged group was significantly lower than that in control embryos; however, the supply of PGs into the medium was effective in preventing the decrease in mitochondrial MP. In the aged+PGs group, mitochondria maintaining high MP were localized on the surface layer of the blastomeres as in the control group. While no differences were observed in the expression level of BAX among groups, which is involved in mitochondrial membrane permeability, its localization resembled the distribution of high MP mitochondria in both the aged+PGs and control groups, distinct from that in the aged group. The expression and phosphorylation level of p53, increased in the aged group but decreased in the aged+PGs group to a comparable level as the control group.

Conclusions

Our findings suggest that PGs indirectly suppressed the mitochondrial MT loss and the expression of apoptosis-related factors in embryos derived from in vitro aged oocytes.

P1-17 The Mouse Born after Adipose Stem Cell Mitochondria Supplementation Show Normal Reproductive System

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Introduction

We successfully obtained offspring from fertilized eggs by injecting mitochondria derived from adipose stem cells (ASC) simultaneously with ICSI into vitrified-and-warmed murine oocytes (Udayanga et al., 2022), and the first generation (F1) and second generation (F2). However, the normality of the resulting mice has not been sufficiently validated. In this study, in order to assess the effect on ASC mitochondria supplementation on female reproductive performances, we compared the ovarian follicles numbers in F1 and F2 generations with those of naturally bred females.

Material and Method

ASC derived mitochondria were injected into vitrified-and-warmed murine (C57BL/6JJmsSlc) oocytes simultaneously with ICSI, and the zygotes were transferred into foster mothers. The born males were mated with wild type (WT) females after growth to obtain the first generation. A second generation was also obtained in the same manner. The primordial, primary, secondary, antral follicles were counted on four ovarian serial sections (H&E stained) of fifteen-week-old female mice (F1 n=3, F2 n=3). As a control, the number of ovarian follicles in commercially available same age WT females (WT n=3) born by natural mating were measured. The follicle numbers were standardized into available follicle number per um^3 in each generation and WT females and then they were compared by using two-way analysis of variance.

Results

The average numbers of primordial follicles in WT, F1 and F2 were 4.3±0.9, 4.2±1.0, 4.7±1.4 (e-06/um^3, mean±sd), with no significant difference. The average number of primary follicles were 8.5±2.8, 10.2±3.1, 11.9±4.6 (e-07/um^3), the secondary follicles were 6.3±1.0, 7.9±5.0, 7.2±3.5 (e-07/um^3), and the antral follicles were 5.7±1.6, 5.5±2.1, 6.6±2.1 (e-07/um^3), and no significant difference was observed.

Conclusion

Data of the present study suggested that ASC mitochondria supplementation into oocyte may not have adverse effects on mouse female reproductive performances in transgenerational manner.

P1-18 Genetic Mutations in Japanese Women with Recurring Assisted Reproductive Technology Failure

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Introduction

Reproductive aging presents a significant obstacle to successful pregnancy, leaving many patients infertile and experiencing recurrent assisted reproductive technology (ART) failures. Approximately 20% of infertility cases remain idiopathic. Recent studies have linked specific gene mutations to fertilization failure and early embryonic arrest. This research focuses on patients with repeated implantation failures and low blastocyst development rates, aiming to identify potential gene mutations that may contribute to compromised embryo development.

Material & Methods

25 infertile women who had experienced IVF/ICSI failure more than 5 times, with blastocyst formation rates below 10%, and 10 staff members who had conceived and delivered naturally were enrolled. Informed consent was obtained from all participants. Genomic DNA was extracted from whole blood samples, and whole-exome sequencing was performed. High-impact homozygous variants with allele frequencies ≤0.20 in the 1000 Genome EAS, absent in control subjects with natural pregnancies and deliveries, were identified. The Human Genetic Variation Database (HGVD) was used, and the Hardy-Weinberg equation was applied to evaluate potential candidates for recurrent ART failures caused by genetic mutations.

Results

57 high-impact homozygous gene mutations were identified, and among them, significant differences in allele frequencies were observed between 8.3KJPN and the patients for *ADAM33* ($p=0.009$), *CEP89* ($p=0.012$), *CRIPAK* ($p<0.001$), *LGALS9B* ($p<0.001$), *PDZRN3* ($p=0.001$), *RAET1E* ($p=0.007$), and *SPATA31A3* ($p=0.045$). The Hardy-Weinberg equilibrium for each gene based on data from HGVD was calculated. Patients exhibited significantly lower Hardy-Weinberg equilibrium compared to HGVD for *ADAM33* ($p<0.001$), *CEP89* ($p=0.033$), *OR2T29* ($p=0.003$), *OR52J3* ($p=0.0497$), *RABL2A* ($p<0.001$), *RNF17* ($p=0.0499$), *SPATA31C1* ($p=0.030$), and *WWTR1* ($p<0.001$).

Conclusions

In this study, 13 gene mutations associated with repeated ART failures were identified. Among these genes, *RNF17*, a piRNA pathway gene, and *WWTR1*, a Hippo signal pathway gene, were the most likely causes of repeated ART failures. These findings may serve as potential diagnostic markers for patients with recurrent ART failures, offering insights into the genetic basis of female infertility.

P1-19 Migration Speed of Nucleolar Precursor Bodies in Pronuclei Affecting In Vitro Fertilization-derived Human Embryo Ploidy Status, Pregnancy, Live Birth, and Miscarriage

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Introduction

Nucleolar precursor bodies (NPBs) are essential for embryo development after fertilization, which suggests they might be involved in forming centromeric chromatin. We previously demonstrated that the NPBs that migrated faster in ICSI-derived zygotes had a higher chance of developing into a blastocyst and then into a baby. However, relationships between NPB migration speed, IVF-derived embryo ploidy status, and clinical outcomes after ART are unclear.

Material & Methods

NPB migration speed, ploidy status, pregnancy, live birth (LB) and miscarriage were retrospectively analyzed in IVF-derived zygotes. The central coordinates of the male and female pronuclei (mPN and fPN), and NPBs (mNPB: NPB in mPN and fNPB: NPB in fPN) were noted at periodic intervals. The migration distances of the NPBs between sequential images were measured to calculate the NPB migration speed.

Results

The migration of mNPBs and fNPBs in euploid embryos was significantly faster than in aneuploid embryos. In multivariate logistic analysis, the mNPB migration speed (OR, 10.2; 95% CI, 1.90–54.90; $P < 0.01$) and age of the patient providing the oocyte (OR, 0.8; 95% CI, 0.64–0.98; $P = 0.03$) were associated with ploidy status. Using receiver operating characteristics (ROC) curve analysis to determine the optimum mNPB migration speed for classifying ploidy status, the cutoff value was found to be 3.65 μm/h (AUC, 0.78; 95% CI, 0.62–0.93). The migration speeds of mNPBs and fNPBs were significantly faster in patients with LB than in those with no pregnancy. When the ability of mNPB migration speed to classify LB was examined using ROC curve analysis, the AUC was 0.83 (95% CI: 0.69–0.96). Conversely, the mNPB migration speeds in patients who had miscarriages, or a biochemical pregnancy were significantly slower than in patients with LB. When zygotes were categorized based on the cutoff values determined for euploidy, the proportion of FHM+/LB−, GS+/FHM−, hCG+/GS−, and no pregnancy zygotes, with mNPB migration speed >the cutoff value, was very low (12.5%, 21.4%, 21.4%, and 21.1% respectively, LB: 78.9%).

Conclusions

This study demonstrates that zygotes with a fast NPB migration speed possess a developmental potential for LB regardless of the insemination method that produced them. The mNPB migration speed is a novel predictor of ploidy status and LB. It may have clinical value for embryo selection and is an attractive option as a marker for noninvasive human embryo selection.

P1-20 Development of a Physiological Intracytoplasmic Sperm Injection Reagent Compatible with Glass-bottom Dishes and Capable of Sustained Binding of Human Spermatozoa

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Introduction

Currently available commercial products for sperm selection during physiological intracytoplasmic sperm injection (PICSI) are widely used but have some disadvantages. This study compared PICSI with a self made hyaluronic acid (smHA) reagent with PICSI with SpermSlow to potentially circumvent these limitations.

Material & Methods

This study used spermatozoa that were isolated by density-gradient centrifugation and swim up procedures (N = 10 per group) to quantify the binding of the reagents to spermatozoa on plastic- or glass-bottom dishes. Furthermore, we investigated the relationship between PICSI reagents and clinical outcomes after assisted reproduction with PICSI (N = 103).

Results

The smHA reagent demonstrated extremely stable binding to human spermatozoa. The binding time of spermatozoa was significantly longer in the smHA reagent than in SpermSlow on both types of dishes (plastic: 60.0 ± 0.0 min vs. 2.7 ± 5.9 min, $P < 0.001$; glass: 60.0 ± 0.0 min vs. 2.5 ± 1.8 min, $P < 0.001$). PICSI with smHA reagent and PICSI with SpermSlow demonstrated no significant difference in normal fertilization rate (78.1% [146/187] vs. 74.5% [216/290]). The frequency of blastocyst development from the PICSI-derived zygote was not significantly different between PICSI with the smHA reagent (69.3% [79/114]) and PICSI with SpermSlow (61.4% [102/166]). In addition, the rates of biochemical pregnancy, clinical pregnancy, fetal heart movement, live birth or ongoing pregnancy, and miscarriage were not significantly different between PICSI with the smHA reagent (13/40 [32.5%], 13/40 [32.5%], 10/40 [25.0%], 8/40 [20.0%], and 5/13 [38.5%], respectively) and PICSI with SpermSlow (22/44 [50.0%], 13/44 [29.5%], 10/44 [22.7%], 9/44 [20.5%], and 4/13 [30.8%], respectively). Therefore, assisted reproduction/ICSI success was not affected by the HA reagent used for sperm isolation.

Conclusions

PICSI with smHA reagent and with SpermSlow demonstrated similar clinical outcomes. Sperm binding to the smHA reagent was not reduced over a 60-min time course. SmHA reagent may shorten and simplify PICSI procedures because it may be used with any dish material, allowing for easy spindle visualization or intracytoplasmic morphology assessment. Moreover, the shape of the adhesive surface is adjustable.

P1-21 Evaluation of Physiological Intracytoplasmic Sperm Injection in the Sibling Study

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Introduction

A method of selecting sperm that will adhere to hyaluronic acid (HA) during intracytoplasmic sperm injection (ICSI) has been devised as physiologic ICSI (PICSI). It is known that mature sperm express receptors for adhesion to the HA due to reconstruction of the protoplasmic membrane. In fact, it has been confirmed that sperm that can adhere to HA are mature sperm, and it has been reported that their DNA is highly normal. However, previous studies on the clinical outcomes of PICSI have shown inconsistent results. Therefore, the purpose of this study was to evaluate the clinical usefulness of PICSI using sibling oocytes.

Material & Methods

The 625 cycles in which two or more mature oocytes were obtained in ICSI cycles were included, and the sibling oocytes of the same cycle were equally divided between conventional ICSI (n=1786) and PICSI (n=1713) methods. Sperm selection was based on sperm morphology and motility in the conventional method, while adhesion to HA was taken into account in the PICSI method. The evaluation items were fertilization and embryo development rates, as well as clinical pregnancy and miscarriage rates after a single frozen-thawed blastocyst transfer. All study participants provided informed consent and the study design was approved by the ethics committee of the IVF Nagata Clinic, Fukuoka, Japan.

Results

The normal fertilization rates for the conventional and PICSI methods were 81.5% and 80.7%, respectively; the abnormal fertilization rates were 7.4% and 7.6%, respectively; the cleavage rates were 97.5% and 97.5%, respectively; the blastocyst rates were 63.6% and 66.8%, respectively, and good blastocyst rates were 37.1% and 38.1%, respectively. There were no significant differences in any of the endpoints. There were also no significant differences in clinical pregnancy (35.9% vs. 44.3%) and miscarriage (24.6% vs. 20.7%) rates after a single frozen-thawed blastocyst transfer between the two groups.

Conclusions

In the patients in this study, the clinical outcomes of PICSI were equivalent to those of the conventional method, and the clinical benefit of PICSI was not demonstrated. In the future, it will be necessary to examine the conditions under which PICSI is effective.

P1-22 Comparison of the Number of Usable Embryos in Four Patient Groups Based on POSEIDON Criteria

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Introduction
Previous studies have demonstrated that higher egg retrieval and fertilization rates are associated with increased pregnancy success. However, the impact of poor ovarian responders (POR) on these outcomes remains relatively unknown. Therefore, our study aims to investigate whether there are differences in the number of 'usable embryos' based on the Patient-Oriented Strategies Encompassing Individualized Oocyte Number (POSEIDON) criteria.

Materials & Methods
This retrospective study utilized ART data from the CHA Fertility Center, Gangnam, covering the period from January 2021 to February 2022. A total of 2396 retrieval cycles were collected from 1790 patients. The study population was divided into four groups based on the POSEIDON criteria: Group 1 (age < 35; AMH ≥ 1.2 ng/mL), group 2 (age ≥ 35; AMH ≥ 1.2 ng/mL), group 3 (age < 35; AMH < 1.2 ng/mL), and group 4 (age ≥ 35; AMH < 1.2 ng/mL). For each group, we compared the number of 2PN and usable embryos, along with their usable rates.

Results
The POR group accounted for 22% of the total. Across all retrieval cycles, there was a strong positive linear correlation between the number of oocytes and 2PNs/usable embryos. On average, 62% of oocytes developed into 2PNs, and 26% of oocytes were transferred and/or vitrified as usable embryos. The younger POSEIDON groups (I and III) had higher numbers of both 2PN and usable embryos compared to the older groups (II and IV). However, the usable rate decreased with the number of eggs retrieved for all groups except group III.

Conclusions
Regardless of age and AMH level, the number of 2PNs and usable embryos increases with the number of oocytes retrieved. Additionally, the age factor has a greater impact on the number of 2PNs and usable embryos than AMH levels. Standardized, graded classification systems like POSEIDON help predict cycle fertilization and the likelihood of having usable embryos per cycle.

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P1-23 The Correlation between the Angle of Meiotic Spindle and Dysmorphic Pronuclear and Its Effects on Clinical Outcomes

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Introduction
Recent studies have shown that the angle of spindle in MII oocyte could be used for the assessment of human embryo quality. And the dysmorphic pronuclear (PN) is associated with embryo development. However, the correlation between the angle of spindle and dysmorphic PN have not been studied. In present study, we investigated the relationship between the angle of spindle and the dysmorphic PN, and the effect of its correlation on clinical outcomes.

Material & Methods
Data were retrospectively collected from polscopy and time-lapse system performed from March 2019 to March 2022. We analyzed 230 zygotes from 65 patients. We measured the angle of spindle prior to ICSI using a Polscope. Oocytes were divided into two groups according to the angle of MII spindle (<30°group=148, ≥30°group =82) and were cultured in Embryoscope. The dysmorphic PN (differences more than 4μm between PN size, diameter of PN smaller than 20μm or diameter of PN lager than 30μm, nonjuxtaposed) were analyzed by measuring the horizontal diameter at same focal plane before PN fade. Also, the number of multinucleated blastomeres (MN) and abnormal division (direct division and not available cell number due to poor quality) in 2-cell embryo were checked.

Results
The rate of dysmorphic PN (<30°group; 20.3% vs. ≥30°group; 47.6%, $p<0.05$) was significantly increased in ≥30°group. And the rate of MN (20.9% vs. 24.4%) was increased in ≥30°group. The rate of abnormal division (8.8% vs. 15.9%, $p<0.05$) was statistically increased and the rate of good quality (65.5% vs. 48.8%, $p<0.05$) and utilization (66.9% vs. 52.4%, $p<0.05$) were significantly decreased in the ≥30°group compared with<30°group. When only embryos from the <30°group were selected for transfer, the pregnancy rate was 61.9%. But when only embryos from the ≥30°group were transferred, the pregnancy rate was 33.3%, respectively ($p<0.05$).

Conclusions
This study could help predict embryos that are more likely to become pregnant at the earliest time point by enabling noninvasive judgement of embryos. And, it could be especially useful to assess zygote quality in countries with strict policies. Also, the combined method of dysmorphic PN and standard morphological assessment may assist in selecting embryo for the single embryo transfer. This is the first study that has analyzed the correlation between spindle angle and dysmorphic PN.

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P1-24 Live-cell Imaging of Mouse Preimplantation Embryos Using a Simple Closed Glass Capillary Method

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Introduction

Live-cell imaging is a popular method for analyzing mammalian preimplantation embryos, but it often requires expensive equipment and skilled techniques. We previously developed a simply and costly embryo-culture system in a sealed tube that does not require a CO_2 incubator. In the present study, we developed a new live-cell imaging system using our previous culture method and a glass capillary.

Material & Methods

First, we determined how to seal the embryos into the glass capillary to observe their development. Next, we cultured mouse zygotes (ICR strain) derived from IVF using several commercial glass capillaries in a thermoplate. Finally, we performed live-cell imaging of the zygotes developing into blastocysts by Glass Capillary Live-cell imaging method (GCL method), comparing their developmental rate, cleavage speed, and offspring rate to those obtained using a dedicated equipment. In this study, we just used a stereomicroscope, simple digital camera for general use, thermoplate and Switch Bot Plug, which controlled the emission of light. Additionally, we established fluorescence imaging for short-term observations.

Results

We placed zygotes in the glass capillary, which could be easily observed under a stereomicroscope or inverted microscope when glass capillary was submerged in water or oil. By placing the air bubbles in the glass capillary and sandwiching the embryos between them, we were able to keep embryos in one place. By warming the glass capillary with a thermoplate, we were able to achieve blastocyst development (95.8%) and healthy pups (33.9%) in high rate, which were comparable to the control. When we tried live-cell imaging using the GCL method, 81.5% of zygotes developed into blastocysts despite effect light on embryos. In addition, developmental speed from zygote to blastocyst and offspring rate cultured by GCL method were equivalent to the control. Fluorescence imaging was also succeeded to observe embryos without affecting embryonic development and shot clearly enough to analyze localization of fluorescence in embryonic cell.

Conclusions

We successfully developed a simple and cost-effective live-cell imaging method, which has comparable developmental rates and speeds relative to a conventional time-lapse imaging machine. The GCL method utilizes tools that already exist in institutions researching mammalian embryos, making it an accessible option for laboratories with limited research funding.

P1-25 Factors Affecting Tripronuclear (3PN) Incidence in ICSI Cycles: A Retrospective Analysis

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Introduction
Tripronuclear (3PN) formation, characterized by the presence of three pronuclei in the fertilized egg, is an abnormal phenomenon during ICSI cycles. This study investigated factors contributing to the occurrence of 3PN embryos.

Material & Methods
Data from 3568 ICSI cases conducted between January 2020 and August 2022 were retrospectively analyzed. Cases were excluded if they involved conventional IVF, OPU failure, maturation failure, unfertilization, or oocyte banking. The incidence of 3PN was assessed based on patient age and the specific oocyte induction protocols used. Furthermore, the impact of 3PN on stimulation duration, implantation rate, maturation rate, fertilization rate, and good embryo rate was examined. Statistical significance was evaluated with a two-sided p-value < 0.05.

Results
Among all 3568 ICSI cases, 515 (14.4%) cases exhibited one or more 3PN zygotes. The analysis revealed no significant difference in 3PN incidence among different age groups: 15.1% (33/218) for ages 23 to 29 years, 13.7% (188/1372) for ages 30 to 37 years, 14.4% (188/1302) for ages 38 to 42 years, and 15.7% (106/676) for ages 43 to 51 years. However, the incidence of 3PN was notably lower in low stimulation protocols utilizing natural cycle, letrozole, or clomiphene citrate. Moreover, the group with 3PN experienced statistically longer stimulation duration compared to the group without 3PN (15.32 ± 4.8 vs. 14.4 ± 2.9, $p < 0.05$), and the implantation rate was significantly lower in the 3PN group (37.9% vs. 51.8%, $p < 0.05$). No significant differences were found in maturation rate, fertilization rate, or good embryo rate between the groups with and without 3PN.

Conclusions
The results of this retrospective analysis suggest that low stimulation protocols, such as natural cycle, letrozole, or clomiphene citrate, may reduce the occurrence of 3PN during ICSI cycles. Additionally, prolonged stimulation duration is associated with an increased risk of 3PN formation and reduced implantation rates.

P1-26 Separation Efficiency of a New Sperm Separation Device to Minimize Sperm Damage

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Introduction
The quality of sperm is a crucial factor that directly affects the success and health of offspring in assisted reproductive technology (ART). Sperm separation is a critical step in improving sperm quality for both research and clinical purposes. In this study, we assess the efficacy of a new sperm separation device in recovering motile sperm without DNA damage and functional competence, in comparison to conventional methods.

Materials and Methods
The semen samples collected from 50 men who underwent semen analysis were divided into three groups and subjected to sperm separation using three different techniques: the sperm separation device (SSD), density gradient (DG) separation, and swim-up (SU) techniques. All groups were evaluated for sperm concentration, motility, viability by eosin-nigrosin staining, membrane maturity by hyaluronic acid-binding assay (HBA), and DNA fragmentation or damage by halosperm test.

Results
Compared to conventional methods, the novel device demonstrated remarkable simplicity and the shortest processing time as it does not require separate steps for centrifugation or washing. Furthermore, the difference in the concentration of recovered motile sperm, based on the semen condition or the proficiency of the researchers, was more stable compared to conventional methods. Additionally, SSD resulted in a significantly lower rate of sperm DNA fragmentation(SSD; 19.0±8.2, SU; 24.0±7.5, DG; 74.3±6.4) and higher membrane maturity when compared to DG or SU-selected sperm.

Conclusions
The ideal sperm separation technique should be able to isolate motile sperm while minimizing damage to non-physiologically altered cells, removing dead sperm and other cells, eliminating toxic or bioactive substances such as reactive oxygen species, and be quick, easy, and cost-effective. In this regard, a new sperm separation device holds great promise as an effective and efficient sperm separation method that meets various requirements. By improving the success rate of ART, this device can contribute to the birth of healthy offspring. The SSD separation method was proven to be an efficient and reliable way to prepare sperm when compared to DG and SU techniques. However, further research is needed to evaluate the safety and efficacy of SSD for clinical use in assisted reproductive technologies.

P1-27 Does the Site of Blastocyst Hatching in a Single Vitrified-warmed Blastocyst Transfer Affect Pregnancy Outcomes?

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Introduction
The blastocyst hatching from the zona pellucida (ZP) is necessary for implantation into the uterus and achieving a successful pregnancy. Cryopreservation can cause ZP hardening, inhibiting hatching and leading to pregnancy failure. Although it is well known that different species have different blastocyst hatching sites, little is known about the relationship between these locations and the success of a pregnancy. Therefore, our study investigates pregnancy outcomes after single vitrified-warmed blastocyst transfer (SVBT) from various hatching sites.

Material & Methods
A retrospective study of SVBT cycles was conducted at the CHA Fertility Center Gangnam. 316 cycles transferred hatching blastocysts and analyzed them. According to the hatching site, embryos were divided into three groups: $0°\leq\theta\leq30°$, $30°<\theta\leq60°$, and $60°<\theta\leq90°$. The angle (θ) is measured based on the hatching site to the midpoint of the ICM. And according to ICM and TE grades, blastocysts were split into two groups. The relationship between the hatching site and pregnancy outcomes was examined using chi-square. Statistical significance was defined as $P < 0.05$.

Result
Among the 316 blastocysts, 67.4% were classified as $0°\leq\theta\leq30°$, 20.3% as $30°<\theta\leq60°$, and 12.3% as $60°<\theta\leq90°$. Regardless of the hatching site, there was no statistically significant difference in the positive hCG (56.8%, 60.9%, and 53.8%, respectively) and clinical pregnancy rate (51.2%, 50.0%, and 48.7%, respectively). There was no statistically significant difference, even when blastocyst grades were considered. Positive hCG (63.5%, 62.9%, and 64.7%, respectively) and clinical pregnancy rates (58.5%, 48.6%, and 64.7%, respectively) were not statistically significant in good quality blastocysts ($p>0.05$). Additionally, regardless of the hatching site, positive hCG (37.0%, 31.5%, and 45.5%, respectively) and clinical pregnancy rates (29.6%, 27.8%, and 36.4%, respectively) in poor quality blastocysts were not statistically significant ($p>0.05$).

Conclusions
We found that the proportion of blastocysts that hatched from the site $0°\leq\theta\leq30°$ was significantly higher than that from other sites ($p<0.05$). However, the hatching site had little impact on pregnancy outcomes. Therefore, it is not advised to consider the blastocyst's hatching site when choosing an embryo to transfer in SVBT cycles.
However, these findings help embryologists better understand the dynamics of embryos during that process.

P1-28 A Novel Alarm System to Detect Leakage of Liquid Nitrogen from Embryo Cryopreservation Container

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Introduction

The cryopreservation of embryos stands as a fundamental part in assisted reproductive technology. Strict maintenance of cryopreservation container is crucial. Liquid nitrogen (LN_2) container consisted of vacuum layer insulated aluminum wall. At many instances, breakage of the vacuum layer causes drastic evaporation of LN_2 and resulting in devastating consequences. (This phenomenon is referred to as a vacuum failure.) Previously, it was demonstrated that initial frost on the container lid and subsequent condensation manifested within 15 min following vacuum failure induction. Therefore, we utilized this phenomenon to invent new device to detect container accidents. In the present investigation, we deliberately induced vacuum failure in a cryopreservation container and meticulously recorded the surface temperature variations over time. Moreover, we assessed the efficacy of a monitoring system designed to identify abnormal container's surface temperature.

Method

A 10-liter container was completely filled with LN_2, while a data logger temperature sensor, equipped with an error notification feature via e-mail, was affixed to the surface of the container. The temperature of the container's surface was continuously recorded at one-minute intervals after the vacuum failure induction and e-mail notifications were reviewed until the LN_2 completely evaporated. The experiment took place under room temperature condition (about 24℃). The data logger was programmed to dispatch an email notification during the surface temperature descended below 20℃, with a time interval of 3 min.

Result

The surface temperature started to drop 3 min after the induction of vacuum failure, and reached below 20℃ after 6 min (19.1℃), below 10℃ after 11 min (8.8℃), and the lowest temperature of -0.5℃ was observed after 93 and 99 min. The temperature was 5.6℃ 400 min after the vacuum failure, when the LN_2 completely evaporated. The first alarm e-mail was received at 6 min after the vacuum failure induction. After that, a total of 132 alarm e-mails were received until the end of the measurement.

Conclusion

In previous alarm systems for cryopreservation containers such as using level sensors and weight measuring, notification is not sent until a significant reduction of LN_2 occurs after a vacuum failure. In contrast, the system in this study, monitoring the container surface temperature enabled to notify abnormality immediately after the vacuum failure induction and before a significant reduction of LN_2. Furthermore, the receipt of repetitive emails was considered very effective because initial email alerts may go unnoticed at times. These findings underscore the efficacy of surface temperature monitoring as an effective approach for detecting container accidents in cryopreservation.

P1-29 A Study That Investigated Changes in the Perception of Patient Safety of Embryologists with less than Two Years of Experience through a Survey after Patient Safety Education

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Introduction
With the development of reproductive medicine, various procedures are being developed, and the risk to safety is also increasing. For this reason, ART errors cannot be avoided completely. For embryologists who are new to IVF, IVF work is unfamiliar and the risk of safety accidents is high. The CHA Fertility Center conducted its own patient safety education program to investigate the effectiveness of education through the perception of patient safety of embryologists with less than two years of experience.

Material & Methods
This study was conducted through a survey of 30 embryologists who worked at five clinics of CHA fertility centers. The 30 embryologists are those who have been in the IVF laboratory for less than two years. The patient safety management system was created by the CHA fertility Center itself. From August 2021 to December 2022, safety confirmation training was conducted for 16 months. All embryologists were anonymously surveyed twice with 32 questions. The first survey was conducted in April 2022, and the second survey was in December 2022.
Answers were scored using a 5-point Likert scale (1=strong disagree, 5= Strong agree).
The survey was conducted in 32 items in 10 categories. The response was conducted in five steps for each item. It asked 1) whether to learn the standard safety manual, 2) changes in safety awareness before and after training, changes in work performance, 3) compliance with seven safety instruction (double checks) for each work part, 4) difficulty level of education, 5) the effect of education and 6) suggestions for safety confirmation were asked.

Results
All the embryologists with less than two years of experience showed improved patient safety awareness after education.
In the first survey, 23 under 2 years embryologists responded and 22 responded in the second survey. The mean score for work satisfaction was 3.4/5 in the first and 3.1/5 in the second survey, and the work satisfaction remained unchanged. The perception of patient safety confirmation was 3.2/5 before training and 4.0/5 after training in the second survey, which improved the perception of safety confirmation after training($P<0.001$). Most of CHA IVF lab. under 2 years embryologists were well aware of the guidelines (first 4.2/5 and second 4.4/5) to know that seven safety instructions (double check, outloud, RI witness, monitor, document, information) should be followed well.

Conclusion
According to the study, patient safety confirmation education on assisted reproductive technology is effective for embryologists who are new to work to improve patient safety awareness and performance as an embryologist.
Safety errors can be prevented through systematic system development and repeated safety training.

P1-30 Morphokinetics Parameters as Prognostic Tools: Identifying High-Quality Embryos for Successful Outcomes

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Introduction

Pregnancy is the outcome of bidirectional communication between the endometrium and the developing embryo. The generation of a competent embryo is a crucial initial step toward successful childbirth. In this study, a time-lapse imaging system was employed to identify critical points during various fertilization events, serving as biomarkers for early embryo assessment.

Materials and Methods

A retrospective analysis of time-lapse images was conducted on 194 zygotes obtained from 73 patients who underwent IVF with minimal stimulation between January 2019 and October 2022. The morphokinetics analysis covered the period from immediate post-fertilization to just before cleavage, aiming to assess the morphological and temporal characteristics of the 2PN zygotes. A comparison was made between high-quality and low-quality embryos, focusing on their morphological features (categorized into 14 groups) and temporal attributes (categorized into 5 groups).

Results

A total of 194 zygotes were analyzed, of which 109 embryos (56.2%) developed into high-quality blastocysts, while 85 embryos (43.8%) developed into low-quality blastocysts. The high-quality embryos exhibited significantly smaller overall zygote size (20650.4 μm^2 vs. 21172.5 μm^2, p=0.013), cytoplasmic size (10034.8 μm^2 vs. 10204.6 μm^2, p=0.018), and cytoplasmic size at nuclear emergence (9609.3 μm^2 vs. 9752.67 μm^2, p=0.038) compared to low-quality embryos. Additionally, high-quality embryos demonstrated faster nuclear appearance time (6.7 vs. 7.1, p=0.05) and nuclear fading time (22.7 vs. 23.9, p=0.009) than low-quality zygotes.

Conclusion

Our findings suggest that the morphokinetics parameters observed during fertilization could serve as valuable predictors for enhancing the selection of a more competent embryo. We observed significant morphological and temporal distinctions between high and low-quality embryos, underscoring the importance of early embryo assessment.

Funding

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P1-31 Does Day 4 Morula Embryo Transfer Provided a Pregnancy Outcome Compatible with Day 5 Blastocyst Embryos in Fresh IVF/ET Cycles of Young Women?

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Introduction

The morula is the point at which embryonic gene expression begins and represents a critical stage in pre-implantation embryo development. It is also well known that the embryo migrates into the uterine cavity on the 4th day after fertilization. In particular, compared with day 5 ET, it can be exposed to the natural environment in utero for a maximum period of time before implantation, and the exposure time to in vitro culture is short. Moreover, it appears to be an alternative day to perform ET, such as improving the flexibility and planning of IVF clinics. However, the use of morula transfer at day 4 has received little attention, and data on morula ET compared to blastocyst ET are extremely limited.

Material & Methods

This retrospective study analyzed all cycles of fresh embryo transfer on days 4 and 5 from January 2020 to October 2022 at the CHA Fertility Center in Seoul Station. As a subgroup, we analyzed pregnancy outcomes in women younger than 35 years of age who underwent embryo transfer on days 4 and 5 of an IVF/ET cycle. IVF cycles using donor oocyte, TESE sperm, PGT, or those lacking follow-up were excluded. A total of 1,406 cycles were analyzed, with 510 cycles for day 4 morula transfers and 896 cycles for day 5 blastocyst transfers. As subgroups, we analyzed 153 cycles for 4-day morula transfer and 254 cycles for 5-day blastocyst transfer in young women (<35 years of age). All women underwent standard IVF protocols following usual individualized practice in our IVF clinic. Chi-square test was used to evaluate the differences between the patient cohorts.

Results

There were no statistically significant differences in clinical and ongoing pregnancy rate between fresh embryo transfer on days 4 and 5 (40.4% vs. 46.5%, $p>0.05$ and 43.5 vs. 47.9%, $p>0.05$, respectively). However, in a subgroup of young women, both pregnancy and clinical pregnancy rates were significantly higher on day 4 of morula. (71.2% vs 55.3%, 60.8% vs 47.1%, $p<0.05$). Ongoing pregnancy rates were slightly higher in day 4 ET than in day 5 ET, but there was no significant difference. (52.9% vs. 41.3% $p>0.05$)

Conclusions

For young womens, day 4 morula ET appears to be an alternative day to perform embryo transfer if day 5 blastocyst ET is not possible. It is considered an easy and applicable methods to improve the flexibility and planning of IVF clinics.

P1-32 Comparison of Clinical Pregnancy Rates in Fresh Cycle, Surplus Embryo Freezing Cycle, and Selective Freezing Cycle in Single Blastocyst Transfer

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Introduction

We hypothesize that the reason for pregnancy failure in fresh transfer is that ovarian stimulation alters endometrial receptivity due to a superphysiological level of steroid effects on endometrial maturation. Therefore, as an alternative to conventional fresh embryo transfer, all freezing strategies have been proposed to improve pregnancy rates. In addition, increasing pregnancy rates after frozen-thawed embryo transfer have encouraged wider implementation of a freezing-all (elective freezing of all embryos) strategy in assisted reproductive technology treatment. However, it is unclear whether these strategies are also helpful in blastocyst single embryo transfer.

Material & Methods

This retrospective study included infertile patients aged 25 to 48 years old who underwent single blastocyst transfer at Seoul Station Cha Hospital between January 2020 and October 2022. This study only included first cycle transfer from our center. IVF cycles using donor oocyte, TESE sperm, PGT, or those lacking follow-up were excluded. Embryo transfer was divided into three groups: A. Fresh transfer, B. Frozen embryo transfer of surplus embryos remaining after fresh transfer, C. Frozen embryo transfer of embryos selectively frozen by the all-freezing strategy. The number of cycles complying with the inclusion criteria was 3228 cycles. Fresh embryo transfer (ET) was 873 cycles, subsequent frozen embryo transfer of surplus embryos was 225 cycles, and frozen embryo transfer following a freez-all strategy was 2130 cycles.

Results

The mean (±SD) ages across group A, B and C were 37.4 (±3.6), 37.3 (±3.0) and 37.1(±3.4) years, respectively. The clinical pregnancy rates (48.1% versus 55.6% versus 62.8%) and biochemical pregnancy rates (59.0% versus 65.3% versus 73.0%) were significantly higher in group C than in group A. The ongoing pregnancy rate was the highest in group C compared to groups A and B, but there was no significant difference. (38.8% versus 46.7% versus 52.7%)

Conclusions

In single blastocyst transfer, all freezing strategies are thought to improve clinical pregnancy rates compared to fresh transfer and surplus embryo frozen embryo transfer.

P1-33 Artificial Intelligence(AI)-based Analysis of Blastocysts in Patients Showing Low-risk Noninvasive Prenatal Testing (NIPT) Levels or Early Pregnancy Loss

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Introduction
There are few studies on the correlation between NIPT results or early pregnancy loss outcomes in patients and AI-based morphokinetics and morphology parameter analysis. This study aims to confirm the possibility of selecting healthy embryos by analyzing the blastocysts of patients with low-risk NIPT results or early pregnancy loss through AI-based analysis.

Materials and Methods
A retrospective study was performed from January 2021 to January 2023. Time-lapse images of 60 embryos were obtained from single blastocyst transfer and categorized into two group: ongoing pregnancy with low-risk NIPT(group1,n=50) and early pregnancy loss (group2,n=10) with chromosomal normality(CN)(group2a,n=5) and abnormality(CA)(group2b,n=5). Embryos were cultured in a time-lapse incubator(Embryoscope+) until day 5 and analyzed using both iDAScore and KIDScoreD5. Group1(over 10weeks) was screened through noninvasive analysis for chromosomal(21,18,13 and Sex chromosome) disorders, while group2(below 10weeks) underwent karyotyping for CA from abortus. We compared the morphokinetics, morphology parameters, and the score from the deep learning system between the two groups.

Results
The mean age was 36.43±2.53 yrs in group1 and 36.72±3.43 yrs in group2(p=0.380). Significant differences were observed in the iDAScore(6.59±1.69 vs. 7.6±0.76, p=0.098) and KIDScoreD5(7.29±1.75 vs. 8.92±0.51, p=0.022) between group1 and group2b, respectively. During the early stage of embryo development, the morphokinetic parameters in group2b were faster than those in group1 (tM:85.36±7.31 vs. 78.82±6.27,p=0.030). However, after the morula stage, the speed of blastocyst expansion in group2b was slower than group1(tB-tEB:8.18±3.49 vs. 10.07±3.97,p=0.132). While comparing the iDAScore values, group1 accounted for 35% at a low score of 6 points or less, however the blastocyst showed good morphology. On the other hand, group2 did not fall in this range.

Conclusions
Morphokinetic parameters showed faster development in the early pregnancy loss group with chromosomal abnormality compared to the ongoing pregnancy group with low-risk NIPT results. However, the speed of blastocyst expansion was slow. Even if the iDAScore or KIDScoreD5 values are low, the NIPT results show that the probability of chromosomal abnormality is low if the morphological quality of the blastocyst is good. Furthermore, this study suggests that it might offer useful results for patients who could not undergo PGT-A.

P1-34 Telomere Length Determines the Mitochondrial DNA Content in the Blastocyst Stage Embryos

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Introduction
Telomere and number of mitochondria are markers of oocytes and embryo quality, and targets of reproductive aging. We recently showed that telomere length (TL) is closely correlated with number of mitochondria in the blastocyst stage in mice (Mitochondrion 2023). Here we address the hypothesis that TL is a decisive factor in regulating the mitochondrial DNA copy number (Mt-cn) in the blastocyst stage.

Material & Methods
We produced bovine embryos using oocytes derived from a slaughterhouse. Oocytes were aspirated from the antral follicles and subjected to in vitro maturation and fertilization. Eighteen hours after insemination, presumptive zygotes were cultured in vitro up to the blastocyst stage (day 7 post insemination).The culture medium was supplemented either with telomerase inhibitor (10μM TMPyP4 diluted in water) or siRNA targeting TERT during the culture period. We determined TL and Mt-cn in the embryos using real-time quantitative PCR (qPCR) using external standard. Prior to qPCR, we determined the embryos' total cell number. The total cell number was then used to calculated TL and Mt-cn in the blastomere. Zona-free zygotes were transfected with siRNA using lipofectamine 2000. Effect of siRNA was examined using dual luciferase assay.

Results
We produced bovine blastocysts using frozen thawed semen derived from six bulls. We observed significant positive correlation between TL and Mt-cn in the cohort blastocysts derived from all the bulls. Addition of telomerase inhibitor in the in vitro culture medium reduced the developmental rate, TL, and Mt-cn of the blastocysts. In addition, we observed significant positive correlation between TL and Mt-cn in both control and TMPyP4 treated embryos. siRNA treatment reduced both TL and Mt-cn in the blastocysts compared to the blastocysts developed in the same culture conditions. Furthermore, we observed significant positive correlation between TL and Mt-cn in both control and siRNA treated embryos.

Conclusion
TL regulates the Mt-cn in the blastocyst stage embryos.

P1-35 Specific miRNAs in Bovine Follicular Fluids Underlying Background of Good Blastocyst Yields

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Introduction
Oocyte and embryo quality differs significantly among donors, this study examined a hypothesis that the miRNA contains in bovine follicular fluids (FF) determine not only oocyte maturation but also embryonic development.

Material & Methods
Bovine ovaries were collected from a slaughterhouse. Oocyte-cumulus cell complexes were individually aspirated from the antral follicle (3–6 mm in diameter). Ovaries were rated based on the ability of enclosed oocytes to develop to the blastocyst stage; FFs were collected from the ovaries and defined as Good- (top 2) or Poor- (bottom 2) FF. These FFs were prepared from three series of ovaries. FFs were added to in vitro maturation (IVM) or in vitro culture (IVC, at 18–48 h post insemination) at a concentration of 10% or 1%, respectively. Basal media of IVM and IVC were 199 medium and synthesized oviductal fluid, respectively. The extracellular vesicle (EV) was isolated from the FF, RNA was extracted using a kit (SeraMir Exosome RNA Amplification Kit), and miRNAs were analyzed using small RNA sequencing. Effects of the miRNA mimics were verified using dual luciferase assay. Intake of miRNA mimic in EVs by the granulosa cells (GCs) were examined following co-incubation of GCs or embryos with EV enclosing fluorescent conjugated miRNA mimics. The miR-151-3p, -425-5p, and control mimics were mixed in Opti-MEM and added to the culture medium with lipofectamine 2000. In embryo treatment, the zona pellucida was removed using pronase.

Results
Supplementation of either IVM or IVC medium with a Good-FF improved embryonic development compared that with a Poor-FF. The miR-151-3p and -425-5p were detected in the EVs from Good-FF with significantly higher frequency compared to that from the Poor-FF. The effect of the miRNA was verified via dual luciferase assay. EV-miRNA secreted from Cy3-miRNA mimic-transfected GCs was taken up by either GCs or embryos. Both the miR-151-3p and -425-5p mimic treatment during IVM or IVC improved embryonic development to the blastocyst stage.

Conclusions
miRNA in FFs is crucial for not only oocyte quality but also embryonic development and is a possible factor for individual differences in assisted reproductive technology outcomes.

P1-36 Developmental Competence of Bovine Oocytes Cultured with Mesenchymal Stem Cell Culture Medium Following in Vitro Fertilization

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Introduction
It has been shown that co-culturing mammalian oocytes with mesenchymal stem cells, such as adipose-derived stem cells, or adding stem cell-derived conditioned medium during in vitro maturation (IVM) improves the developmental competence of post-fertilization embryos. However, serum is often added when culturing cells. Therefore, it has been suggested that experimental results may be influenced not only by the effects of cells or their secretory factors but also by the effects of serum. In this study, we investigated whether R:STEM (Rohto Pharmaceutical, Osaka, Japan), which was developed for serum-free culture of mesenchymal stem cells, can support the culture of bovine oocytes.

Methods
IVM was performed for 21 hours using R:STEM or TCM-199, and the maturation rate of oocytes was examined. In addition, in vitro fertilization was performed using post-IVM oocytes and cryopreserved-thawed sperm, and the fertilization rate and subsequent embryo development rate were measured.

Results
There were no significant differences in the maturation rate of oocytes matured with R:STEM or TCM-199 (86.3±0.9% vs. 77.9±5.6%), fertilization rate (59.0±0.8% vs. 47.6±1.9%), polyspermy rate (37.6±3.2% vs. 24.5±3.9%), cleavage rate (97.5±1.4% vs. 91.7±1.4%), blastocyst formation rate (20.0±4.1% vs. 25.0±4.1%), and blastocyst cell number (188.9±6.9 vs. 175.6±6.6).

Conclusion
The results of this study showed that mesenchymal stem cell culture medium can support the developmental competence of bovine oocytes to a similar extent as TCM-199. Further experiments co-culturing oocytes with stem cells are planned for future investigations.

P1-37 Relationship between Delayed First Mitotic Division and Duration of Fertilization Events for Human Embryos

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Introduction
Early embryo cleavage is an essential indicator of embryo health, which is associated with an increased blastocyst formation rate, superior morphology, and high implantation potential. Conversely, delayed first mitosis is a predictor of adverse embryonic developmental effects both in vitro and in vivo. During fertilization, several events occur between the entry of the sperm into the oocyte and the first mitotic division. However, a limited number of studies have focused on the association between these events and the first mitotic division. In the present study, we examined the relationship between fertilization events and investigated its association with the delay in the first mitotic division.

Material & Methods
To estimate the timing between fertilization events, time-lapse images of intracytoplasmic sperm injection-derived embryos (n = 114) were acquired. The fertilization events were divided into the following time intervals: sperm injection to second polar body extrusion (phase 1), second polar body extrusion to juxtaposed female/male pronucleus (phase 2), juxtaposed female/male pronucleus to pronuclear envelope breakdown (phase 3), and pronuclear envelope breakdown to the first mitotic division (phase 4). Correlation analyses of the time interval of each phase and between the period from sperm injection to the first mitosis were performed. In addition, the correlation between phases was also examined. The Spearman's rank correlation coefficient (ρ) was used for assessing correlations, with a $p < 0.05$ considered statistically significant.

Results
The whole first mitotic period was strongly positively correlated with the time interval of phase 3 ($\rho = 0.72$, $p < 0.01$) and moderate positively correlated with phase 1 ($\rho = 0.36$, $p < 0.01$) and phase 2 ($\rho = 0.21$, $p < 0.05$) time intervals. No correlation between the duration of the first mitotic division and phase 4 was found ($\rho = 0.14$). There were weak positive and negative correlations between phase 1 and phase 4 ($\rho = 0.25$, $p < 0.01$) and between phase 2 and phase 3 ($\rho = -0.39$, $p < 0.01$), respectively.

Conclusions
We found that a delayed first mitotic division during fertilization is correlated with a prolonged interval from the juxtaposition of the female/male pronucleus to pronuclear envelope breakdown. In addition, fertilization phases between fertilization events were weakly related to each other.

P1-38 Assessing the Effect of Lengthening or Shortening the Duration of Pronuclear Junction Formation on the Development of Human Embryos in Vitro

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Introduction
The time interval between the formation and breakdown of the pronuclear junction varies widely among individual zygotes. Chromatin polarization and clustering at the junction plane are essential for proper chromosome segregation during the first mitosis and subsequent embryo development. However, the relationship between the duration of junction plane maintenance and embryo development is not fully understood. The objective of this study was to investigate the impact of the duration of pronuclear junction formation on the development of human embryos in vitro.

Material & Methods
The duration of pronuclear junction formation was measured in 183 zygotes using time-lapse monitoring. The period to pronuclear junction formation was classified into four groups: short (<600 min), medium (600–840 min), long (840–1080 min), and very long (>1080 min) time intervals. The proportion of blastulation between groups was compared. In addition, the logistic regression model was used to estimate the adjusted odds ratio (AOR) of blastulation at a 95% confidence interval (CI), using the medium-time interval (600–840 min) as the reference group. The multivariate logistic regression model was adjusted for maternal age, insemination technique, and total motile sperm count as potential confounders. Ryan's test for multiple comparisons of proportions was performed, with a $p < 0.05$ considered statistically significant. Independent factors were considered significant if the 95% CI of the AOR did not include 1.

Results
The proportion of blastulation in the short-time group was 44%, a value significantly lower than that in the medium-time group (72%). The incidence of blastocysts in the very long-time group was 26%, a value significantly lower than those in medium- and long-time groups (72% and 65%, respectively). Blastulation failure was more likely to occur by lengthening or shortening the junction formation time, showing AOR values of 0.27 (95% CI: 0.10-0.71, $p < 0.01$) and 0.14 (95% CI: 0.04-0.43, $p < 0.01$) in short- and very long-time groups, respectively.

Conclusions
These results provide evidence that the duration of pronuclear junction formation is a critical factor for in vitro embryo development and deviations from optimal time intervals may decrease the blastocyst rate.

P1-39 A Study on Effective Laser Assisted Hatching and Vitrification Freezing Time for In Vitro Fertilization

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Objective
This study was conducted to investigate the efficacy of laser-assisted hatching(LAH) and various vitrification freezing times for embryonic development and blastocyst cell numbers.

Methods
First, 2-cell and 8-cell embryos were collected by flushing out the oviduct. In the control groups, they were vitrified for 8 or 10 minutes without LAH. The LAH groups underwent quarter laser zona thinning-assisted hatching before vitrification (4, 6, and 8 minutes or 4, 7, and 10 minutes, respectively). After incubation, double-immunofluorescence staining was performed.

Results
The hatched blastocyst rate 72 hours after the 2-cell embryos were thawed was significantly higher in the 2LAH-ES8 group (33.3%) than in the other groups ($p<0.05$). In the control-8 group (22.1±4.6), the cell number of the inner cell mass(ICM) was higher than in the LAH groups ($p<0.05$). The number of trophectoderm cells was higher in the 2LAH-ES6 group (92.8±8.9) than in the others ($p<0.05$). The hatched blastocyst rate 48 hours after the 8-cell embryos were thawed was higher in the 8LAH-ES4 group (45.5%) than in the other groups, but not significantly. The inner cell mass cell number was highest in the 8LAH-ES7 group (19.5±5.1, $p<0.05$). The number of trophectoderm cells was higher in the 8LAH-ES10 group (73.2±12.1) than in the other groups, but without statistical significance.

Conclusion
When LAH was performed, 2-cell embryos with large blastomere had a lower hatched blastocyst rate when the exposure to vitrification solution was shorter. Conversely, 8-cell embryos with small blastomere had a higher hatched blastocyst rate when the exposure to vitrification solution was shorter.

Keywords: Mouse embryo, Laser assisted hatching, Vitrification, Blastocyst, In vitro fertilization

P1-40 Overnight Sperm and Oocyte Co-Incubation versus a Few Hours of Co-Incubation for Fertilization in Conventional Insemination while Using a Time-Lapse Culture System

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Introduction

Traditionally during IVF, sperm and oocytes are co-incubated overnight for conventional fertilization. When utilizing time-lapse for embryo culture, the oocyte should be placed into the time-lapse incubator soon after exposure to sperm for capturing images that show critical characteristics in embryo development. Common practice is to co-culture oocytes and sperm before the oocyte is isolated and placed into the time-lapse culture system. Several hours of co-incubation may be required to allow sperm to interact with and to penetrate the oocyte. This study is to compare traditional overnight versus a few hours of co-incubation for fertilization in laboratory outcomes.

Materials & Methods

Three groups with different durations of co-incubation were assigned for insemination. 3 hours post-retrieval + 3 hours of co-incubation (A), 2 hours post-retrieval + 4 hours of co-incubation (B) and overnight co-culture (C). At the time of retrieval, oocytes were rinsed in insemination medium + 5 mg/ml human serum albumin and placed into organ culture dishes with 1.0 ml of the same media and 1.0 ml oil overlay. Approximately 0.250 x 10¬¬6 total motile sperm were added to each dish with up to six oocytes and co-incubated for the designated exposure time. For groups A and B, cleaned oocytes were placed into an EmbryoScope® for culture and photo documentation. Oocytes in Group C were isolated from co-culture the following morning thereafter to continue embryo culture in groups utilizing benchtop incubators. Rates of fertilization, blastulation and good quality of blastocyst were compared between groups.

Results

Number of retrievals were 107, 122 and 142 in groups A, B and C respectively. Patient age (33.1, 34.8. 35.2), maturity of oocytes per retrieval (1556/1906=81.6%, 1399/1799=77.8%, 1644/2001=82.2%) and average number of oocyte per retrieval (17.8, 14.7 and 14.1) were not statistically different between all groups. There was no significant difference in normal (2pn) fertilization rates (70.0%, 67.5%, 79.4%), nor in rates of blastulation (71.2%, 73.3%, and 68.2%) and of good quality usable blastocyst (62.9%, 64.7%, 60.6%) in groups A, B, and C. Clinical pregnancy outcomes for both fresh and frozen embryo transfers are under analysis.

Conclusions

There is no significant difference in fertilization rates and embryo developmental potential as evidenced by blastulation and good quality embryo formation in overnight versus 3-4 hours of sperm-oocyte co-culture for fertilization. As little as 3 hours of sperm-oocyte co-incubation may be sufficient for fertilization which is cooperative to take full advantage of time-lapse technology. This study provides a reference for IVF laboratories utilizing time-lapse culture systems when considering insemination protocols as well as in managing laboratory work flow.

P1-41 Selection of Developmentally Competent Oocytes Using Cytoplasmic Granulation Pattern in IVF Cycles

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Cytoplasmic granulation is a rare morphological feature of the oocyte that appears denser than in other regions, and it might be a sign of oocyte cytoplasmic immaturity and used as a predictor for oocyte quality. Some clinical reports showed that granulation adversely affects embryonic development, especially in ongoing pregnancy rates. Thus, we aimed to investigate the relationship between oocyte cytoplasmic granulation and embryo development in IVF cycles.

This study investigated 235 blastocysts in couples with IVF cycles from December 2021 to July 2022. Granulation morphology was categorized into four patterns: fine granulation (FG), central granulation (CG), uneven granulation (UG), and dispersed granulation (DG). The correlations between the granulation pattern and good quality embryo (GQE) rate on day 3 or 5 were analyzed. By the morphological criteria, the GQE rate was defined as an embryo with at least eight blastomeres on day 3, symmetric regular or slightly irregular blastomeres, and less than 10% of fragmentations.

Our results showed that the oocytes with different granulation patterns had no significant difference in fertilization, embryo quality, and total blasturation rates. However, FG group produced numerically the highest blastocyst formation rate on day 5, followed by CG, UG, and DG groups (62.5%, 54.2%, 51.5%, and 0.0%, respectively). Significantly, FG group showed statistically higher blasturation rates on day 5 than UG group (FG vs. UG, $p<0.05$).

Therefore, we suggest that the pattern of the cytoplasmic granulation in the oocyte could be a morphological marker for selecting oocytes having good developmental competence. A better understanding of the fate of embryos derived from granulation oocytes could benefit and improve ART treatment.

P1-42 Treatment with Human GM-CSF Cytokine Improves Pregnancy and Implantation Rates in Poor Responders

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Introduction
Cytokines drive the dialogue between the embryo and endometrium and are increasingly expressed throughout embryo development. GM-CSF is known for its importance in the development of blastocysts and in normal fetal and placental development. It is reported that inclusion of GM-CSF in embryo culture media significantly increases ongoing implantation rates in women who have previously experienced miscarriage. However, it is still unclear whether poor responders of IVF attempts should be another indication for the use of GM-CSF.

Material & Methods
This prospective study was performed between January 2020 and September 2022 at a reproductive center. All patients involved gave written consent, and institutional review board approval was granted. This study includes 150 non-treatment and 155 treatment couples. A prospective study was conducted including patients with poor responders in our center. In the treatment cycles, embryos were cultured for 3 days in EmbryoGen (Origio, Denmark). ET was performed on Day 3 in BlastGen (Origio, Denmark). Clinical pregnancy and ongoing implantation rates were compared, p-value of <0.05 was considered statistically significant.

Results
The average female age, number of oocytes retrieved, embryo developmental stage and number of embryos transferred did not vary significantly amongst the groups. Fertilization and cleavage rates did not differ. However, Clinical pregnancy rates (65/150 (41.9%) vs 50/155 (32.3%), and ongoing implantation rates (18.6 ± 3.3 vs 15.2 ± 3.6) were higher in EmbryoGen and BlastGen treatment group compared to control, but these differences did not reach statistical significance.

Conclusions
The findings of this study indicate that GM-CSF treatment improves the rates of pregnancy and ongoing implantation rates in patients with poor responders. However, this treatment does not seem to completely resolve the patients with poor responders. Further investigations are necessary to determine the effects of GM-CSF treatment of the culture conditions.

P1-43 Association between Turner Syndrome and Premature Ovarian Insufficiency Patients with Chromosomal Abnormalities

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Objective
Premature Ovarian Insufficiency (POI) is a condition where menopause occurs before the age of 40. It shows resistance to various infertility treatments, and one of its causes is known to be chromosomal abnormalities. Turner Syndrome is a sex chromosome abnormality represented by 45,X, occurring at a frequency of about 1 in 1000 female births. Clinically, it is characterized by short stature, gonadal dysgenesis, and distinctive physical features. It often presents with high levels of gonadotropins and amenorrhea, leading to challenging infertility. While natural pregnancies are rare, if they occur, there is a high risk of pregnancy complications due to heart and aortic diseases. Some patients have confirmed diagnoses of pure Turner Syndrome and attend clinics, while others with minimal physical features are identified as potential mosaic Turner Syndrome patients during the investigation of POI.
In this study, we aimed to investigate the frequency and distribution of chromosomal abnormalities related to Turner Syndrome in patients with POI.

Methods
We retrospectively examined 1797 cases of POI who underwent chromosomal testing (G-banding method) for infertility treatment from January 2009 to July 2021, excluding 7 cases after bone marrow transplantation. We analyzed the number and distribution of cases with chromosomal abnormalities. Additionally, among cases with chromosomal abnormalities, we retrospectively investigated the frequency and distribution of cases related to Turner Syndrome.

Results
Among POI patients, 161 cases (9% of the total) showed chromosomal abnormalities. Details of these 161 cases were as follows: numerical abnormalities in 77 cases (48%), structural abnormalities in 61 cases (38%), a combination of numerical and structural abnormalities in 20 cases (12%), and other abnormalities in 3 cases (2%). Among these chromosomal abnormalities, 83 cases were related to Turner Syndrome, accounting for 51% of the total. Among them, there were 6 cases of pure Turner Syndrome (7%) and 77 cases of mosaic Turner Syndrome (93%). Among cases of mosaic Turner Syndrome, 57 cases (69%) had numerical mosaic abnormalities, and 20 cases (24%) had structural mosaic abnormalities.

Conclusion
The frequency of chromosomal abnormalities among POI patients was 9%. In this study, 51% (83 out of 161 cases) of cases with chromosomal abnormalities were related to Turner Syndrome, and among these, there were 77 cases of mosaic Turner Syndrome. Potential mosaic Turner Syndrome patients were identified, suggesting the need to consider genetic counsseling in advance, including appropriate screening tests for Turner Syndrome, as necessary.

P2-01 Biochemical Analysis of Sperm Antigens Recognized by Sperm-Immobilizing Antibodies in Infertile Women

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Objective
Sperm-immobilizing antibodies have been detected in approximately 3% of the sera from infertile women. These antibodies are thought to be produced as a homologous antibody against sperm and are also considered to cause immune infertility. The primary effect of sperm-immobilizing antibodies is probably to inhibit sperm passage through the female reproductive tract. It is also known that sperm-immobilization antibodies interfere with fertilization and implantation. The purpose of this study is to identify antigens recognized by the sperm-immobilizing antibodies and elucidate the pathogenesis of immune infertility.

Methods
Human sperm proteins were extracted with 0.1% sodium dodecyl sulfate (SDS) from good motile sperm prepared by density gradient centrifugation. Eight patients' sera with sperm-immobilizing antibodies were used for SDS-PAGE and western blotting to detect corresponding antigens. Three serum samples showing marked positive reactions were selected for further examination by two-dimensional electrophoresis and mass spectrometry to identify the target antigen.

Results
All the three sera selected for two-dimensional electrophoresis reacted to multiple spots, not a single spot. Mass spectrometry using particularly strong signal spots (Mr: 51K, pI: 5.6) in serum No.1 and (Mr: 58K, pI: 4.9) in serum No.2 identified as Tubulin beta-4A (TBB4A) and Tubulin beta-4B (TBB4B), respectively. No significant molecule was detected in serum No.3.

Conclusions
This study showed that sperm-immobilizing antibodies recognized a wide variety of sperm molecules. Two molecules, TBB4A and TBB4B, were independently identified as the target antigens. As both TBB4A and TBB4B are major components of tubulin that forms microtubules in the sperm tail, the antibodies present in infertile patients plausibly inhibit sperm motility in female reproductive tracts. Further examinations are necessary to confirm whether tubulin components are a major target for sperm-immobilizing antibodies.

P2-02 Clinical Significance of Peritoneal Sperm Recovery Test (PSRT) in Infertile Women under Laparoscopic Investigation

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OBJECTIVE
To clarify the significance of PSRT in laparoscopic investigation, the postoperative pregnancy rate of timed intercourse (TI) or intrauterine insemination of husband's semen (IUI) was evaluated.

MATERIALS AND METHODS
From January 2009 to December 2019 in Yuuai Medical Center, 115 infertile women under 40 y.o. among 148 patients who were treated with laparoscopy to search the causes of infertility, were tested by PSRT. Preoperatively these 115 patients were examined by hysterosalpingography, showing their oviducts patent unilaterally or bilaterally for excluding the patients with bilateral tubal obstruction. Their partners' spermiograms were normal. The average age of 115 patients was 34.4 The average infertility period was 3.8 years. Concerning PSRT a few hours before surgery the patients were treated with IUI. During the surgery their sperms were recovered from the Douglas' pouch for checking the motile sperm. If there were motile sperms, they were diagnosed as PSRT positive. Without motile sperms they were defined as negative. After laparoscopic investigation, they were examined by hysteroscopy with selective catheterization into the bilateral fallopian tubes for injecting Indigocarmine saline solution to check the patency, next injecting Lipiodol, and lastly saline water to flush the fallopian tubes. Postoperatively, they were treated with TI or IUI within a year.

RESULTS
There were 73 PSRT positives (63.5 %). The average age of the positive group was 33.8 y.o. and the negative group was 35.2 y.o. The comparative study of postoperative pregnancy rate within a year between these two groups revealed the positive group showed 34.2% (25 from 73) and the negative group indicated 16.7% (7 among 42) (P value = 0.043; Pearson's chi-square test). The average period required for pregnancy in the positives was 3.6 months, and in the negatives was 4.6 months. This clarified the pregnancy rate in the positives was significantly higher than the negatives.

CONCLUSIONS
Our results revealed the infertile women with PSRT positive generated a significantly high pregnancy rate than the negatives. This suggests the PSRT can be clinically significant test to estimate the postoperative treatment success with TI or IUI. And the selective catheterization by use of Lipiodol may improve the oviductal miliue. Therefore, the laparoscopic investigation with PSRT and the selective tubal catheterization with Lipiodol might be recommended before ART treatment.

P2-03 Ultra-fast Vitrification: Minimizing Toxicity of Cryoprotective Agents and Osmotic Stress in Mouse Oocyte Cryopreservation

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Introduction

While oocyte cryopreservation has seen significant advancements through vitrification, challenges remain concerning the toxicity of high-concentration cryoprotective agents (CPAs) and osmotic stress. To address these issues, reducing the exposure time to high-concentration CPAs can alleviate toxicity and minimize the width of the cell's shrinkage and swelling curve to decrease osmotic stress. In this context, we evaluated ultra-fast vitrification (UF-VIT) through observation of subcellular organelles in oocytes.

Material & Methods

We compared fresh mouse oocytes from 7-week-old B6D2F1 mice (control) with two experimental groups: UF-VIT without equilibration and C-VIT with 8-15 minutes of equilibration. Each group contained 50 mature mouse oocytes. We observed the survival rate and functionality (fluorescence intensity and distribution) of endoplasmic reticulum (ER) and mitochondria (MT) using confocal microscopy. Additionally, we analyzed the morphology of the meiotic spindle and chromosomes in both 2D and 3D.

Results

Compared to the control, C-VIT-treated oocytes showed significant differences in post-warming survival rate ($p < 0.05$), ER distribution (equatorial part: $p < 0.01$; cortical part: $p < 0.01$), ER fluorescence intensity ($p < 0.001$), MT distribution ($p < 0.001$), mitochondrial membrane potential ($p < 0.001$), and MT fluorescence intensity ($p < 0.001$). UF-VIT-treated oocytes showed significant differences in ER fluorescence intensity ($p < 0.01$) and MT distribution ($p < 0.001$) compared to control oocytes. There were no significant differences in ER parameters between the two vitrification methods; however, UF-VIT-treated oocytes had better MT results (distribution: $p < 0.01$; mitochondrial membrane potential: $p < 0.001$; fluorescence intensity: $p < 0.001$). No significant differences in meiotic spindle and chromosome morphology changes among the three groups were observed.

Conclusion

Our research results revealed that the widely used C-VIT method in oocyte cryopreservation can cause damage to cell organelles due to CPA toxicity and osmotic stress. In contrast, UF-VIT showed minimal damage to cell organelles, with only 1 minute of CPA exposure in the non-vitrified solution. Additionally, the freeze-thaw process had no effect on the meiotic spindle and chromosomes. These findings indicate that UF-VIT can overcome the drawbacks of C-VIT, specifically CPA toxicity and osmotic stress, and contribute to advancements in oocyte cryopreservation.

P2-04 Application of Reconstructed Decellularized Mouse Ovarian Tissue Transplantation

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Purpose

In recent years, autotransplantation of cryopreserved ovarian tissue before cancer treatment has been performed for the recovery of fertility. However, it has been reported that if minimal residual disease (MRD) is present in the ovarian tissue, a risk of cancer recurrence could occur. We have planned to reconstruct ovarian tissue for safe transplantation, where we isolated follicles from different strains of ovarian tissue and implanted with decellularized ovarian tissue. Here, we report the study on reconstructed decellularized mouse ovarian tissue.

Method

We removed the ovarian tissue from ICR mice and then immersed them in PBS containing 0.1% sodium dodecyl sulfate (SDS) for 24 hours, followed by a 30-minute treatment with DNase (1 mg/mL) at 40 units/mL, and decellularized by washing in PBS for 24 hours. We compared the decellularized mouse ovarian tissue with untreated tissue by HE and Masson's trichrome staining. For ovarian tissue reconstruction, we then isolated follicles from B6D2F1 mouse ovarian tissue using a 31G needle under microscope with Multipurpose Handling Medium. We introduced the collected follicles into decellularized tissue cut using an automatic tissue slicer. We covered them with 20μL of Matrigel, or added 20μL of sodium alginate solution followed by contacted with calcium ions to generate cross-linking and gelation to reconstruct the ovaries. Then, we partially removed the ovaries of SCID mice and transplanted these reconstructed ovarian tissues into the same site. Transplanted SCID mice was mated with ICR mice, and confirmed whether offspring derived from B6D2F1 were born. When reconstructed ovarian tissues were implanted two mice out of four mice left one ovary as controls without removal of the other ovary.

Results

In the untreated mouse-derived cortical tissue, both cytoplasm and nuclei were present, however, in the decellularized tissue, only extracellular matrix (ECM) containing collagen fibers remained with no cytoplasm and nuclei. Four transplanted SCID mice underwent ovulation induction on the 26th day after transplantation, followed by mating with ICR males two days later. In all four mice, vaginal plugs were confirmed. On the 16th day after mating, three out of the four mice became pregnant underwent cesarean section. A total of six offspring were born, however, none of them were derived from B6D2F1.

Conclusion

In this study, it was demonstrated that ICR mouse ovarian tissue can undergo decellularization, retaining the extracellular matrix (ECM) and showing the potential to serve as a scaffold for ovarian reconstruction. However, the absence of offspring derived from B6D2F1 suggests that the ovarian function of the reconstructed ovaries with the introduction of follicles could not recover. For ovarian function restoration, it is considered to be necessary for ovarian stromal cells to infiltrate and remodel the decellularized tissue. In the future, histological evaluation of the transplanted reconstructed ovarian tissue will be conducted.

P2-05 Radiofrequency Identification Tag System Improves the Efficiency of Closed Vitrification for Cryopreservation and Thawing of Bovine Ovarian Tissues

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Introdution

A radiofrequency identification (RFID) tag system was designed to streamline cryopreservation and thawing procedures. This study evaluated the usefulness of the RFID tag system for improving the efficiency of cryopreserving/thawing bovine ovarian tissue by the closed vitrification protocol. We already reported this in the Journal of Assisted Reproduction and Genetics.

Methods

Six participants carried out closed vitrification and thawing of bovine ovarian tissues procedures using either the conventional or the new RFID tag method, and the time required to perform each step of the respective methods was measured. After normality of data was confirmed by the Shapiro-Wilk test, the significance of differences was assessed by the unpaired t test.

Results

When closed vitrification was performed, the time required for each step showed a significant difference between the two methods ($t(4) = 2.938$, $p = 0.042$, $d = 2.40$), and the total cryopreservation time was 11 min shorter using the RFID tag system. When thawing was performed, the time required for each step also showed a significant difference between the two methods ($t(4) = 2.797$, $p = 0.049$, $d = 2.28$), and the total thawing time was 2 min shorter using the RFID tag system.

Conclusions

The RFID tag system tested in this study seems to be suitable for managing biological samples stored in liquid nitrogen. Adoption of an RFID tag system by fertility centers may not only improve the efficiency of cryopreserving/thawing reproductive tissues but could also reduce human error.

P2-06 Investigation of the Optimal Culture Time for Warmed Bovine Ovarian Tissues before Ovarian Tissue Transplantation

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Introduction
Ovarian tissue cryopreservation by vitrification is an effective technique, but there are still many unresolved issues related to the procedure. The aim of this study was to investigate the optimal culture time of post-warmed ovarian tissues and their viability before ovarian tissue transplantation. We already reported this in the Biology of Reproduction, 2022.

Material & Method
The bovine ovarian tissues were used to evaluate the effect of post-warming culture periods (0, 0.25, 0.5, 1, 2, 5, and 24 h) in the levels of residual cryoprotectant, LDH release, ROS generation, gene and protein abundance, and follicle viability and its mitochondrial membrane potential.

Results
Residual cryoprotectant concentration decreased significantly after 1 h of culture. The warmed ovarian tissues that underwent between 0 and 2 h of culture time showed similar LDH and ROS levels compared with fresh non-frozen tissues. The anti-Mullerian hormone transcript abundance did not differ in any of the groups. No increase in the relative transcript abundance and protein level of Caspase 3 and Cleaved-Caspase 3, respectively, in the first 2 h of culture after warming. On the other hand, an increased protein level of double stranded DNA breaks (gamma-H2AX) was observed in post-warmed tissues disregarding the length of culture time, and a temporary reduction in pan-AKT was detected in post-warming tissues between 0 and 0.25 h of culture time. Prolonged culture time lowered the percentage of viable follicles in warmed tissues, but it did not seem to affect the follicular mitochondrial membrane potential.

Conclusions
In conclusion, 1–2 h of culture time would be optimal for vitrified-warmed tissues before transplantation.

P2-07 Current Status and Issues of Fertility Preservation Therapy in Oncofertility Therapy

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Objective
To analyze the actual situation of oncofertility outpatients at single hospital and to clarify the effectiveness of each fertility-preserving treatment.

Methods
A retrospective study of 1457 patients who visited the oncofertility outpatient clinic from 2010 to 2022 was conducted from medical records.The study included patient background, underlying disease, method of fertility preservation and outcomes by method of preservation.

Results
Out of 1457 outpatients, 1207 (186 males, 906 females and 115 children) wanted to preserve their fertility. The fertility preservation rate was 92.4% (172/186) in men, 49.1% (411/837) in women and 60.0% (69/115) in children, and the methods of preservation in women were oocyte cryopreservation in 131 cases, embryo cryopreservation in 196 cases and ovarian tissue cryopreservation (OTC) in 99 cases (with overlaps). Until 2015, our hospital performed OTC for adult women, but from 2015 onwards, we decided to apply it to women under the age of 35. Most of the patients undergoing OTC in recent years are children. Breast cancer was the most common underlying disease in female patients undergoing fertility preservation, accounting for approximately 67.2% of the total. The proportion of patients using fertility preservation was 7.0% (12/172) sperm, 4.6% (6/131) oocytes, 35.7% (70/196) embryos, and 8.2% (14/168) ovarian tissues. In the 70 cases in which preserved embryo transfers were performed, the pregnancy rate per cycle was 30.0% (42/140 cycles), the pregnancy rate per patient was 57.2% (40/70) and the live birth rate was 35.7% (25/70). Ovarian tissue transplantation was performed in 14 patients, with improvement in menstrual irregularities and increased the number of oocytes retrieved.The pregnancy rate per patient was 28.6% (4/14) and the live birth rate 14.3% (2/14).

Discussion
Despite the paucity of reports on pregnancy outcomes after fertility preservation therapy, the preserved embryo transfers in this study showed results comparable to general infertility. Further long-term follow-up studies are necessary to verify the pregnancy outcomes of fertility-sparing therapy. In addition, it is important to demonstrate that they do not affect the recurrence of the primary disease or life prognosis.

P2-08 Oocyte Cryopreservation for Children and Adolescent Girls with Predicted Premature Ovarian Insufficiency

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Objective
In recent years, oocyte cryopreservation has become increasingly popular. The number of cases of oocyte cryopreservation used as a fertility preservation therapy has been increasing every year. However, reports of oocyte cryopreservation in children and adolescents are extremely rare, and its safety and efficacy have not been established. In this study, we investigated the feasibility of oocyte cryopreservation for children and adolescents based on our own experience.

Methods
We performed a retrospective review of the medical records of pediatric and adolescent patients who underwent oocyte freezing with the approval of our hospital's ethics committee.

Results
Five patients were included in the study, and a total of nine oocyte retrievals were performed. The median age was 14 years (12-17), and the primary diseases included two cases of Turner syndrome, one case of precocious puberty, one case of aplastic anemia, and one case of recurrent ovarian cancer. The patients had basal FSH levels of 6.0 mIU/mL (<0.3-14.3) and serum AMH levels of 1.26 ng/mL (0.04-4.7). Three short and six antagonist protocols of controlled ovarian stimulation were used, with a median of 10 days (2-14) from the start of stimulation to egg retrieval and a total gonadotropin dose of 1650 IU (525-3000). Only in the case of precocious puberty, follicular development was observed during spontaneous follow-up, and the oocyte retrieval was performed urgently according to the antagonist protocol, but the others were scheduled. Oocyte retrieval was determined at the maximum follicular diameter of 21mm (18-26) and serum estradiol level of 835 pg/mL (156-3854) and acquired 6.0 oocytes (0-11), 5 mature oocytes (0-10), and a maturation rate of 70.8% (50.0-90.9). All patients underwent transvaginal oocyte retrieval without complications.

Conclusion
Although there is a need to further evaluate the effectiveness of this method in terms of pregnancy outcome and perinatal prognosis, our results suggest that oocyte cryopreservation can be safely performed in pediatric and adolescent patients.

P2-09 Safety and Pregnancy Outcome of Interruption of Endocrine Therapy to Conceive for Hormonal Receptor-positive Breast Cancer Patients

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Objective
In patients with hormonal receptor-positive breast cancer, ovarian function is impaired by chemotherapy, and/or long-term endocrine therapy (ET), which could lead to missed opportunities for pregnancy and childbirth. In addition, fertility preservation (FP) prior to cancer treatment has been widely used in recent years, but there are very few reports on pregnancy outcomes in Japan. Furthermore, international randomized controlled trials have been conducted on the safety of interrupting ET to conceive, but the results are inconclusive.
In this study, we investigated pregnancy rates and cancer recurrence in young breast cancer patients who wanted to become pregnant during ET and evaluated the safety and efficacy of ET interruption.

Subjects
A retrospective study was conducted of 56 breast cancer patients who visited our oncofertility outpatient clinic between 2013 and 2022, and who interrupted ET to attempt pregnancy.

Results
The median age of patients at ET interruption was 39 (29-47) years, and 41 (73.2%) underwent FP with embryo cryopreservation. The pregnancy rate was 62.5% (35/56) and the live birth rate was 42.8% (24/56). Twenty-two cases were term and there were no fetal morphological abnormalities. In addition, recurrence was observed in 6.5% (3/46).

Discussion
The results of this trial showed that interruption of ET with a pregnancy attempt did not increase the recurrence rate, and no problems with the perinatal prognosis were noted. Interruption of endocrine therapy may be a way to avoid age-related infertility in cancer patients of reproductive age. However, there were some cases of relapse after ET interruption, which suggests the importance of shared decision-making, where doctors and patients share information and decide on the course of treatment, including the risk of relapse.

P2-10 A Study on Obtaining Mature Oocytes through Organ Culture and Follicle Development in 3-week-old Mice

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Introduction
Fertility preservation in young cancer patients is gaining attention due to its potential to improve cancer remission rates and enhance quality of life. Ovarian cryopreservation is one of the methods that provide the possibility for future pregnancies. However, concerns remain regarding the risk of cancer recurrence caused by minimal residual tumor cells in the transplanted ovaries. An alternative approach involves isolating follicles from ovaries and culturing in vitro, inducing maturation, and obtaining fertilizable mature oocytes without the need for ovary transplantation. In this study, we conducted a model experiment by using prepuberal mouse ovaries to assess the effectiveness of combining organ culture with follicle development to obtain mature oocytes.

Material & Methods
Prepuberal mouse ovaries were collected from 3-week-old B6D2F1 mice and cut into pieces for organ culture using a cell culture insert. Growing follicles were isolated from the cultured ovaries under a microscope using a 30G needle and encapsulated in Matrigel. The Matrigels were further cultured for 4-9 days in the media supplemented with FSH (for in vitro growth). Developing follicles were selected based on oocyte size and the presence of the germinal vesicle, and were then transferred to IVM (in vitro maturation) medium containing hCG. The number of oocytes that underwent germinal vesicle breakdown (GVBD) and reached the MII stage was determined.

Results
Organ culture for more than 4 days resulted in the degeneration of most follicles within the ovaries; therefore, the optimal duration of organ culture was estimated to be 2-4 days. A total of 192 primary and secondary follicles were isolated from 24 ovaries, and 99 of these follicles (51.6%) were subjected to IVM, with 56 (29.2%) undergoing GVBD and 14 (7.3%) reaching the MII stage.

Conclusions
The present study suggests that it is possible to obtain fertilizable mature oocytes by a combination method of organ culture and follicle development. The rate of achieving MII oocytes was not as high as expected. Future investigations are necessary to refine the culture conditions and explore supplementary factors. Furthermore, the fertilization capability of the mature oocytes induced by this method should be assessed.

P2-11 A Comparative Study on in Vitro Maturation Rate between Ovarian Tissue Oocytes and Punctured Follicle-derived Oocytes in Our Institute

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Purpose
In recent years, oocyte cryopreservation through ovarian tissue oocytes - in vitro maturation (OTO-IVM) has been performed for fertility preservation. It has been reported that punctured follicle-derived oocytes (p-FDO) and OTO consist of follicles at different developmental stages. In this study, we compared p-FDO with OTO about in vitro maturation rates.

Methods
Oocytes were laparoscopically collected from one ovary, while the other was removed for ovarian tissue cryopreservation (OTC). Follicles were manually punctured using a syringe with 23G needle, and oocytes were aspirated if follicles were present from removed ovary. The retrieved immature oocytes were in vitro cultured for maturation as p-FDO. The ovarian cortex was thinly sliced using scissors or scalpels for OTC, and the cortical sections were cryopreserved. Cumulus-oocyte complexes (COC), which detached from the ovarian tissue during this process, were collected and cultured as OTO for IVM. Medical records were retrospectively reviewed to determine patients' mean age, maturation rates of both p-FDO and OTO, and the number of frozen oocytes.

Results
Forty-four patients underwent unilateral oophorectomy for fertility preservation, with a mean age of 19.1 years (range: 2 to 43 years). Among these patients, 25 underwent IVM. A total of 46 COCs were collected from ovarian tissues, and oocytes that matured to MII through IVM were frozen. No oocyte was inseminated with sperm. The maturation rate for p-FOD was 35.8% (66/184), and oocytes could be frozen in 16 out of 25 cases. The maturation rate for OTO was 19.5% (9/46), and oocytes could be frozen in two out of six cases. Furthermore, immature oocytes were grouped into three types according to the quantity of cumulus cells as follows: naked oocytes, small COSs with 3–10 layers of cumulus cells, and large COSs with more than 10 layers of cumulus cells. The maturation rate of naked oocytes, small COSs, and large COSs were 20%, 17.6%, and 21.0% respectively.

Conclusion
The maturation rate of p-FDO was significantly higher than that of OTO ($p=0.034$). This discrepancy could be attributed to the advanced developmental stage of the punctured follicle-derived oocytes. Even in cases with residual cancer lesions in frozen ovaries, a live birth could be achieved without concern for cancer recurrence because of no need for reimplantation of ovarian tissue. Oocytes cryopreservation by OTO-IVM at the time of OTC is expected to improve fertility preservation outcome.

P2-12 The Role of Death Receptor Signaling Pathways in TM4 Sertoli Cell Avoidance of Apoptosis during Lipopolysaccharide- and Interleukin-18-induced Inflammatory Conditions

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Introduction

Lipopolysaccharide (LPS) initiates infectious acute inflammation, and interleukin (IL)-18 is an inflammasome-mediated cytokine. We previously established that endogenous IL-18 induces testicular germ cell apoptosis during acute inflammation when plasma IL-18 levels are high. Furthermore, high-dose recombinant IL-18 (rIL-18) induced Leydig cell apoptosis. The blood–testis barrier formed by Sertoli cells protects testicular germ cells from exogenous and endogenous harmful substances. However, the effect of LPS and IL-18 on Sertoli cells has not been elucidated.

Material & Methods

We stimulated TM4 cells, a mouse Sertoli cell line, with LPS (200 or 1000 ng/mL) or rIL-18 (0.1–100 ng/mL) to induce Leydig cell apoptosis based on our previous study. We assessed caspase 3 cleavage (apoptosis marker) and the mRNA expression of inflammatory cytokines and apoptotic pathway markers (*Tnfr1, Fasl, Fas, Fadd*) following stimulation.

Results

LPS stimulation increased *Il6* mRNA. *Tnfα* mRNA was increased after 1 h and subsequently decreased to baseline within 18 h with 200 ng/mL LPS treatment. However, *Tnfα* mRNA showed no increase following 1000 ng/mL LPS treatment but was decreased 12 and 18 h after treatment when compared with controls. *Fas* mRNA was increased after 1 h and remained elevated 24 h after LPS treatment. *Fasl* mRNA was decreased after 6 or 24 h of LPS stimulation with wide variability between the sample replicates when examined at other timepoints. LPS barely had an effect on *Tnfr1* or *Fadd* mRNAs. Cleaved caspase-3 showed no increase after LPS treatment for up to 24 h. *Il18* mRNA did not increase following treatment with either concentration of LPS. *Il18r1* mRNA decreased after LPS treatment in a concentration-dependent manner. Treatment with rIL-18 increased *Il6* and *Il18r1* mRNAs and induced inflammation. *Tnfα* mRNA remained unchanged following rIL-18 treatment, and *Tnfr1* mRNA decreased. High-dose rIL-18 (100 ng/mL) increased *Fasl* mRNA but had no effect on *Fas* or *Fadd* mRNAs. Western blot for cleaved caspase-3 revealed that rIL-18 treatment failed to induce TM4 cell apoptosis.

Conclusions

These results revealed that Sertoli cells demonstrate tolerance to apoptosis in response to infectious inflammatory stimulation (tested using LPS) and inflammasome-mediated cytokine stimulation (tested using IL-18). Compared with other testicular cell components, Sertoli cells may resist inflammation and play a predominant role in protecting testicular homeostasis.

P2-13 ZP Bounded Sperm Injection: How to Efficiency of Clinical Outcome in Art

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A total of 129 oocytes an Intracytoplasmic sperm injection (ICSI) treatment cycles with at least one immature oocyte collected were studied. Immature oocytes were used for the preparation of zona pellucida (ZP)-bound sperm. In fertile men, the average ejaculate contains 107 X 10^6 sperm, of which approximately 50% have progressive motility. ZP-induced acrosome reaction (AR) is motile sperm selected by density gradient centrifugation are incubated with one or two immature oocytes for more than at least 30 minutes at 37 ℃ in 5% CO2 in air. After incubation, the immature oocyte transferred to fresh medium drop and the ZP-bound sperm were then removed from the surface of the immature oocytes through a tightly bound glass pipette. The isolated ZP-bound motile sperm with normal looking morphology were subsequently used for ICSI. The ICSI was performed using either the ZP-bound sperm (test group) or conventional (control group). Fertilization rate was calculated from the total number of oocytes fertilized normally divided by total number of mature oocytes injected. A single blastocyst was transferred per embryo transfer cycle. Clinical pregnancy rate was calculated as the number of ETs performed.

P2-14 The Effect of Sperm DNA Fragmentation on Reproductive Outcomes and Embryonic Euploidy in Male Factor ICSI Cycles

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Introduction
The study aimed to investigate the role of sperm DNA fragmentation (SDF) on embryonic development in the intracytoplasmic sperm injection (ICSI) cycle for male factor infertility with a sperm morphology of <4%. We also investigated the correlation between SDF and embryonic aneuploidy.

Materials & Methods
This is a retrospective cohort study of 101 ICSI cycles in which sperm DNA fragmentation was performed at fertility center of CHA Gangnam from March 2019 to October 2022. Patients who underwent a combined IVF/ICSI cycle, natural cycle, ovarian factor to eliminate female factor and fresh ET or freezing at day 3, 4 were excluded. Data were divided according to SDF rate into two groups: ≤30% low SDF (n= 64) and >30% high SDF (n=37). The effect of SDF on fertilization, embryo development and euploidy rate was compared.

Results
There were no differences in basic characteristics except male age, BMI and female BMI between ≤30% low SDF and >30% high SDF. The levels of SDF were not affect the fertilization (76.41% vs 71.72%, p=0.123) or good cleavage (47.96% vs 41.35%, p=0.106) rate, but significantly effect on the blastocyst (41.55 vs 33.65, p=0.049) and usable blastocyst (30.87% vs 23.08%, p=0.036) rate. There was no significant effect on embryonic euploidy according to SDF levels, but there were high aneuploidy rates in higher SDF.

Conclusions
Our results indicate a negative association between SDF levels and reproductive outcomes. High DNA fragmentation significantly prevents successful blastocyst development. The high SDF may also affect the risk of aneuploidy embryos. This not only implies that the SDF should be considered when assessing semen quality for successful blastocyst development, but also suggests the need to improve the SDF prior to the ART procedure for subsequently better blastocyst formation.

Funding information
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P2-15 Sperm DNA Fragmentation Affects Embryonic Developmental Capacity But is not Correlated with Embryonic Aneuploidy Outcome by PGT-A

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Introduction
There is still no consensus on whether IVF results are involved in sperm DNA fragmentation index (DFI) evaluation, and it is still a subject of debate. However, it has also been reported that the degree of sperm DNA fragmentation correlates with embryogenesis abnormalities and is involved in chromosomal abnormalities. Therefore, we report the effect of DFI evaluation on IVF performance by examining the results of embryogenesis and aneuploidy analysis using PGT-A.

Material & Methods
Between January 2018 and March 2023, we investigated 56 married couples who requested DFI at the time of semen analysis and underwent IVF. A DFI test was performed at the first semen examination and divided into 3 groups: 15% or less (low, n=35), 15-30% or less (moderate, n=13), 30% or more (high, n=5). On the day of egg retrieval, ICSI was performed on 1006 mature oocytes, normal fertilization was confirmed on the day after insemination, and blastocyst culture was performed to verify the embryonic development potential between each group. 278 embryos that reached the blastocyst freezing criteria were vitrified. The trophectoderm cells of 216 blastocysts of PGT-A subjects were biopsied and NGS analysis was performed to analyze embryonic chromosome aneuploidy.

Results
The average age of the husband and wives who underwent IVF after the DFI examination was 42.3±4.2 years old and 41.0±2.4 years old. Normal fertility rates after ICSI in low, moderate, and severe DFI were 85.5%, 84.5%, and 83.1%, with no significant difference. Similarly, the blastocyst freezing rates were 35.4%, 26.3%, and 29.6%, and tended to decrease as the DFI value increased ($p<0.05$). In addition, the results of aneuploidy analysis of PGT-A target embryos were euploid 22.4%, 16.3%, 18.2%, aneuploid 65.4%, 69.4%, 54.5%, and statistically significant differences between groups were observed.

Conclusions
DFI assessment does not affect performance at the stage of fertilization. Higher sperm DNA fragmentation tended to lower developmental potential to blastocysts. On the other hand, PGT-A-induced chromosomal aneuploidy was shown to be independent of DFI assessment. This suggests that sperm DNA imperfections have a negative impact on the developmental process during the early stages of division, but during late stages of division, the ability to repair DNA works to differentiate into blastocysts with normal developmental potential.

P2-16 Association between Reduced Muscle Mass and Obesity on Various Metabolic Risk Factors and Fracture Risk in Mid-aged Korean Women

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Introduction

The aim of this study is to investigate effects of reduced muscle mass and obesity on various metabolic risk factors and fractures risk in middle age Korean women.

Material & Methods

Retrospectively, the study reviewed medical records of 1,775 female patients who had visited Pusan National University Hospital for routine health screenings from 2010 to 2016. The patients were divided into four groups: group 1, non-sarcopenic, non-obese (NS-NO); group 2, non-sarcopenic, obese (NS-O); group 3, sarcopenic, non-obese (S-NO); and group 4, sarcopenic, obese (S-O) population. Each of the patients was assessed through self-report questionnaires and individual interview with a healthcare provider. The Fracture Risk Algoritham (FRAX) tool was used for bone fracture risk.

Results

Postmenopausal women composed 68.0%, 78.4%, 60.1% and 67.1% in groups 1, 2, 3 and 4, respectively, compared to 68.5% in the overall patients ($p<0.001$). Statistical analysis of various parameters for metabolic and cardiovascular risks further showed that more patients tended to have hypertension in S-O group (patient % with hypertension in NS-NO, NS-O, S-NO and S-O groups as 15.2%, 36.7%, 10.1% and 46.3%, respectively; $p<0.001$). Metabolic syndrome, type 2 diabetes and hyperlipidemia were more prevalent in NS-O and S-O groups, which was statistically higher in the remaining two groups. FRAX score was also significantly higher in NS-O and S-O groups than NS-NO and S-NO groups (4.2 and 3.9 versus 3.8 and 3.6, respectively; $p=0.001$).

Conclusions

When combined with obesity and menopause, patients with reduced muscle mass showed dramatically increased risks to hypertension and diabetes; especially, patients with comorbid obesity and sarcopenia had the highest prevalence of hypertension. Regarding fracture risk, individual cardio-metabolic risks and menopause were more significant than the presence or absence of obesity or sarcopenia.

P2-17 Timing of Hormone Therapy and Its Association with Cardiometabolic Risk in Primary Ovarian Insufficiency Mouse Model

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Introduction

The present research examines how different timing and duration of initiation impact cardiovascular and metabolic indicators in a young mouse model with premature ovarian insufficiency (POI) induced by 4-vinylcyclohexene diepoxide (VCD).

Material & Methods

The study utilized 50 four-week-old mice to establish a mouse model of POI. These mice were then randomly divided into five distinct groups: a control group without any intervention (NT); a group that received a high-fat diet (HFD); a group that received delayed estradiol treatment (T1); a group that received on-time and continuous estradiol treatment (T2); and a group that received on-time estradiol treatment but with an early termination (T3). Cardiovascular risk and metabolic parameters were measured.

Results

The groups T1, T2, and T3, which were subjected to hormone therapy (HT), displayed notably lower blood glucose levels compared to the group NT, despite having similar body weights. Additionally, all HT groups showed significantly reduced serum total cholesterol and insulin levels compared to the NT group, with group T2 exhibiting the most substantial improvement. Concerning serum low-density lipoprotein-cholesterol, only group T2 demonstrated a statistically significant reduction compared to groups NT, T1, and T3. Furthermore, aortic thickness increased significantly, and fibrotic changes in the intima worsened in the NT group. However, these adverse effects were significantly mitigated in the HT groups, particularly in group T2. Finally, serum pro-inflammatory cytokines were significantly lower in the HT groups compared to the NT group, with group T2 exhibiting the lowest levels among all the groups.

Conclusions

Administering on-time, continuous estradiol treatment immediately after biologic estrogen deprivation significantly reduced metabolic and cardiovascular risks in a sexually immature, estrogen-naïve female mouse model of POI. This treatment approach was associated with decreased serum levels of pro-inflammatory cytokines, indicating its potential benefits in POI management. The study emphasizes the importance of considering the timing and duration of hormone therapy in the course of POI treatment.

P2-18 Supplementation of Oocyte Maturation Medium with Acetic Acid Affect Metabolism and Epigenetics of Oocyte and Cumulus Cells on Porcine

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Introduction
Short chain fatty acids (SCFAs) including acetate are generated by the gut microbiota through fermentation. Acetate is a major source of energy for the cells and could be important for the cell metabolism including cholesterol synthesis and epigenetics. Also, it protects cells against the oxidative stress and has anti-obesity effects. It is present at a higher level in follicular fluids, but little is known about its effect on the oocytes and embryos. The purpose of this study was to determine the effects of supplementing the in vitro maturation (IVM) medium with acetic acid on the oocytes.

Material & Methods
We collected cumulus-oocyte-complexes (COCs) from porcine ovaries and then cultured them in IVM medium containing 0, 0.1, or 1 mM acetic acid. After parthenogenetic activation, the zygotes were cultured up to the blastocyst stage. In the first experiment, we determined the lipid and ATP content, mitochondrial DNA copy number (Mt-cn) and membrane potential (MMP), and reactive oxygen species (ROS) levels (only oocytes) in the oocytes and 4-cell stage embryos. In the second experiment, we performed RNA-seq of the oocytes and cumulus cells and subsequently gene ontology analysis and KEGG pathway analysis. In the third experiment, we determined the expression level related to lipid metabolism (fatty acid synthase (FASN), acetyl coa carboxylase (ACC) and, phosphorated-ACC (P-ACC)), acetylation (acetyl lysine, AMP-activated protein kinase (AMPK), phosphorated-AMPK (P-AMPK) and Sirtuin 1 (SIRT1)), and DNA methylation (5-methylcytosine (5-mC)) in the oocytes and 4-cell stage embryos by immunofluorescence.

Results
Acetic acid treatment significantly decreased lipid and ATP content, MMP and the ROS levels in the oocytes, but did not affect Mt-cn. Moreover, it improved the blastulation rate. The RNA-seq of the cumulus cells revealed that glycolysis gets activated. RNA-seq of the oocytes revealed that cytosine methylation and the AMPK signaling pathway get upregulated. Acetic acid increased the expression levels of P-ACC, P-AMPK and SIRT1, but decreased expression of FASN in the oocytes. It did not affect the 5mC level in the oocytes but decreased the levels in the 4-cell stage embryos.

Conclusions
Acetic acid improves the oocyte developmental potential by changing the metabolism of each oocyte and cumulus cells and changes level of DNA methylation in the early embryos.

P2-19 The Effect of Fursulthiamine on Glucose Tolerance in a DHT Induced PCOS Mouse Model

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Introduction

PCOS is known to be associated with insulin resistance as well as defects in insulin secretion. Hyperinsulinemia in PCOS leads to androgen excess, which then cause arrest in antral follicle development. Pyruvate dehydrogenase (PDH) is a key component for the regulation of glucose uptake and metabolism and it is present in a form of pyruvate dehydrogenase complex (PDC). The PDC in normally active in most tissues in the fed state, and its activity is suppressed by pyruvate dehydrogenase kinase (PDK) under different conditions. PDK4 is known to suppress PDC under starvation, diabetes, and obesity. Previous study found that PDK4 is increased in human PCOS and as a result, PDH phosphorylation was decreased. Fursulthiamine is a derivative of thiamin (vitamin B1), often used for vitamin B1 deficiency. It is also known that fursulthiamine is a PDH activator. We aim to evaluate the effect of fursulthiamine on glucose level in a dihydrotestosterone (DHT) induced PCOS mouse model.

Materials and methods

C57BL/6J wild-type female mice were obtained and kept under standard animal housing conditions in accordance with the National Institutes of Health guidelines for the Care and Use of Experimental Animals. At postnatal d 19, mice of comparable body weight were randomly divided over three treatment groups (control, DHT treated, DHT treated with fursulthiamine; 6-7 mice per group) and were implanted subcutaneously with a 90-day continuous DHT release pellet. These pellets contained 2.5 mg of DHT (daily dose, 27.5ug). Control mice received a placebo pellet. Mice were killed at the end of the treatment period (90 day). Body weight was determined at the start and end of treatment. In addition, at the end of the 90 day treatment period, blood samples were taken from the heart.

Results

The body weight of both DHT treated and DHT treated with fursulthiamine was higher than the control group (22.93 g, 22.21g respectively vs. 20.34 g, $p<0.05$). There was no significant difference in terms of blood glucose among three groups (116.75 mg/dl control, 113.13 mg/dl DHT treated, 113.13 mg/dl DHT treated with fursulthiamine). Statically analysis of intraperitoneal glucose tolerance test-area under curve (IPGTT-AUC) glucose level revealed no significant difference between three groups.

Conclusion

In conclusion, fursulthiamine did not improve glucose level compare to the control in a DHT induced PCOS mouse model.

P2-20 Is There a Necessity to Process Preimplantation Genetic Aneuploidy Testing (PGT-A) of Embryos in Single Frozen Blastocyst Transfer for Patients Aged ≥ 40 years?

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Introduction

Currently, the proportion of women who are delaying childbearing until their late 30s and early 40s has increased significantly for a variety of reasons, including increased education and woman employment, career goals, and paucity of social incentives to support parenthood. As a result, the number of women over the age of 40 seeking infertility treatment is rapidly increasing. In particular, reducing complications such as miscarriage and multiple gestations and a shorter time invested to achieve a pregnancy are crucial aspects in IVF of advanced-age women. The embryonic aneuploidies increase exponentially with advancing-maternal-age, ranging from 30-50% up to 37 years to 80% in women ≥42 years. Consequently, the use of PGT-A is the only clinical strategy for patients with advanced maternal age and is widely used as a useful add-on, but its effectiveness remains controversial.

Material & Methods

All Frozen Embryo Transfer (FET) cycles that underwent a single blastocyst transfer from January 2020 through October 2022 at CHA Fertility Center Seoul Station were included. This was a retrospective study designed to compare the embryo implantation and ongoing pregnancy rates with (n=402) and without PGT-A (n=2689) according to maternal age ranges (<40 and ≥40 years of age). There were 3091 FET cycles that underwent single blastocyst transfer during the study time period: 151 cycles with PGT-A and 2219 cycles without PGT-A in <40 years, 251 cycles with PGT-A and 470 cycles without PGT-A in ≥40 years. Trophectoderm (TE) biopsy of about 5-10 cells was performed on day 5/6 followed by vitrification. PGT-A was performed using next generation sequencing. The transfer of thawed embryos of with PGT-A and without PGT-A was conducted into the uterine cavity under ultrasound control. Chi-squared test was used for statistical analysis and significance was considered if $p<0.05$

Results

There were no statistically significant differences in implantation and ongoing pregnancy rate between with and without PGT-A in FET cycles that underwent single blastocyst transfer (50.5 vs. 49.4%, $p>0.05$ and 49.0 vs. 48.3%, $p>0.05$, respectively). However, the implantation rate of single embryos transferred in PGT-A cycles was significantly greater than without PGT-A cycles in the patients older than 40 years (58.6 vs. 26.2%, $p<0.05$). Also, ongoing pregnancy rates were significantly greater between the PGT-A cycles and without PGT-A cycles (49.4 vs. 14.3%, $p<0.05$). There were no statistically significant differences in implantation and ongoing pregnancy rate between with and without PGT-A in the patients younger than 38 years (57.7 vs. 52.8%, $p>0.05$, and 55.6 vs. 51.6%, $p>0.05$, respectively).

Conclusions

For single frozen blastocyst transfer in women over 40 years of age, PGT-A is considered to be the most useful clinical strategy to reduce complications such as miscarriage and multiple gestations and a shorter time invested to achieve a pregnancy.

P2-21 Characteristics of Endometrial Stem Cells According to Fertility: Exploring the Dynamic Interplay for Optimal Embryo Receptivity

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Background & Objectives
The human endometrium is composed of highly regenerative tissue experiencing around few hundreds of menstrual cycles of shedding and repairing during reproductive life of a women. This regenerative capacity has a stem cell basis, which undergoes phases of growth, differentiation, decidualization and breakdown during menstruation. Endometrial decidualization is the foundation for a healthy pregnancy by which the uterus adapts to better implantation. Recently, cell therapy has been identified as an ideal alternative for treating a wide range of infertility related clinical disorders including the employment of endometrial mesenchymal stem cells (EnMSC). Since cell therapy is, dynamic, interactive, and personalized further cellular level research is required for improving the clinical procedure and effectiveness of this strategy. According to that, our objective is to in-vitro validated the differentiation and decidualization capacity of Human EnMSC towards improved endometrium receptivity.

Method
The present study received ethical approval from the Institutional Review Board (IRB) at Chungnam National University Hospital. Approval was obtained on November 31, 2022 [CNUH 2022-11-002]. Human EnMSCs were isolated and characterized based on expression of multipotency surface markers and their capacity of differentiation into adipocytes, osteocytes and chondrocytes. In addition, expression of pluripotency markers and decidual cell differentiation, which is a characteristic of endometrium, was also confirmed.

Result
Based on the relative gene expression of cellular pluripotency genes, decidualization-related genes, three-lineage differentiation-related genes, and endometrial receptivity genes, the expression of pluripotency markers was higher in younger age groups, but there was no difference in multipotency marker expression. In addition, it was found that the endometrial stem cells of young women who had no fertility problems had better decidual differentiation ability.

Conclusion
We identified differences in endometrial stem cell characteristics according to age and fertility, and we infer that there may be a correlation between endometrial receptivity associated with implantation and early pregnancy. In addition, experiments on decidua and aging are underway, and stem cell treatment using young and normal endometrial stem cell effective factors is under study.

P2-22 Evaluation of Lipopolysaccharide and Interleukin-6 as Diagnostic Biomarker for Chronic Endometritis

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Introduction

Chronic endometritis (CE) is an inflammatory condition of the endometrium associated with unexplained infertility and repeated implantation failure in gynecology. CE be accompanied by symptoms such as pelvic pain, irregular genital bleeding; however, it is often asymptomatic and difficult to diagnose. Lipopolysaccharide (LPS) has been implicated in reproductive inflammation, and some studies have shown its effects on hormonal response and reproductive tissue in domesticated animals. However, the pathogenic mechanism of CE remains unknown, and there is no consensus on its diagnostic criteria. In this study, the effects of LPS on inflammatory response and reproductive function of the human endometrium were investigated to elucidate the mechanisms of CE using endometrial tissues.

Material & Methods

We investigated whether LPS affected cytokine production and cell proliferation in the endometrium tissue using *in vivo* and *in vitro* experiments from human to mouse models. Endometrial curettage tissues and blood samples were collected from patients with suspected CE (n=13) and controls who were not suspected of CE (n=15) from the Obihiro ART Clinic. To establish CE animal models using mouse, the mice were performed LPS challenge into control (n=5) with PBS and LPS (n=5) groups which was administering LPS (1 mg/ml), once a week for 2 weeks.

Results

LPS administration stimulated proliferation maker of Ki67 in EM-E6/E7 cells derived from human endometrial gland. In the mouse model of CE, the expression of Ki67 was significantly lower in the LPS group compared to the control group ($P < 0.05$). Furthermore, the presence of CD138, a chronic marker of CE, exhibited increased signals in the uterine endometrium of mice that were administered LPS. The expression of inflammation makers, TLR4 and IL-6 were significantly higher in patients with CE than in control patients ($P < 0.05$) and high LPS concentrations were detected in CE patients.

Conclusions

We revealed that the inflammatory signaling evoked by LPS leads to the onset of CE, since LPS stimulates the inflammatory responses and cell cycle in the endometrium. These findings are important for uncovering the mechanism of CE. Furthermore, we identified LPS and IL-6 in CE, suggesting that these are appropriate diagnostic criteria for CE.

P2-23 The Study of Art Therapy using Mandala for Chronic Pain of Endometriosis

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Introduction
The endometriosis is the extra-uterine existence of endometrial tissue. Usually it is in peritoneal cavity, so it bleeds in peritoneal cavity and adhered with adjacent organs. Therefore, it produces the pelvic pain and it need the medical or surgical treatment. However, the pain is not controlled completely after these treatments. Therefore, other supportive treatment is needed. Art therapy can be used for this purpose, because recently many studies were reported that the pain of endometriosis is related to stress or psychological problem.

Material & Methods
In the eleven patients who have remained pain after medical or surgical treatment for endometriosis, they were treated by art therapy as mandala therapy.

Results
After this therapy, more than 2 VAS (Visual Analog Scale) is significantly decreased in 7 of 11 patients ($P<0.05$) although VAS is not decreased in 4 of 11 patients.

Conclutions
Therefore, I concluded mandala art therapy is helpful for the remained pain after medical or surgical treatment for endometriosis. However, further study is needed by the study of more big sample and long duration.