Altered DNA Methylation of IRS-1 Does not Correlate with Gene Expression and HOMA-IR in PCOS

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Introduction

Polycystic ovary syndrome (PCOS) is a multifactorial disease, resulted from interaction of various genetic predispositions and environmental factors, especially chronic low-grade inflammatory condition caused by insulin resistance. Insulin resistance and hyperinsulinemia occurred in 50-75% of PCOS patients, regardless their body mass index. PCOS sufferers showed peripheral insulin resistance similar to type 2 diabetes in which there is a 35-40% decrease in insulin-mediated glucose uptake. Insulin Receptor Substrate-1 (IRS-1) has already known to play important role in glucose uptake and therefore closely associated to insulin resistance in PCOS. DNA methylation, as a mechanism able to control gene expression, hold an important role in synthesizing IRS-1 protein. This study aimed to determine the profile of insulin receptor substrate-1 gene methylation and analyze its effect on non-obese PCOS patients.

Material & Methods

Thirty-one women with PCOS, under reproductive age 18-35 years old, and have lean body mass index (BMI<23) were assessed. In control group, twenty-seven women with same BMI but signed no PCOS criteria were included. DNA and RNA were collected from subjects' whole blood. DNA were then assessed for methylation status using pyrosequencing. IRS-1 expression was examined from isolated RNA samples using real-time qPCR. We also measured glucose fasting levels and insulin fasting levels from subjects' serum which then calculated into HOMA IR.

Result

IRS-1 methylation in lean PCOS group was found statistically lower ($p<0.05$) compared to control, 97.20% (0.75) and 97.63% (0.48) respectively, showed that alteration occurred in women suffered with PCOS. However, we analyzed that this alteration did not affect IRS-1 gene expression, as its level in case and control group was not statistically different. Fasting insulin levels between lean PCOS (6.7 (2.6 - 12.9)) and control (5 (1.2 – 23.6)) also showed no statistical difference and therefore gave no difference in HOMA IR value ($p<0.05$) between case and control group. Statistical test also showed that methylation level of IRS-1 did not correlate with its expression level as well as HOMA IR.

Conclusion

Altered methylation occurred in IRS-1 promoter region in PCOS. However, it was not correlated with IRS-1 expression and HOMA-IR. Further comprehensive analysis of IRS-1 epigenetic modifications and its role in the PCOS, especially in lean subjects, is still needed.

Genetic Variants and Epigenetic Modifications of the FTO Gene in Lean PCOS

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Background
The development of polycystic ovary syndrome (PCOS) and its exact pathophysiological mechanism is still poorly understood, but environmental and genetic factors are likely involved. Fat-mass and obesity-associated gene (FTO) influence susceptibility to PCOS. We, therefore, aimed to examine the genetic and epigenetic role of the FTO gene in PCOS.

Methods
We recruited forty women with PCOS as cases and forty healthy women as controls. Peripheral whole blood was obtained from all subjects, and genomic DNA and total RNA were extracted. SNP rs9939609 of the FTO gene was determined by sequencing. The DNA methylation level was carried out using the methylation-specific polymerase chain reaction (MSP) method. A quantitative real-time PCR (qPCR) was used to measure the mRNA expression of the FTO gene.

Results
Compared to the control groups, PCOS groups had a higher proportion of variant genotype AA, and the difference was statistically significant (X2=17,48, $p<0.001$). Prevalence of the A allele was significantly higher in PCOS women than controls (X2= 20.42, $p<0.001$). The odds ratio and 95% confidence interval for the A allele of FTO rs9939609 was 2.03 (1.47-2.77).
One-way analysis of variance (ANOVA) was performed to examine the methylation levels of the FTO gene. The results showed a significant difference in the methylation levels of the FTO gene between lean control, lean PCOS, obese control, and obese PCOS ($p=0.011$). We adjusted both PCOS women and controls according to their BMI into lean and obese groups for further analysis. There was a significant difference between the lean group and obese group in women with PCOS and healthy controls ($p=0.038$ and $p=0.013$, respectively).

Conclusion
The present results demonstrated that gene variants of rs9939609 of the FTO gene are significantly associated with and directly affect PCOS. Furthermore, the significant differences in DNA methylation levels of the FTO gene between lean and obese women play a crucial role in developing PCOS. Genome sequence variations and changes in DNA methylation patterns in the FTO gene substantially increase the risk of PCOS.

Cellular Senescence and Senolytics in PCOS Pathology

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Introduction

Cellular senescence, is a permanent state of cell cycle arrest induced by multiple stresses. Senescent cells contributes to the pathogenesis of various diseases, due to the altered secretory profile termed as senescence-associated secretory phenotype (SASP), including pro-inflammatory cytokines. Senolytics, a class of drugs that selectively eliminate senescent cells were discovered and the combination of Dasatinib + Quercetin (DQ) has been extensively used as a senolytic. Polycystic ovarian syndrome (PCOS) is one of the most common endocrine disorders in reproductive aged women, but its pathology and treatment strategy remain unclear. We hypothesized that cellular senescence plays a pivotal role in pathology of PCOS and aimed to investigate whether DQ treatment has beneficial effects on the pathogenesis of PCOS.

Material & Methods

We obtained ovaries from women who underwent hysterectomy for uterine cancer and granulosa cells (GCs) from patients who underwent oocyte retrieval for IVF. The expression of senescence markers (P21, P53, $P16^{INK4a}$ and γH2AX) were examined by immunohistochemistry (IHC) and qPCR. We also stimulated human GCs by testosterone, and treated with DQ. The accumulation of senescent cells and a SASP factor interleukin-6 (IL-6) were examined by western blotting, ELISA and SA-β-gal staining. Using 3-week-old female Balb/c mice, PCOS model mice were established by daily injection of dehydroepiandrosterone (DHEA) for 20 days, and treated with DQ or vehicle. The ovaries were obtained to examine the expression of senescence markers by IHC and the morphological change by histological analysis.

Results

The expression levels of senescence markers were significantly higher in GCs from PCOS patients and PCOS model mice, compared to the control groups. In human GCs, senescence markers and IL-6 were upregulated by testosterone stimulation and DQ treatment mediated the upregulation. In addition, in PCOS model mice, DQ treatment mitigated the upregulated expression of senescence markers and IL-6 in the GCs and improved the ovarian morphology, specifically, reduced the number of atretic follicles.

Conclusions

We confirmed that cellular senescence occurs in PCOS ovary. Our results indicated that hyperandrogenism induced cellular senescence in GCs. DQ treatment reduced the accumulation of senescent GCs and improved the ovarian morphology of PCOS mice, suggesting that cellular senescence is novel target in treatment of PCOS.

Epigenetic Clock Age Prediction based on 853.307CpG Sites in White Blood Cells and Cumulus Cells of Women with Infertility

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Background and Aim

In recent years, trends toward delayed parenthood has become increasingly common, thus has been associated with increased prevalence of infertility related to advanced female age. DNA methylation has been known as informative biomarkers of aging in human. A predictive model or epigenetic clock analysed the methylation pattern that used to accurately predict the chronological age of a subject. This study aimed to assess the accuracy of the "epigenetic clock" concept in reproductive age women undergoing fertility treatment by applying the age prediction algorithm in peripheral white blood cells (WBC) and cumulus cells (CC).

Methods

A prospective cohort study was carried out involving 32 infertile women undergoing ovarian stimulation between January – June 2022. The samples were grouped according to a poor (less than 5 oocytes retrieved) or good responder (>5 oocytes) response to ovarian stimulation. We collected CC and WBC for both group. DNA was isolated from peripheral blood samples and CC. Bisulfite conversion was then performed and a DNA Methylation Array was utilized to measure DNA methylation level throughout the genome. Ratio tests were performed to assess the relationship between predicted age, chronological age and ovarian responses.

Results

From 32 samples we found that 16 samples categorized as poor responder and were found to be older than good responder (38.7 plus minus 3.4 years vs 32.3 plus minus 3.8 years). We compare 9 methylation age model and found Hovart model was the consistent model for CC and WBC samples comparing to chronological age. Both poor and good responder have a negative correlation with chronological age, however the good responder patients have a strong correlation with r = 0.90 and p value <=0.05. Based on Pearson correlation of good response patients, we found a positive correlation with methylation age from follicle and oocyte count, oocyte morphology score and fertilized oocyte count. For the poor response patients the Pearson correlation showed a negative correlation with follicle and oocyte count, morphology, and fertilized count. Patients with poor ovarian responses did not affect the epigenetic clock based on study in WBC samples.

Conclusion

This study indicated that the epigenetic algorithm accurately predicts age when applied to WBCs but not to CCs.

Keyword : Cumulus Cells, DNA Methylation, Epigenetic, Infertility

Genes and Viruses should be Considered in Assisted Reproduction to Optimise the Success Rate of in Vitro Fertilisation

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Introduction

In recent years the birth rate in Japan has been steadily declining despite effective assisted reproductive techniques. The Japanese government is studying ways of dealing with this problem. In 1997, we published findings demonstrating that a human Herpes virus could be hindering success of IVF (1).

Material and Methods

A human Herpes virus was identified with a DNA sequence similar to Equine abortion virus, which causes abortion in mares, based on the comparison of genetic sequences by the fast-dot programme (2). Samples of semen, and blood from failed IVF were tested using a specific nested polymerase chain reaction (PCR) test developed to detect small quantities of viral DNA. Further research showed that the presence of the virus was linked to certain genetic traits, specifically HLA Cw3.

Results

Couples had no symptoms and unexplained infertility was implied. When the PCR test was positive antiviral drug allowed successful pregnancies and birth. A retrospective study published in1998 showed that two different Human Leucocyte Antigens (HLA) types were linked to either a positive or negative viral test (3).

Conclusion

In couples with unexplained infertility who tried IVF several times, the cause was found in some to be due to the presence of a virus. There were no symptoms of Herpes Simplex Virus. It was found that a positive test for Herpes Simplex virus, was associated with HLA Cw3. All HSV positive females had HLA-Cw3. Treatment with the antiviral drug acyclovir allowed IVF to succeed.

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The Expression of miR-372 and miR-661 in Human Blastocyst Culture Media as a Potential Biomarker for Non-Invasive Preimplantation Genetic Testing for Aneuploidy

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Introduction

Embryo selection using embryo biopsy has been the gold standard for increasing implantation efficiency in In Vitro Fertilization (IVF). Although PGT is a gold standard for embryo quality assessment, the trophectoderm biopsy procedure could be invasive to the embryo. Therefore, a new, ideal technology with the potential as a noninvasive biomarker should perform. The embryos produce cell-free DNA (cfDNA) in the extracellular environment, including RNA, DNA, and microRNAs (RNAs). In the past few years, miRNAs expressions have been detected in embryo culture media and differ between euploidy and aneuploidy embryos. Previous studies have reported that miRNAs have many roles in intracellular communication, including implantation. Therefore, we tried to identify miR-372 and miR-661 in spent media culture human embryos according to the embryos' chromosomal status and investigate whether secreted miRNAs could be used as a noninvasive biomarker to increase the implantation rate in the IVF program.

Material & Methods

Sixty-seven blastocyst culture media were collected from IVF patients, followed by pre-implantation genetic testing for aneuploidy (PGT-A). On day 5, after Intracytoplasmic sperm injection (ICSI), the embryos that reached the blastocyst stage were biopsied for aneuploidy testing using Next Generation Sequencing (NGS). The embryos were frozen after biopsied, and the spent embryo culture media were collected for miRNAs expression using quantitative real-time polymerase chain reaction (RT-qPCR) analysis.

Results

We found that miR-372 and miR-661 expression were detected in blastocyst media culture. Both expressions were highly expressed in aneuploidy embryos than media from euploidy embryos. Nevertheless, a statistical difference exists between miR-372 and miR-661 in euploidy and aneuploidy embryos ($p<0.05$). The relative expression of miR-372 was 1.64 fold, and miR-661 was 1.5 fold higher in aneuploidy embryo culture media compared with an embryo with a normal chromosome. Based on the validation, the sensitivity values were 71.4% for miR-372 and 53.8% for miR-372 and mir-661 in detecting aneuploidy in blastocyst embryos.

Conclusions

Based on the results, this study concluded that miR-372 and miR-661 could be potential non-invasive biomarkers for Pre-implantation Genetic Testing.

Metabolomic Analysis of Follicular Fluid in Women with Endometriosis: a Prospective Study

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Introduction

Endometriosis (EMS) is a benign gynecologic disease defined as ectopic proliferation of endometrial gland and stroma. Although the strong relationship between EMS and infertility is well known, its mechanism is still a conundrum. Recently, metabolomics has been spotlighted as a tool to elucidate the etiology, pathophysiology and mechanism of various diseases. Despite follicular fluid (FF) provides the microenvironment for follicular development and affects the quality of oocytes, there are only a limited number of metabolomic studies analyzing FF in EMS. The aim of this study is comparing the metabolomic and microbiome composition of FF of unilateral ovarian EMS with non-EMS patients.

Material & Methods

Ten women receiving oocyte retrieval were enrolled prospectively from July 2021 to July 2022 at Seoul National University Bundang Hospital. Five patients were diagnosed with unilateral EMS and the other five patients were non-EMS control group. In EMS group, FF from EMS-affected ovary was collected. Targeted quantitative metabolomics kit, which can detect 188 metabolites, and twenty bile acid (BA) quantification kit are used for metabolomic analysis. Multivariate analysis (principal component analysis) was performed to identify discriminative the differences of composition.

Results

There were six metabolites with statistical differences. In EMS group, acylcarnitine propenoylcarnitine (C3:1) was significantly increased, whereas amino acid valine, alanine, acylcarnitine butyrylcarnitine (C4), butenylcarnitine (C4:1), and phosphatidylcholine diacyl C 38:3 (PC aa C38:3) were significantly elevated in non-EMS control group. Since antimullerian hormone level and the presence of DOR showed significant difference between EMS group and non-EMS group, the correlation with these factors and the six metabolites were performed. Valine was showed statistically significant positive correlation with AMH and C3:1 and valine had negative and positive correlation with DOR, respectively. Also, the BA kit analysis did not show any statistical difference between EMS and non-EMS patients.

Conclusions

The different levels of acylcarnitines, amino acids, and glycerophospholipids suggest that endometriosis has altered mitochondria energy metabolism in cellular level. The gut microbiome may not affect the pathophysiology of follicular development in EMS since BA kit did not show significantly different patterns.

A Safe and Effective Strategy to Improve the Development Capacity of Oocytes; Autologous Mitochondria Supplementation from Adipose Stem Cells

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Introduction

The development potential of cryopreserved oocyte remains markedly lower than for fresh oocytes. The deleterious effects of oocyte cryopreservation on the developmental potential of oocytes may be attributed to the increased intracellular oxidative stresses and concomitant damage to the mitochondrial structure and function that might resemble the advanced-age oocytes We confirmed that supplementation of mitochondria-derived from adipose stem cells (ASC) simultaneously with ICSI into cryopreserved-thawed murine oocytes enhances the post-fertilization development capacity (Udayanga et al., 2022). This study investigated the potential for the occurrence of transgenerational aberrant phenotypes in the offspring resulting from the mitochondrial supplementation to oocytes.

Materials and Method

The cryopreserved-thawed oocytes were supplemented with ASC mitochondria concurrent with intracellular sperm injection. Thereafter, we have developed three generations of offspring from the embryos developed after mitochondria supplementation. The breeding potential, body growth, total histopathological parameters, hematological parameters, average activity patterns and body temperature changes in both male and female animals of these three generations' were compared with same-age wildtype (WT) animals.

Results

Upon mating with WT counterparts, male and female animals of all three generations were able to produce averagely comparable offspring as a reference to WT animals. And the body growth patterns up to 8 weeks in all three generations were not significantly different from WT animals. All the major organs of all three generations, including the brain, heart, liver, Kidney, lungs, ovaries, and testis, had not shown any significant histopathological abnormalities. Further, no significant difference was found in hematological parameters compared to WT counterparts. The continuous average activity pattern and the body temperature changes for one week were comparable with WT counterparts.

Conclusion

These results suggest that ASC mitochondria supplementation could enhance the embryo's developmental potential and not manifest any heritable abnormal conditions in a transgenerational manner. Thus, we suggest that ASC mitochondria supplementation could be a promising and safe approach for mitochondrial transplantation therapy to improve the development potential of oocytes that have compromised mitochondrial performances.

The Role of Cellular Senescence in Chemotherapy-induced Primary Ovarian Insufficiency (POI)

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Recent studies elucidated pivotal roles of cellular senescence, a phenomenon characterized by the permanent cell growth arrest, in various pathology of disease. Moreover, several promising senolytics, which induce apoptosis of senescent cells, are developing. With the improvements in survival rates of cancer, young female cancer patients are faced with chemotherapy-induced POI, which leads to infertility. In the present study, we investigated whether cellular senescence is involved in cyclophosphamide (Cy)-induced POI and whether senolytics rescue ovaries from damage induced by Cy.

Human granulosa cells (GCs) were aspirated from the patients undergoing oocyte retrieval. C57BL/6J male and female mice were purchased for experiments.

The expression of cellular senescence markers, p16, p21, p53 and γH2AX, was examined in the ovaries harvested from POI model mice with or without a senolytic, Dasatinib/Quercetin (DQ), and in primary cultured human GCs treated with 4-HC, an active metabolite of Cy, with or without DQ, by immunohistochemistry and by Western blotting, respectively. Furthermore, morphology of the ovaries and the response to superovulation were examined in DQ treated POI model mice. Last, the fertility of POI model mice with or without DQ were evaluated by mating test.

The expression of cellular senescence markers was significantly up-regulated in GCs of POI mice and in human GCs treated with Cy, while treatment with DQ abrogated the Cy-induced expression of these markers of cellular senescence. Administration of Cy resulted in significant loss of primordial and primary follicles, with a concomitant increase in secondary and preovulatory follicles. This phenomenon, so called ‘Burn-out', was partially recovered by co-treatment with DQ. Moreover, in superovulation test, POI model mice ovulated more ova in short term and fewer in long term due to “Burn Out", while administration of DQ suppressed the tendency. Last, the fertility of POI model mice decreased faster than control with aging, and administration of DQ improved fertility.

In conclusion, we demonstrated that cell senescence is activated in granulosa cells of the Cy-induced POI ovary. Accumulation of senescent cells in ovary contribute to the fertility failure, and the administration of senolytics, which induced senescent cell to apoptosis, could partially recover ovary function Targeting senescent cells with senolytics might serve as a promising strategy against Cy-induced damage in the ovary.

The Association between miR-183 with Adhesion and Apoptosis Gene in Endometriosis Tissue

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Introduction

MicroRNAs (miR) are postulated to play a role in normal biological processes, while their mis-expression has been associated with numerous diseases. It is still debatable whether miR-183 has a potential role in mediating the adhesion and apoptosis of endometriosis on its pathogenesis. This study aimed to measure the correlation between miR-183 and gene expression that regulates apoptosis and adhesion mechanism that may be linked to the pathogenesis of endometriosis.

Material & Methods

Fifty-eight subjects, including 26 control subjects, participated in this study. We collected ectopic endometriosis and endometrial samples. For the control, the sample was taken from endometrial tissue through pipelle biopsy. RNA was extracted from all tissues using RNA mini kit, and the expression was assessed using quantitative-real time PCR. Relative mRNA and miRNA expression were presented using the formula of the Livak method. The data were statistically analyzed using SPSS.

Results

The expression of Caspase3, Survivin, ITG1B, and Cadherin (adhesion- and apoptosis-related gene) were calculated using the relative expression method. We found no significant difference in caspase3, Survivin, ITG1B, and cadherin expression between eutopic and ectopic endometriosis tissues of women with endometriosis compared to healthy endometrium. No correlation was found between the expression level of miR-183 and Caspase3, Survivin, ITG1B, and Cadherin in both tissue types.

Conclusion

Despite the difference in expression levels of miR-183, there was no significant association between miR-183 with specific adhesion and apoptosis gene in endometriosis tissue.

Influence of X-ray Exposure during Hysterosalpingography on the Quality of Oocytes in IVF

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Introduction
Hysterosalpingography (HSG) plays a crucial role in reproductive medicine by evaluating the condition of the fallopian tubes and uterus. Nevertheless, it is ignored that HSG entails the exposure of the female reproductive organs to X-rays. It is suggested that X-rays, upon absorption by tissues, generate free radicals, thereby having a detrimental influence on the quality of oocytes. The present study aims to investigate the impact of X-ray exposure during HSG on subsequent laboratory parameters in IVF, such as the number of oocytes retrieved, maturation, fertilization, and embryonic development rates.

Material & Methods
A total of 1458 oocytes were analyzed in this study. Among these, 990 oocytes were retrieved from 70 women (89 cycles) who underwent HSG prior to IVF, while 468 oocytes were obtained from 45 women (57 cycles) without HSG. The X-ray exposure during HSG was measured as reference air kerma (RAK) (mGy). The subjects were categorized based on RAK levels: No-HSG (IVF without HSG), HSG with Low-RAK (RAK < 16.23), and HSG with High-RAK (RAK ≥ 16.23). A comparison of the number of oocytes retrieved, maturation, fertilization, and embryonic development was conducted among the three groups. Furthermore, multivariable analyses were performed to explore the impact of X-ray exposure on laboratory outcomes in IVF.

Results
A significant difference was recognized in the fertilization rate among the three groups (No-HSG: 71.6%, Low-RAK: 80.5%, High-RAK: 78.3%). Notably, the rate of good-quality blastocyst formation in the High-RAK group (46.2%) was significantly higher compared to both the Low-RAK group (35.3%) and the No-HSG group (32.4%). Multivariable analyses demonstrated that X-ray exposure was associated with higher rates of fertilization, blastocyst development, and high-quality blastocyst development after adjusting for patient age, body mass index (BMI), ovarian stimulation protocols, and fertilization methods. No association was observed between X-ray exposure and the number of oocytes retrieved or maturation rate.

Conclusions
The present study suggests that X-ray irradiation on female reproductive organs during HSG has the potential to enhance oocyte competencies instead of exerting detrimental effects.

Outcomes of Biphasic in Vitro Maturation Treatment on Non-obese Women with Polycystic Ovary Syndrome: Does Phenotype Have Any Impact?

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BACKGROUND

The biphasic in vitro maturation (CAPA-IVM) system is currently an alternative assisted reproductive technique for women with polycystic ovary syndrome (PCOS) due to its proven advantages compared to in vitro fertilization. For the manifestations of PCOS, four different phenotypes with diverse clinical complexities might affect IVM treatment outcomes. Additionally, it should be noted that the Eastern Asian PCOS phenotype is commonly non-obese. Therefore, this study was the first to evaluate the effects of PCOS phenotypes on non-obese women undergoing CAPA-IVM treatment.

MATERIALS AND METHODS

This retrospective cohort study included 498 PCOS patients undergoing CAPA-IVM treatment between April 2019 and December 2022. The study included 140 patients with phenotype A (28.11%), 17 patients with phenotype C (3.41%), and 341 patients with phenotype D (68.48%). Cycles were performed at IVFMD, My Duc Hospital, Ho Chi Minh City, Viet Nam. The primary outcome was the live birth rate after the first embryo transfer. Several secondary outcomes were also collected.

RESULTS

Patients were young and non-obese. The common phenotypes were A and D. The number of oocytes retrieved was significantly higher in group C than in groups A and D (26, 19.5, and 16, respectively); however, the number of MII cultured did not differ. After the first transfer, phenotype A had a significantly higher positive pregnancy rate than D, while the miscarriage rate was considerably lower in phenotype D. Consequently, live birth rates were comparable among groups (37.86%, 41.18%, and 36.66%, respectively). Multivariable logistic regression analysis was also developed, which revealed no impact of phenotypes on live birth. Ovarian hyperstimulation syndrome did not occur in any cases as expected.

CONCLUSION

Acceptable live birth rates could be achieved from CAPA-IVM. Moreover, preliminary results showed no impact of PCOS phenotypes on CAPA-IVM treatment outcomes in non-obese PCOS women. Still, it was worth noting that there might be the roles of hyperandrogenism on the miscarriage rate in non-obese women, which should be taken into account and further investigated.

Keywords: biphasic-IVM, CAPA-IVM, polycystic ovary syndrome, phenotypes, non-obese

Morphological Assessment Alone is a Sufficient Criterion for Selecting Transferred Blastocysts in a Single Blastocyst Transfer

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Objective
Recently, numerous investigations have been conducted on the efficacy of morpho-kinetic assessment of embryos. Nevertheless, these studies have solely substantiated in pregnancy prediction. There are few studies scrutinizing its clinical efficacy, such as embryo assessment to diminish embryo transfer cycles needed to achieve pregnancy. In the present study, we examined the clinical effectiveness of Eeva™, a morpho-kinetic embryo evaluation, for blastocyst selection for transfer as compared to conventional morphological evaluation.

Methods
A total of 217 participants were enrolled. These individuals underwent their first IVF oocyte retrievals between March 2019 and December 2020, and subsequently underwent a frozen-thawed single blastocyst transfer. The participants were divided into two groups: Group G, in which the embryos were cultured in a time-lapse incubator (Geri), and evaluated by Gardner score and Eeva™ technology, and Group D, in which the embryos were cultured in a conventional dry incubator and evaluated morphologically by Gardner score. We conducted a comparative analysis of clinical pregnancy, miscarriage and live birth rates between the two groups. Additionally, on 168 clinically pregnant cases, the proportion of the cases who achieved clinical pregnancy following the first embryo transfer and the number of transfer cycles required for clinical pregnancy between the two groups were compared.

Results
There were no significant differences in clinical pregnancy rates (83/187, 44.4% vs. 98/203, 48.3%), miscarriage rates (12/83, 14.5% vs. 21/98, 21.4%), and live birth rates (69/187, 36.9% vs. 76/203, 37.4%) between group G and D. Among clinical pregnancy cases, no significant differences were observed in the proportion of cases attaining clinical pregnancy following the initial embryo transfer (55/77, 71.4% vs. 65/91, 71.4%) and the number of cycles required to achieve clinical pregnancy (1.42 ± 0.78 vs. 1.44 ± 0.88) between group G and D.

Discussion
No significant differences were observed between Eeva™ and conventional method. Eeva™ could not be substantiated. Hence, evaluating blastocysts solely on their morphology is deemed satisfactory at present time. In future studies, we will examine particular situations necessitating morpho-kinetic assessment.

Outpatient Treatment of Ectopic Pregnancies: Chorionic Villi Targeted (CV-T) Therapy with Transvaginal Local Injection of Absolute Ethanol and Its Efficacy in 267 Cases

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Introduction

Newer diagnostic methods have resulted in earlier detection and an increase in the percentage of cases without rupture ectopic pregnancies (EP). For nearly four decades, methotrexate has been the main mode despite many challenges of nonsurgical treatment for EPs. The purpose of this research is to develop a method that has a rapid pharmacological effect, can be determined in a short time, can be administered repeatedly if necessary, and can be applied to patients with high β-hCG levels and positive fetal heart beat. (FHB).

Material & Methods

Chorionic villi-targeted (CV-T) therapy which involves injection of absolute ethanol (AE) into the lacunar space to treat EPs and evaluated its efficacy of this method were examined in 267 cases of EP, including 113 with positive FHB.

Results

From April 2006 to October 2022, 267 patients with EP received CV-T therapy with AE injection. A total of 226 patients had conceived through IVF treatment. Of 267 patients, 113 were FHB-positive and 247 (92.5%) were successfully treated with CV-T therapy. The occurrence sites of the EPs were fallopian tube (214cases), interstitial fallopian tube (18cases), cervix (29cases), Cesarean section scar (3cases) and peritonea (3cases). The mean level of the initial serum β-hCG was 26,450.5±32,529.9 mIU/ml and the mean level of serum β-hCG 2 hours after AE injection was 20,219.9±22,215.2 mIU/ml , the mean decrease rate of serum β-hCG was 19.5±9.9 %. The average number of AE injection(s) required was 1.54 times, and the average dose was 3.49 mL In all but one patient with CP, theβ-hCG levels decreased by an average of 17.5% after 2 hours of AE injection, by 50% within 3 days, and by less than 10% of the initial value within 14 days. Furthermore, β-hCG levels reached <10.0 mIU/mL within 36 days and took only 40 days to reach <1.0 mIU/mL. After the treatment, no rupture of the fallopian tubes, bleeding from the injection site, or infection, was observed. However, in 117 (57.5%) out of 214 patients with fallopian tube pregnancies, symptoms of peritoneal irritation were observed in case AE leaked into the abdominal cavity. 20 patients (7.5%) of the failures were due to misdiagnosis of the EPs implantation site by ultrasonography and required laparoscopic surgery.

Conclusion

CV-T therapy with AE injection be an effective treatment for EPs with the potential to replace conventional surgical interventions and medical treatment using methotrexate.

Effect of Laparoscopic Surgical Correction on Chronic Endometritis and Pregnancy Rates Post in Vitro Fertilization

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Introduction

To assess the prevalence of chronic endometritis (CE) in patients with infertility and hydrosalpinx or peritubal adhesions and to examine the effects of laparoscopic surgical correction (LSC) on CE and pregnancy rates post VF-ET.

Material & Methods

A total of 438 patients, known to have hydrosalpinx (n=194) or peritubal adhesions (n=244), undergoing IVF treatment between April 1, 2018 and September 30, 2020 were included in the study. CE was diagnosed through CD138 PCs. The diagnosis of CE was deemed positive if the number of CD138 PCs was ≥ 5 in 20 non-overlapping visual fields per HPF. Hysterosalpingography, magnetic resonance imaging, and transvaginal ultrasonography were used to diagnose the hydrosalpinx or peritubal adhesions. LSC were performed on patients with CE. IVF-ET was performed after recovery from LSC.

Results

All the 89/194 patients with CE and hydrosalpinx underwent laparoscopic salpingostomy and/or fimbrioplasty, and 64 (71.9%) further underwent proximal tubal occlusion. All the 35/244 patients with CE and peritubal adhesions underwent laparoscopic adhesiolysis and/or fimbrioplasty, and 19 (54.3%) further underwent proximal tubal occlusion. CD138 PC levels after LSC decreased to <5 in 70 of 124 patients (56.5%) in one menstrual cycle and decreased to <5 in all cases within 6 months. Of the 66 patients who underwent a single BT, 57 delivered (cumulative LBR: 86.3%). Of the 124 patients who underwent LSC between April 1, 2018 and September 30. 2020, the outcome of the 66 patients' IVF-ET cycle after LSC was evaluated after exclusion of 11 patients who did not undergo or discontinued IVF-ET and 47 patients aged ≥43 years. The follow-up data were collected on September 30, 2022. Patients underwent a single BT, and 57 delivered (cumulative LBR = 86.3%, 57/66). The cumulative LBR of patients with cured CE after LSC was significantly more than the 320 patients who received antibiotic therapy and 811 patients who were D138-negative (cumulative LBR = 86.3% [57/66] vs. 38.4% [120/320], 31.8% [258/811]; P < .0001 and P < .0001, respectively). There was no significant difference between the antibiotic therapy group and the D138-negative group: 38.4% [120/320] versus 31.8 [258/811] (P= 0.078).

Conclusion

LSC improved CE without antibiotic therapy, improving the CP and LBR after IVF-ET. Based on our research results, the cause of CE may not be a bacterial infection but cytokine-induced endometritis due to pathological fluids.

Unraveling the Protective Interplay between PGK1 and ITI-H4 in Recurrent Pregnancy Loss

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Introduction

Recurrent pregnancy loss (RPL) has been linked to specific proteins in the blood, as evidenced by our previous proteomics research. One significant protein of interest is inter-α-trypsin inhibitor heavy chain 4 (ITI-H4). The present study aims to uncover novel substrates of ITI-H4 and explore their functional roles in the inflammatory response underlying RPL.

Material & Methods

To identify potential binding partners of ITI-H4, JEG3 and HEK293T cells were transfected with ITI-H4 and its short isoform for immunoprecipitation. Differential gel analysis, followed by matrix-assisted laser desorption/ ionization time-of-flight mass spectrometry (MALDI-TOF/MS), was employed. Immunoprecipitation and GST pull-down assays confirmed the direct or indirect binding between ITI-H4 and phosphoglycerate kinase 1 (PGK1). Subsequently, protein expression of PGK1 was evaluated in the sera of RPL (n=60) and control patients (n=32) using western blotting. The impact of PGK1 overexpression on inflammatory cytokines and related signaling pathways was verified through RT-PCR and western blotting.

Results

Building upon our previous study, we established that kallikrein B1 (KLKB1) mediates the cleavage of ITI-H4, resulting in the formation of ITI-H4 short isoform that plays a role in modulating the balance between pro- and anti-inflammatory cytokines. In this study, we successively identified a novel substrate of ITI-H4, PGK1, which not only upregulates ITI-H4 expression but also protects it from KLKB1 cleavage. Furthermore, our investigation into the influence of PGK1 on pro-inflammatory cytokine levels revealed its downregulation through the suppression of the JAK2/STAT3 signaling pathway.

Conclusions

The interplay between ITI-H4 and PGK1 prevents the cleavage of ITI-H4 by KLKB1, leading to reduced production of the anti-inflammatory ITI-H4 short isoform. These findings shed light on the potential mechanisms underlying RPL and provide valuable insights into novel therapeutic targets for managing inflammatory responses associated with RPL.

Niacin Improves Developmental Competence of in Vitro Grown Porcine Oocyte

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Introduction

In vitro culture systems have been developed for growing oocytes to study their growth and explore the potential use of growing oocytes as a source of fertilizable ova. However, the viability and developmental competence of in vitro grown oocytes are still low. It has been reported that niacin improves cellular functions, such as energy metabolism, antioxidant capacity, and mitochondrial biogenesis, by increasing intracellular NAD+ content. In the present study, we investigated the effect of niacin on the in vitro growth of porcine oocytes obtained from early antral follicles.

Methods

Porcine growing oocytes were cultured in a medium containing 0-10 mM niacin for 12 days. To assess oocyte viability and growth, their morphology and ooplasm diameter were measured every 2 days. After culture, oocytes were stained with Nonyl Acridine Orange (NAO) and Mitotracker Orange (MTO) to examine mitochondrial mass and activity, and fluorescence intensity was captured using a confocal microscope. Furthermore, the in vitro grown oocytes cultured with 10 mM niacin were evaluated for maturation, fertilization, and developmental competence. Additionally, the intracellular GSH content of mature oocytes was assessed by staining them with Thiol Tracker violet.

Results

Niacin did not affect the viability and growth of porcine oocytes, regardless of the concentration. Niacin did not improve the fluorescence intensity of NAO and MTO in oocytes cultured with concentrations up to 1 mM. However, 10 mM niacin significantly increased the relative fluorescence intensity of NAO, suggesting an increase in mitochondrial mass. Moreover, while meiotic maturation of oocytes was not affected, 10 mM niacin significantly improved the fertilization rate and blastocyst formation rate after ICSI. The intracellular GSH content of in vitro grown oocytes in both the control and 10 mM niacin groups was significantly lower than that of in vivo grown oocytes. The intracellular GSH content of in vitro grown oocytes slightly increased with 10 mM niacin treatment.

Conclusions

The data from this study demonstrate that the presence of 10 mM niacin during in vitro oocyte growth accelerates mitochondrial biogenesis and supports the fertilization and developmental competence of growing porcine oocytes.

Nucleolar Variations in IVF Embryos: Implication for Prenatal Development and Birth Outcomes

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Introduction

In vitro fertilized (IVF) and in vitro cultured embryos are particularly susceptible to adverse environmental influences. After embryo transfer, this susceptibility can lead to reduced implantation rates and an increased risk of preterm birth, low birth weight and congenital anomalies. After fertilization embryonic nucleoli known as nucleolus precursor bodies (NPBs) undergo a transformation process to become functional somatic type nucleoli. The aim of this research is to discern and characterize embryos based on nucleolar morphology and its potentiality to predict the future offspring.

Materials and Methods

To clarify the IVF-specific nucleolus morphology at different embryonic developmental stages we used IVF and in vivo fertilized/ in vitro cultured (IVVc) ICR mouse embryos. Nucleolus size, number and morphology were determined by immunostaining, using a nucleolus marker anti-NOPP140 antibody. Hence we categorized 4-cell stage embryos based on nucleolus number for embryo transfer to predict the offspring.

Results

The immunostaining with anti-NOPP140 antibody showed differences between IVF and IVVc embryonic nucleoli from the zygotic stage. The nucleolus size was determined from the ring shape of the NOPP140 immunostaining. Above all, at the 4-cell and morula stage, IVF embryos exhibited larger nucleolus size compared to IVVc embryos. At the blastocyst stage, IVF embryos nucleolus showed more compact nucleolus whereas IVVc embryos showed more reticulated pattern. At the 4-cell stage, the nucleolus of IVF mouse embryos exhibited a dominant presence of single nucleolus in each blastomere, whereas IVVc 4-cell stage embryos showed mostly multiple nucleolus. The 4-cell stage embryo transfer, revealed that embryos with single nucleolus exhibited a higher birth rate in compared to embryos with multiple nucleoli. Additionally, a significant difference was observed in the ratio of body weight to placental weight between single nucleolus IVF embryos and their multiple nucleolus counterparts, indicating a potential influence of nucleolar status on prenatal development.

Conclusions

Together all the evidence indicates that the preimplantation IVF embryonic nucleolus at the 4-cell stage could be a potential marker for predicting future offspring.