A large-scale, prospective cohort study to investigate the association between environmental exposures and behavioral factors and infertility (success rate of assisted reproductive technology)

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Research Proposal Summary

Trial Title: A Large-Scale, Prospective Cohort Study Exploring the Association Between Environmental Exposure and Behavioral Factors and Infertility (Success Rate of Assisted Reproductive Technology)
Research Objective: This study aims to clarify the reasons for the failure of assisted reproductive technology (ART) in infertile patients in Hunan Province, to achieve early identification and prediction of risk factors, and thus improve the success rate of ART.
Experimental Design: is a prospective cohort study, aiming to collect data from 5,000 participants (male to female ratio 1:1) . Experimental procedure: 1. To establish a large-scale, prospective cohort of infertile patients to provide basic data for studying the association between environmental, biological, and social factors and ART success rates. 2. Analyze the association between exposure to environmental factors (such as environmental pollutants related to plastic products) (internal exposure) and infertility (ART success rate) to provide a scientific basis for formulating environmental protection policies. 3. To explore the impact of lifestyle and social factors on the success rate of ART in infertile patients, and to provide guidance for improving public reproductive health literacy. 4. To construct a predictive model for adverse pregnancy outcomes in infertile couples undergoing ART, providing new insights for improving the success rate of ART in infertile patients.

Selection criteria:

(1) A. Women aged 18 to 46 who use their own eggs or men aged 18 to 55 who use their own sperm; B. Patients who meet the diagnostic criteria for infertility; C. Patients with a clear history of persistent infertility; D. Patients who voluntarily participate in the program and sign an informed consent form;

Exclusion criteria:

(1) A. Individuals undergoing artificial insemination with any of the following contraindications to ARTs : a. Impaired sperm-egg fertilization due to fallopian tube factors in the woman. b. The woman has an acute infection of the reproductive or urinary system or a sexually transmitted disease. c. The woman has a genetic disease, a serious physical illness, or a mental or psychological disorder. d. A history of birth defects in infants confirmed to be caused by female factors. e. The woman has been exposed to teratogenic doses of radiation, toxins, or drugs and is currently under the influence of these factors. f. The woman has a history of alcoholism, drug abuse, or other unhealthy habits. B. Individuals undergoing first-generation or second-generation IVF with any of the following contraindications to ARTs: a. Either the donor of gametes has an acute infection of the reproductive or urinary system or a sexually transmitted disease, or has a history of alcoholism, drug abuse, or other unhealthy habits. b. Either the donor of gametes has been exposed to teratogenic doses of radiation, toxins, or drugs and is currently under the influence of these factors. c. The woman receiving donated embryos/eggs suffers from an acute infection of the reproductive or urinary system or a sexually transmitted disease, or has a history of alcoholism, drug abuse, or other unhealthy habits. d. The woman's uterus is not capable of carrying a pregnancy or she

has a serious physical illness that prevents her from conceiving. C. No embryo transfer was performed after egg retrieval; D. More than 180 days have passed since frozen embryo transfer was performed after egg retrieval;
Statistical methods: Lasso regression was used, and a penalty function was constructed. Variables were selected based on the lambda value that minimized the error, achieving subset shrinkage and avoiding overfitting. ART failure in infertile patients was used as the outcome indicator, and the selected indicators were used as predictors. Algorithms such as Cox proportional hazards regression, random forest (RF), extreme gradient boosting (XGBoost), and Naive Bayes (NB) were employed to construct a risk prediction model.

Research Text

1. Research Background

Along with economic progress, a series of problems, such as the use of many chemical products, excessive destruction of the ecological environment, various chemical (biological) pollution of human living environment, use of various genetically modified food technologies, large doses of pesticide residues in food, and abuse of antibiotics, seriously threaten human fertility. Infertility is becoming increasingly serious. It is estimated that the infertility rate of Chinese women is as high as 15-20% (400,000-500,000) and the infertility rate of men is as high as 10-12% (450,000) [1]. The incidence of infertility is rising year by year worldwide, bringing heavy psychological and economic burdens to many families. As a result, human assisted reproductive technology (ART) has emerged, which can make up for the regret of couples who cannot have offspring in the traditional way, and at the same time make a certain contribution to the genetic improvement of genetic diseases. This has a profound impact on the entire human society.

In vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) are the main ART methods for fertilizing eggs. With the advancement and widespread application of ART, the proportion of ART-born babies is increasing. Currently, the number of ART babies born globally each year has exceeded 350,000, and the cumulative number of IVF babies has reached 5 million. The proportion of ART newborns in Europe is 0.2%-3.9%. According to statistics, as of 2011, the proportion of ART newborns in China has reached 1.013% [2]. However, the success rate of ART in most reproductive centers hovers around 40%, and the delivery rate is still only 20%-30% [3]. Among them, the high rate of spontaneous abortion is one of the key reasons affecting the live birth rate. The causes of spontaneous abortion are extremely complex. Even after comprehensive medical examination, the causes of many spontaneous abortions remain unknown. Therefore, it is necessary to comprehensively explore the possible risk factors for early spontaneous

abortion [4]. Most of the attention in the past has been focused on the impact of clinical factors on infertility and antiretroviral therapy, rather than environmental factors such as ambient air pollutants and new environmental pollutants. Previous studies have analyzed air pollutants exposed to by ART participants and found harmful associations between pregnancy failure and particulate matter [5, 6] and nitrogen dioxide [7, 8] in ambient air pollutants. With the widespread use of new materials, new environmental problems have also emerged. Studies have suggested that parental exposure to new pollutants, such as phthalates , phthalates, and nanoplastic particles, is associated with adverse pregnancy outcomes [9-11]. In addition, a US study suggests that improving lifestyle and social factors, including diet, can effectively improve ART success rates [12].

However, China has not yet conducted comprehensive and in-depth research on the impact of environmental factors, lifestyles, and social factors on infertility (ART success rate). Therefore, we plan to conduct a large-scale, prospective cohort study to explore the association between environmental exposures and behavioral factors and infertility.

2. Research Objectives

This study aims to clarify the reasons for the failure of assisted reproductive technology (ART) in infertile patients in Hunan Province , to achieve early identification and prediction of risk factors , and thus improve the success rate of ART .

2.1 . Establish a large-scale, prospective cohort of infertile patients to provide basic data for studying the association between environmental, biological, and social factors and ART success rates .

2.2 . Analyze the association between exposure to environmental factors (such as

environmental pollutants related to plastic products) (internal exposure) and infertility (ART success rate) to provide a scientific basis for the formulation of environmental protection policies.

2. 3. To explore the impact of lifestyle and social factors on the success rate of ART in infertile patients , and to provide guidance for improving public reproductive health literacy.

2.4 . To construct a predictive model for adverse pregnancy outcomes in infertile couples undergoing ART , providing new insights for improving the success rate of ART in infertile patients .

3. Research Design

3.1 . Research on the Construction of an Infertility Cohort in Hunan Province

This study aims to establish a cohort of infertile patients in Hunan Province, including those who visited Xiangya Third Hospital in Changsha, Hunan Province from December 2024 to December 2034. Study participants will be selected based on clearly defined inclusion and exclusion criteria. Baseline data collection will cover multiple dimensions, including sociodemographic characteristics, lifestyle behaviors, basic health status, physiological and biochemical indicators, and internal exposure measurements to environmental factors. Follow-up will be conducted from the start of the study through reproductive care, pregnancy, delivery, and childbirth to observe the ART live birth rate of infertile patients. This will provide data support for research on the identification, early prediction, intervention, and prevention of ART risk factors in infertile patients.

3.2 . Research on risk factors for ART failure in infertile patients

Based on the infertility cohort and database established in Content 1 of Hunan Province, this study uses a multivariate analysis method to explore the association between relevant environmental, lifestyle, and social risk factors and ART success rate, thereby identifying risk factors for ART failure and comparing the differences among different risk factors. This provides an evidence basis for effective

interventions such as early prediction , reduction, or elimination of non-clinical factors leading to ART failure rate.

3.3 . Research on the Construction and Evaluation of Predictive Models for ART Failure in Infertile Patients

Building upon Contents 1 and 2, this study uses ART failure in infertile patients as the outcome indicator and sociodemographic characteristics, basic health status, environmental exposure, lifestyle behaviors, and physiological and biochemical indicators as potential predictive factors. It combines traditional regression analysis and machine learning algorithms to construct a risk prediction model for ART failure in infertile patients . The model is then validated internally and externally, and its performance is evaluated based on discrimination, calibration, and predictive ability.

4. Research Content

4.1 . A cohort study of infertility in Hunan Province

4.1.1 . Baseline Survey

4.1.1.1 . The research units selected were Xiangya Third Hospital and the Department of Health Toxicology, School of Public Health, Central South University. The above-mentioned hospitals are provincial key clinical specialties in the field of ART for infertility. The Department of Health Toxicology, School of Public Health, Central South University has a foundation for research on exposure to relevant environmental risk factors and related populations, and has a good foundation for cooperation, which is conducive to the development of the infertility cohort.

4.1.1.2 . Inclusion criteria for study subjects:

(1) Infertility cohort

①Inclusion criteria : A. Women aged 18 to 46 using their own eggs or men aged 18 to 55 using their own sperm; B. Patients meeting the diagnostic criteria for infertility; C. A clear history of persistent infertility ; D. Willingness to participate in the program and signing an informed consent form; E....

② Exclusion Criteria: A. Individuals undergoing artificial insemination with any of the following contraindications to ARTs: a . Female partner has impaired sperm-egg fertilization due to fallopian tube factors. b. Female partner suffers from acute infection of the reproductive or urinary system or sexually transmitted diseases. c. Female partner suffers from genetic diseases, serious physical illnesses, or mental or psychological disorders. d. History of birth of an infant with congenital defects confirmed to be caused by female factors. e. Female partner has been exposed to teratogenic doses of radiation, toxins, or drugs and is currently within the period of their effects. f. Female partner has a history of alcoholism, drug abuse, or other unhealthy habits. B. Individuals undergoing first-generation or second-generation IVF with any of the following contraindications to ARTs: a. Either partner donating gametes suffers from acute infection of the reproductive or urinary system or sexually transmitted diseases, or has a history of alcoholism, drug abuse, or other unhealthy habits. b. Either partner donating gametes has been exposed to teratogenic doses of radiation, toxins, or drugs and is currently within the period of their effects. c. The woman receiving the donated embryo/egg suffers from an acute infection of the reproductive or urinary system, a sexually transmitted disease, or has a history of alcoholism, drug abuse, or other unhealthy habits. d. The woman's uterus is not capable of carrying a pregnancy, or she has a serious physical illness that prevents her from conceiving. C. No embryo transfer was performed after egg retrieval ; D. More than 180 days after egg retrieval, frozen embryos were transferred.

(2) Diagnostic criteria for infertility:

① Infertility: Couples who have been having regular unprotected sexual intercourse for at least one year and have not conceived.

② Primary female infertility: women who have never had a history of pregnancy and have never become pregnant despite not using contraception.

③ Secondary infertility in women : women who have a history of pregnancy but have not conceived for 12 consecutive months without using contraception.

④ Male primary infertility: The male has never impregnated a woman.

⑤ Secondary male infertility: Infertility that occurs after a man has previously impregnated a woman.

⑥ Unexplained infertility: Infertility where the cause cannot be found using standard infertility assessment methods.

⑦ Diagnostic criteria for azoospermia: No sperm was found in the semen after centrifugation and microscopic examination three or more times, while excluding those who do not ejaculate and those who ejaculate retrogradely.

⑧ Diagnostic criteria for oligospermia: sperm concentration in semen analysis is less than 15×10^{6}/ml.

⑨ Diagnostic criteria for asthenospermia: The percentage of progressively motile sperm in semen analysis is less than 32%.

⑩ Diagnostic criteria for teratospermia : The percentage of sperm with normal morphology in semen analysis is less than 4%.

⑪ Polycystic ovary syndrome (PCOS): The most commonly used diagnostic criteria are the Rotterdam criteria proposed in 2003 by the European Society for Reproductive and Embryological Medicine and the American Society for Reproductive Medicine: A. Infrequent ovulation or anovulation; B. Clinical manifestations of hyperandrogenism and/or hyperandrogenemia; C. Polycystic ovarian changes: ultrasound shows ⩾12 follicles with a diameter of 2-9 mm in one or both ovaries , and/or ovarian volume ⩾10 ml; D. Meeting 2 of the 3 criteria and excluding other causes of hyperandrogenism, such as congenital adrenal hyperplasia, Cushing's syndrome , and androgen-secreting tumors.

⑫ Ovarian insufficiency refers to a serum follicle-stimulating hormone (FSH) level greater than 10 U/L or a total of fewer than 5 follicles in both ovaries during the early follicular phase. It can further develop into premature ovarian failure.

⑬ Pelvic and fallopian tube factors are caused by previous infections, surgical procedures, etc., resulting in pelvic adhesions, fallopian tube blockage, hydrosalpinx, and impaired egg-picking function of the fimbriae of the fallopian tubes.

⑭ Endometriosis refers to the growth of uterine glands and stroma outside the endometrium and myometrium of the uterine cavity, causing lesions.

4.1.1.3 . Survey Content: The baseline survey includes questionnaires, clinical physical examination and physiological and biochemical indicators, scale assessments , and the specific type of ART technology ultimately received . Details are as follows:

(1) Questionnaire survey: covering sociodemographic characteristics , baseline health status, lifestyle, environmental factors and medical history, etc., and collecting general information and medical history of both spouses on site.

① Sociodemographic characteristics include gender, age, occupation, education level, and socioeconomic status;

② Baseline health includes: (including age at first attempt to conceive, use of oral contraceptives, and presence of sexual dysfunction)

A. Female:

a. Self-assessment of health status;

b. The woman's menstrual and reproductive history (including age of menarche, regularity of menstruation, and whether menstruation is infrequent (infrequent menstruation generally refers to a menstrual cycle that is longer than 35 days).

c. Contraceptive history (method, frequency);

d. Medical history, medication history, family history, etc.;

e. Obstetric history;

B. Male:

a. Self-assessment of health status: Date of onset of puberty; whether any sexual problems have occurred (including difficulty maintaining an erection, premature ejaculation, or inability to ejaculate).

b. Contraceptive history (method, frequency);

c. Medical history, medication history, family history, etc.;

History of cryptorchidism (undescended testis); whether the patient has undergone vasectomy or other abdominal, pelvic, or scrotal surgery; use of antibiotics or other over-the-counter medications (dosage, frequency); use of illicit drugs such as marijuana, cocaine, or anabolic steroids.

③ Lifestyle behaviors include:

a. Diet and Nutrition: Food frequency questionnaire + whether you have a habit of eating food in plastic bags + use of disposable tableware; frequency of eating fruit peels and vegetables; consumption of canned food; frequency of drinking milk tea; amount of tea and coffee consumed; use of dietary supplements (vitamins); smoking and alcohol consumption (dosage and frequency).

b. Daily routine: Exercise (frequency, duration); daily use of electronic devices; sleep quality (start and end times); nap habits (duration of naps);

c. Work environment: Are there any conditions that are unfavorable to fertility, such as prolonged sitting , high temperature, environmental pollution, radiation, chemical reagents, etc.?

d. Living environment: Exposure to chemicals, pesticides, radiation, or toxins such as lead, especially frequent exposure; exposure to kitchen fumes; whether you have recently lived in a house with new furniture or renovations; whether the surrounding area is close to garbage stations or transportation hubs;

e. Personal care: Use of skincare and cosmetic products (frequency, type of products used); frequency of perming, dyeing, and manicures per year)

④ Environmental factors include:

A. Traditional air pollutants: Data on six traditional air pollutants ($PM_{2.5}$, PM_{10}, SO

$_2$, CO, NO_2, and O_3) were used. The observation exposure period was divided into seven periods according to the IVF treatment process (i.e., 85 days before oocyte retrieval to the day of oocyte retrieval, Gn initiation to the day of oocyte retrieval, oocyte retrieval to the day of embryo transfer, 30 days before frozen-thawed embryo transfer to the day of embryo transfer, embryo transfer to the day of HCG pregnancy test , 85 days before oocyte retrieval to the day of HCG test in fresh cycles, and 30 days before embryo transfer to the day of HCG test in frozen-thawed cycles). Data from the air monitoring station closest to the patient's residence was used as approximate exposure.

B. Environmental pollutants related to plastic products :

Plastic raw materials: Different types of plastic monomers (polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), polystyrene (PS), polyethylene terephthalate (PET), polycarbonate (PC), polyamide (PA), polymethyl methacrylate (PMMA), epoxy resin (EP), polyurethane (PU), polyphenylene sulfide (PPS), polyetheretherketone (PEEK), polytetrafluoroethylene (PTFE), ethylene-vinyl acetate copolymer (EVA) and different particle sizes)

plastic.

Plastic additives : plasticizers, flame retardants, antioxidants, colorants, lubricants, fillers and foaming agents (see Appendix 2 for details).

on factors such as whether the living environment is adjacent to a street or main road, the renovation status of the residence or workplace (historical renovations, new furniture), and whether there are petrochemical plants or waste incineration plants nearby.

(2) Clinical physical examination:

① Physical examination for female patients: routine gynecological examination, vaginal secretion examination, transvaginal ultrasound, ultrasound-guided tubal patency test, sex hormone testing, and detection of antisperm antibodies, anti-endometrial antibodies, anticardiolipin antibodies, and anti-ovarian antibodies. For patients whose cause cannot be determined through other examinations,

hysteroscopy and laparoscopy may be performed. (Follicular fluid, urine, and blood samples should be collected.)

② Physical examination for male patients: Specialized andrological examination, routine semen analysis, sperm morphology examination, mycoplasma examination, chlamydia examination, gonorrhea examination, and antisperm antibody testing. Routine semen analysis: Patients abstain from ejaculation for 2-7 days, collect semen via masturbation, and use a semen analysis system. The analysis report includes semen volume, color, liquefaction time, motility, sperm density, and sperm morphology. (Semen, urine, and blood samples are collected.)

(3) Sample collection and preservation

Sampling containers: Blood, urine, semen, and follicular fluid are collected using glass sampling containers .

Sampling time: a total of 4 times, namely: the first admission physical examination, the day of surgery, the 14th-16th day after surgery, and the 30th day after surgery.

Samples collected: blood, urine, semen (seminal plasma without sperm), follicular fluid, vaginal/ cervical secretions.

Preservation method: Blood is divided into two parts: whole blood is frozen at -80℃ and the supernatant is frozen after centrifugation ; urine, follicular fluid and semen are frozen directly at -80℃ .

(4) Scale assessment:

The Hospital Anxiety and Depression Scale (HADS) , the Social Support Rating Scale , and the Perceived Stress Scale (PSS) were used .

(5) Record the type of ART technology finally accepted.

4.1.2 Follow-up

Participants registered individually or as couples and were followed up from the start of the study through reproductive care, pregnancy, delivery, and childbirth . Follow-up was conducted every three months during the assisted reproductive treatment phase, pregnancy, and the first six months postpartum,

and every two years postpartum . Specific follow-up content and methods are as follows:

(1) Success in the field of ART refers to live birth , which can be obtained through doctor diagnosis or access to medical system records, while recording whether adverse pregnancy outcomes and their types (miscarriage, stillbirth, premature birth, low birth weight infant, macrosomia, congenital abnormalities, stillbirth, neonatal death).

(2) Variable factors such as lifestyle in the baseline indicators: The specific content is the same as in 1.1.3.

1.3 Data Management

A database of infertility cohorts in Hunan Province will be established, and baseline survey information and follow-up information will be managed in a standardized and electronic manner through a project-specific software system. At the same time, original data and relevant biological samples will be retained for verification.

1.4 Quality Control

Establish a standardized survey manual to clearly define survey items and methods. Provide standardized training to surveyors to ensure they use consistent and standardized methods to collect questionnaire information.

4.2 . Research on risk factors for ART failure in infertile patients

To identify risk factors for ART failure in infertile patients , based on previously established data from an infertile cohort in Hunan Province, participants were grouped according to different lifestyle behaviors, health conditions, environmental factors, and other potential risk factors (e.g., if smoking was a potential risk factor, participants were divided into smoking and non-smoking groups). A multivariate Cox proportional hazards model was used to adjust for confounding factors, and differences in pregnancy outcomes were compared between the groups , calculating the risk factor (HR) and 95% confidence interval (CI). An HR > 1 was considered a risk factor, while an HR < 1 was considered a

protective factor. Further comparisons were made regarding the differences in risk factors for ART failure among infertile patients.

4.3 . Research on the Construction and Evaluation of Predictive Models for ART Failure in Infertile Patients

4.3.1 . Screening of candidate predictors

4. 3.1.1. The risk factors for ART failure in infertile patients identified in the above studies were used as candidate predictors, including sociodemographic characteristics, basic health status, lifestyle, physiological and biochemical indicators, and environmental factors.

4. 3.1.2. Screening candidate predictors based on Lasso regression: Given the large number of candidate predictors, direct modeling can easily lead to high complexity. Therefore, the Lasso regression method is adopted. By constructing a penalty function, the lambda value with the smallest error is used as the standard to screen variables, thereby achieving the purpose of subset shrinkage and avoiding overfitting.

4.3.2 . Construction of Risk Prediction Model

Using ART failure in infertile patients as the outcome indicator and selected indicators as predictive factors, a risk prediction model was constructed using algorithms such as Cox proportional hazards regression , random forest (RF), extreme gradient boosting (XGBoost), and Naive Bayes (NB).

4.3.3 . Evaluation and Validation of Risk Prediction Model

4.3.3.1 . Validation Methods: Internal and external validations were performed to evaluate the model's internal and external effectiveness. Internal validation employed a 10-fold cross-validation method, dividing the data into 10 mutually exclusive subsets. Nine subsets were selected as the training set and one subset as the validation set in each iteration, repeated 10 times, and the average value was used as the model performance evaluation result. External validation involved applying the risk prediction model built for this region to other regions to evaluate its performance.

4.3.3.2 . Evaluation Indicators: The predictive model is evaluated based on three aspects: discrimination, calibration, and predictive ability. The discrimination of the model is assessed by calculating the area under the receiver operating characteristic curve (AUC); the calibration curve is plotted to assess the calibration of the model; and the predictive ability of the model is evaluated from multiple aspects, including error rate, sensitivity, and specificity.

4.3.3.3 . Optimal Model Selection: Based on the model discrimination and calibration map results, the optimal prediction model is selected, and finally a prediction model for the risk of ART failure in infertile patients is formed.

4.3.4 Visualization of Risk Prediction Model

By presenting and reporting risk prediction models in the form of scorecards, nomograms, web calculators, or applications, the models can be visualized, thereby forming a simple, convenient, efficient, and easy-to-promote risk prediction system and promoting its application in practice.

5. Data integrity and quality assurance

Researchers should ensure that data is authentic, accurate, complete, and traceable, and should ensure the integrity of basic clinical research documents during retention, avoiding intentional or unintentional alteration or loss.

6. Ethical Statement

The clinical research will adhere to the relevant regulations of the World Medical Association's Declaration of Helsinki and the Ethical Review Guidelines for Biomedical Research Involving Human Subjects. The research protocol will be approved by the ethics committee before the clinical research can commence. The privacy of research participants and the confidentiality of data will be protected throughout the research process.

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