Original Article

Association between Exposure of Rare Earth Elements and Outcomes of *In Vitro* Fertilization-Embryo Transfer in Beijing*

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Abstract

Objective The study aimed to investigate the impact of rare earth elements (REEs) exposure on pregnancy outcomes of *in vitro* fertilization-embryo transfer (IVF-ET) by analyzing samples from spouses.

Methods A total of 141 couples were included. Blood and follicular fluid from the wives and semen plasma from the husbands, were analyzed for REEs using inductively coupled plasma mass spectrometry (ICP-MS). Spearman's correlation coefficients and the Mann–Whitney U test were used to assess correlations and compare REE concentrations among three types of samples, respectively. Logistic models were utilized to estimate the individual REE effect on IVF-ET outcomes, while BKMR and WQS models explored the mixture of REE interaction effects on IVF-ET outcomes.

Results Higher La concentration in semen (median 0.089 ng/mL, P = 0.03) was associated with a lower fertilization rate. However, this effect was not observed after artificial selection intervention through intracytoplasmic sperm injection (ICSI) (P = 0.27). In semen, the REEs mixture did not exhibit any significant association with clinical pregnancy.

Conclusion Our study revealed a potential association between high La exposure in semen and a decline in fertilization rate, but not clinical pregnancy rate. This is the first to report REEs concentrations in follicular fluid with La, Ce, Pr, and Nd found at significantly lower concentrations than in serum, suggesting that these four REEs may not accumulate in the female reproductive system. However, at the current exposure levels, mixed REEs exposure did not exhibit reproductive toxicity.

Key words: Rare earth elements; *In vitro* fertilization; Pregnancy outcomes; Mixture exposure analysis

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INTRODUCTION

Rare earth elements (REEs) are a group of metallic elements with similar structures and the same electron layer[1], including yttrium (Y), scandium (Sc), and 15 lanthanide elements, namely lanthanum (La), cerium (Ce), praseodymium (Pr), neodymium (Nd), promethium (Pm), samarium (Sm), europium (Eu), gadolinium (Gd), terbium (Tb), dysprosium (Dy), holmium (Ho), erbium (Er), thulium (Tm), ytterbium (Yb), and lutetium (Lu)[2,3]. The International Union of Pure and Applied Chemistry (IUPAC) classifies REEs into Light Rare Earth Elements (LREEs) and Heavy Rare Earth Elements (HREEs), including La, Ce, Pr, Nd, Pm, Sm, Eu, and Sc, Tb, Dy, Ho, Er, Tm, Yb, Lu, and Y[4,5].

REEs are widely used in many fields, including agriculture, medicine, electronics, military, energy industries, and other high-tech sectors[3]. This widespread use has increased the environmental exposure risks, which may have potential implications for human health[6]. Numerous studies have documented the widespread occurrence of REEs across various environmental media, including soil, dust, sediments, air, water[7-11], and food[12,13]. Human exposure to REEs can occur through water and food ingestion, inhalation, or dermal contact[2]. Concurrently, the accumulation of REEs has been detected in various biological specimens, including human hair[14], urine[14], nails[15], blood[16,17], semen[11], breast milk[18], placental tissue[19], and brain tissue[17].

Animal studies have revealed the toxic effects of rare earth elements (REEs) on gametes. The injection of a mixture of REE chlorides (La, Ce, Pr, and Nd) before ovulation in female Kunming mice resulted in a decrease in the number of mature oocytes and inhibited their activation[20]. Exposure of male Kunming mice to high doses of $CeCl_3$ led to testicular cell dysfunction and increased[21] whereas the injection of CeO_2 nanoparticles into male BALB/c mice significantly reduced sperm count and motility[22]. This indicates the reproductive toxicity of REEs to some extent.

Cohort studies have also reported an association between REEs and gamete development. A prospective cohort study on assisted reproductive technology demonstrated that elevated levels of La in female serum were associated with an increased risk of clinical pregnancy failure and negatively associated with the number of good-quality oocytes[23]. Human sperm exposed to $CeCl_3$ *in vitro* showed increased oxidative damage[21], suggesting that REEs may be a risk factor for infertility. Furthermore, case-control cohort studies have indicated that elevated levels of La and Sm in female plasma may be associated with the risk of spontaneous preterm birth, whereas higher levels of Pr may be associated with the risk of premature rupture of membranes[24], indicating the potentially deleterious effects of REEs on human embryonic and fetal development.

Previous cohort studies on REEs predominantly concentrated on blood, urine, and hair samples from women[22,25–30]. However, the effect of REE exposure on male reproductive health remains unknown. Additionally, REEs exposure in the environment is heterogeneous; however, there have been no mixed analyses of the effects of REEs on pregnancy outcomes in assisted reproductive populations. In this study, we simultaneously detected REEs in serum and follicular fluid from females and semen from males in couples undergoing *in vitro* fertilization-embryo transfer (IVF-ET) or intracytoplasmic sperm injection (ICSI) to comprehensively investigate the effects of REE exposure on pregnancy outcomes.

METHOD

Study Design and Participants

This study recruited infertile couples who were undergoing their first *in vitro* fertilization (IVF) or ICSI cycle at the Center of Reproductive Medicine, Peking University Third Hospital, Beijing, China, from April to July 2023.

The inclusion criteria were as follows: 1) 20–38 years old, male under 60 years of age, and 2) meeting the diagnostic criteria for infertility (regular intercourse without conception for at least 12 months)[28]. Exclusion criteria were as follows: 1) couples who had poorly controlled diabetes mellitus and hypertension, serious cardiovascular disease, history of malignant tumors, undergone preimplantation genetic testing (PGT); 2) females who had undergone adenomyosis, uterine malformations, endometritis, hydrosalpinx, ovarian or uterine surgery, hyperprolactinemia, congenital adrenal cortical hyperplasia, Cushing's syndrome, and hormone therapy within the past 3 months; 3) males who had severe asthenozoospermia, azoospermia, and undergone microscopic epididymal sperm aspiration (MESA) or testicular sperm extraction (TESE).

When a couple was enrolled, they completed a

baseline questionnaire that included demographics and lifestyle characteristics, eating habits, nutritional intake, alcohol consumption, smoking status, second-hand smoke exposure, and current living and working environments. All participants were informed of the details of the study. Informed consent was obtained to allow us to collect clinical data from medical records and to donate their samples. This study was approved by the Ethics Committee of Peking University Third Hospital (M2022722).

Sample Collection

Controlled Ovarian Hyperstimulation Protocols All women enrolled in the study received individualized controlled ovarian hyperstimulation (COH) treatment following standardized protocols determined by physicians in the outpatient clinic according to their baseline infertility evaluations. 1) In the GnRH-antagonist (GnRH-ant) protocol, gonadotropin (Gonal-F, Pouliquen, or HMG) was administered on the second day of menstruation, and GnRH-ant (Diphereline, Cetrotide, or Ganirelix) was administered for pituitary suppression starting on cycle day 6 or 7, according to follicular growth (when at least one follicle reached a diameter of 12 mm). 2) In the long or super-long GnRH agonist (GnRH-a) protocol, GnRH-a (Triptorelin or Leuprorelin) was administered in the mid-luteal phase or during the menstrual period of the previous menstrual cycle. After 14 or 28 days, gonadotropin (Gn) treatment was initiated following GnRH agonist downregulation. 3) In the short GnRH-a protocol, GnRH-a (triptorelin) was used for pituitary suppression starting on cycle day 2 or 3, and gonadotropin was administered simultaneously.

Follicular Fluid Collection For all female participants, when at least 2 follicles reached 18 mm in diameter, a dosage of 10,000 IU of human chorionic gonadotropin (hCG, Livzon, China, LOT: HCG10000Iu) or 250 μg of recombinant human choriogonadotropin (r-hCG; Eiser, LOT: AZ250 μg) was administered to trigger oocyte maturation. Subsequently, oocyte retrieval was performed 36 hours after hCG injection under the guidance of transvaginal ultrasound *via* a 17–18 g oocyte needle. Follicular fluid samples were collected during oocyte retrieval. Only the first dish of follicular fluid without blood contamination was collected. Subsequently, the follicular fluid samples were centrifuged at 4,000 rpm for 10 min and the supernatants were stored in cryogenic vials (Biosharp, China, LOT: BS-20-ST) at −80 °C immediately.

Serum and Semen Collection Blood samples from females were collected on the second or fourth day of the menstrual period using procoagulant tubes (Sekisui Medical Technology, China). After centrifuging at 3,500 rpm for 10 min, the serum was frozen and stored in a microtube (Axygen, China, LOT: MCT-200-C) at −80 °C until analysis. For male semen collection, a fresh ejaculated semen sample was collected on the day of oocyte retrieval, 2–7 days after abstaining from sexual intercourse. Then the sample was centrifuged at 400 ×*g* for 10 min, and the supernatants were immediately stored in cryogenic vials (Biosharp, China, LOT: BS-20-ST) at −80 °C. The choice between IVF or ICSI was based on the results of the semen analysis conducted on this day.

Information Collection and Assisted Reproductive Outcomes

The following data were obtained from the medical records of female participants: age, height, body weight, body mass index (BMI), infertility type, infertility diagnosis, duration of infertility, and basal serum sex hormone levels. Treatment outcomes of IVF/ICSI were evaluated using a series of indicators. The information included the number of retrieved oocytes, mature oocytes (oocytes that entered metaphase II, MII), normal fertilized oocytes (presented with two pronuclei, 2PN), good-quality embryos (embryos with 6–10 spherical cells and < 20% fragmentation), maturation rate (calculated as the ratio of MII oocytes to retrieved oocytes), and fertilization rate (calculated as the ratio of 2PN oocytes to MII oocytes). Biochemical pregnancy (positive hCG > 25 IU 14 days after embryo transfer) and clinical pregnancy (presence of at least one gestational sac with fetal heartbeat detected on transvaginal ultrasound 30 days after embryo or blastocyst transfer) outcomes were followed up.

Elements Analysis

The samples were analyzed for REE (La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, and Lu) using an inductively coupled plasma-mass spectrometer (ICP-MS, ELAN DRC II; PerkinElmer, USA). To examine serum, follicular fluid, and semen samples, 0.1 mL of each sample was diluted in 2 mL volumetric flasks, then supplemented with 1.8 mL of 1% nitric acid (HNO_3) (UPS, 68%; Suzhou Crystal Clear Chemical Co., Ltd., LOT: 210426227) and 0.1 mL of rhenium (4 ng/mL) (GSB 04-1745-2004, LOT: 228017) were added as internal standard. The mixed solution was shaken well before the measurement. The ICP-MS

was operated under the following conditions: gas flow rate of 0.98 L/min, auxiliary gas flow rate of 1.85 L/min, plasma gas flow rate of 17.5 L/min, radio frequency power of 1,150 W, dwell time ranging from 50 to 100 ms, sample uptake rate of 1.1 mL/min, resolution ratio of 0.7 to 0.9 amu. ClinChek® serum controls for trace elements (order no. 8881) were employed as quality control (QC) samples in the study. Sample analyses were conducted at the Central Laboratory of Biological Elements at the Peking University Health Science Center in China.

Statistical Analyses

Spearman's correlation coefficients were calculated to evaluate the correlations among the REEs. The Mann–Whitney U test was used to compare the REE concentrations across the three types of samples. Bayesian Kernel Machine Regression (BKMR) and Weighted Quantile Sum (WQS) regression models were used to examine the associations between REEs as a mixture and the risks of IVF outcomes. The confounding factor was identified based on prior knowledge, including factors associated with IVF outcomes and REE concentrations in previous research[20,29,30]. Maternal age, BMI, fertilization mode, infertility type, and smoking status were selected as covariates in the adjusted logistic regression, BKMR, and WQS models.

Statistical Model 1: Generalized Linear Regression Model Weighted generalized linear regression models were used to investigate the association between individual LREE concentrations and IVF outcomes[25]. Demographic and behavioral variables, including age, BMI, fertilization mode, infertility type, and smoking status, were incorporated as covariates in the adjusted models. Generalized linear regression models with categorical variables were also used to account for the potential nonlinear correlations between serum REEs and outcomes. Statistical results are presented as estimated odds ratios (*OR*s) and 95% confidence intervals (*CI*s).

Statistical model 2: Bayesian Kernel Machine Regression (BKMR) Model BKMR models were employed to examine mixture effects and potential interactions between REEs and IVF outcomes[26]. Before establishing the model, the concentrations of all elements were log-transformed. Each BKMR model underwent 50,000 iterations for model fitting using the default priors. The BKMR model treated REEs as "exposure", adjusting for covariates similar to logistic models. The probit link function h() was used to examine the association between continuous REE exposure and binary outcomes. This model allowed the identification of the independent effects of individual REEs in addition to the overall combined mixture effect. The association plot provides a visual representation of the individual REEs exposure-response functions. Interactions between REEs were assessed by estimating the changes in outcomes associated with changes in individual REE concentrations. A specific point estimate was calculated for the difference in the outcome levels for the change in individual REE concentrations between the 25th and 75th percentiles. Posterior Inclusion Probability (PIP) was derived to indicate the relative importance of each REE. A higher PIP for a given REE indicates a greater contribution of that element to the effect of mixed exposure on IVF outcomes. The BKMR model was executed using the "bkmr_0.2.2" package in R version 3.5.0.

Statistical Model 3: Weighted Quantile Sum (WQS) Regression Model The cumulative impact of REE mixture components on IVF outcomes was estimated using WQS regression. A threshold of ≥ (100%/*n*) for weights was used to determine the greatest contributors to the overall mixture effect. The established model aimed to estimate the exposure effect of the mixture on IVF outcomes. This study analyzed the association between the mixture exposure (WQS index) of REEs and IVF outcomes and calculated the weight of REEs for the WQS index. A two-sided *P*-value of < 0.05 was considered significant, and 95% *CI*s were calculated. The WQS model was performed using R 3.5.0 with the "gWQS_3.0.5" package.

RESULTS

Demographic Characteristics and Assisted Reproductive Outcomes

A total of 141 couples were enrolled in this study, with 93 undergoing IVF cycles and 48 undergoing intracytoplasmic sperm injection (ICSI) cycles. The demographic characteristics of the study population are presented in Table 1. The baseline hormone levels and semen quality were within normal ranges for these parameters. Following assisted reproductive treatment, the average number of 2PN was 7.8 ± 5.2, the average number of good-quality embryos was 5.6 ± 4.3, and the average fertilization rate was 61.2% ± 22.0%. Ultimately, 74 women (59%) tested positive for hCG, and 57 (47%) presented with clinical pregnancy.

Concentrations of REEs

The concentrations of 14 REEs were examined in the serum and follicular fluid of females and in the semen of males. The REEs with detection rates exceeding 80% included La, Ce, Pr, Nd, and Sm. All were classified as light rare earth elements (LREEs) and further analyzed[27].

La, Ce, Pr, and Nd were detected in all three samples; however, the concentrations of these elements varied in different samples (Table 2). The concentration of REEs in female serum was significantly higher than that in the follicular fluid ($P < 0.001$), suggesting a potential barrier for REEs when entering the follicular fluid from the blood. The concentration in male semen was higher than that in female follicular fluid ($P < 0.001$) and comparable to that in female serum ($P = 0.034$). Sm was only detected in the female serum (Table 3). The Spearman correlation coefficients for all elements are shown in Supplementary Figure S1 (available in www.besjournal.com). The five LREEs (La, Ce, Pr, Nd, and Sm) were positively correlated with each other in serum, and a similar correlation was observed among the four elements (La, Ce, Pr, and Nd) in follicular fluid and semen. La and Ce were positively correlated in all three sample types, with correlations ranging from 0.78 to 0.93. However, there was no correlation between REEs in the serum and follicular fluid of the same female.

Relationship between REE Exposure and Assisted Reproductive Parameters

For females, REE levels in the serum and follicular fluid exhibited no significant correlation with fertilization rate, number of two pronuclei

Table 1. The basic demographic and biological characteristics of the couples

Characteristics	Females	Males
Age (years), mean ± SD	32.0 ± 3.1	33.3 ± 4.0
< 35, *n* (%)	15 (11.0)	98 (69.5)
≥ 35, *n* (%)	126 (89.0)	43 (30.5)
BMI (kg/m^2), mean ± SD	23.2 ± 3.7	26.1 ± 5.0
≤ 25, *n* (%)	104 (73.8)	62 (46.6)
> 25, *n* (%)	37 (26.2)	71 (53.4)
Smoking status, *n* (%)		
Smoker	0	52 (37.4)
Non-smoker	139 (100.0)	87 (63.6)
Infertility type, *n* (%)		
Primary infertility	32 (22.7)	–
Secondary infertility	109 (77.3)	–
AMH (ng/m), mean ± SD	4.3 ± 3.1	–
Basal hormone level, mean ± SD		
FSH (mIU/mL)	6.3 ± 2.1	–
PRL (ng/mL)	14.9 ± 11.1	–
LH (mIU/mL)	3.4 ± 2.1	–
E2 (pmol/L)	111.9 ± 116.8	–
T (nmol/L)	0.8 ± 0.3	–
P (ng/mL)	2.8 ± 19.9	–
Sperm concentration (× 10^6/mL), mean ± SD	–	47.5 ± 3.5
Progressive motility (%), mean ± SD	–	34.0 ± 18.9
Sperm viability (%), mean ± SD	–	28.5 ± 17.4

Note. Data was described as *n* (%) or mean ± SD. SD, standard deviation; BMI, body mass index; IVF, *in vitro* fertilization; ICSI, intracytoplasmic sperm injection; 2PN, two pronuclei.

(2PN), number of good-quality embryos, or clinical pregnancy outcomes. Similarly, REE levels in male semen exhibited no significant correlation with the number of 2PN embryos, the number of good-quality embryos, or clinical pregnancy outcomes (Figure 1 and Supplementary Table S1, available in www.besjournal.com). However, an elevated level of La in male semen (median 0.089 ng/mL, *OR*: 0.79, 95% *CI*: 0.63–0.98, *P* = 0.03) was associated with a lower fertilization rate (Figure 1). Further stratified analysis of ICSI and IVF reveals that the association of La with fertilization rate can be corrected after the artificial selection of sperm through ICSI (*OR*: 1.17, 95% *CI*: 0.88–1.55, *P* = 0.27).

Table 2. The results of IVF and ICSI

Couple/cycle-specific characteristics	Couples
Fertilization mode, *n* (%)	
IVF	93 (66.0)
ICSI	48 (34.0)
No. of oocytes retrieved, mean ± SD	13.0 ± 7.7
No. of 2PN, mean ± SD	7.8 ± 5.2
No. of good-quality embryos, mean ± SD	5.6 ± 4.3
Fertilization rate (%), mean ± SD	61.2 ± 22.0
IVF outcomes, *n* (%)	
HCG test	
Positive	74 (59.2)
Negative	51 (40.8)
Clinical pregnancy	
Yes	57 (47.2)
No	64 (52.8)

Note. Data was described as *n* (%) or mean ± SD. SD, standard deviation; IIVF, *in vitro* fertilization; ICSI, intracytoplasmic sperm injection; 2PN, two pronuclei.

The Mixed Exposure of Rare Earth Elements in Semen Does Not show a Statistically Significant Association with Assisted Reproductive Outcomes

When the other three elements were set at the 25th, 50th, and 75th percentile concentrations of seminal plasma, Pr was positively associated, and Nd was negatively associated with clinical pregnancy (Figure 2A). The dose-response curves for REEs tended to be linear based on the univariate exposure-response curves of individual elements with pregnancy outcomes (Figure 2B). In the bivariate exposure-response function, interactions were found between Nd and La, Ce, and Pr (Figure 2C). Mixed REE exposure to REEs shows not associated with clinical pregnancy outcomes (Figure 2D). The PIP is highest for seminal plasma La at 0.53 (Supplementary Table S2, available in www.besjournal.com). WQS model analysis results indicate no association between mixed exposure and clinical pregnancy (*OR*: 1.03; 95% *CI*: 0.91–1.15; *P* = 0.62). La contributed the most to the mixed index

Table 3. REE concentrations of the IVF-embryo transfer couples

Elements	Sample	Median (IQR)	Mean ± SD	LOD	Detection ratio (%)
Ce (ng/mL)	Serum	0.097 (0.061, 0.167)	0.149 ± 0.163	0.0004	100.0
	Follicular fluid	0.034 (0.020, 0.05)	0.046 ± 0.044	0.0004	100.0
	Seminal plasma	0.246 (0.168, 0.374)	0.289 ± 0.206	0.0004	100.0
La (ng/mL)	Serum	0.080 (0.048, 0.124)	0.107 ± 0.097	0.0006	100.0
	Follicular fluid	0.016 (0.009, 0.027)	0.022 ± 0.021	0.0006	99.3
	Seminal plasma	0.089 (0.054, 0.128)	0.101 ± 0.084	0.0006	97.2
Nd (ng/mL)	Serum	0.144 (0.097, 0.199)	0.172 ± 0.119	0.0003	100.0
	Follicular fluid	0.069 (0.054, 0.101)	0.076 ± 0.034	0.0003	100.0
	Seminal plasma	0.118 (0.080, 0.171)	0.130 ± 0.076	0.0003	100.0
Pr (ng/mL)	Serum	0.026 (0.017, 0.040)	0.033 ± 0.026	0.0001	100.0
	Follicular fluid	0.009 (0.007, 0.012)	0.010 ± 0.006	0.0001	100.0
	Seminal plasma	0.023 (0.013, 0.036)	0.027 ± 0.020	0.0001	100.0
Sm (ng/mL)	Serum	0.050 (0.031, 0.06)	0.053 ± 0.030	0.0004	99.3

Note. Data was described as Median (IQR), *n* (%), or mean ± SD**.** SD, standard deviation; IQR, interquartile range; LOD, limit of detection. REE, rare earth elements; IVF, *in vitro* fertilization.

with a weight of 64% (Figure 2E).

When the remaining elements were set at the 25th, 50th, and 75th percentile concentrations, Sm and Ce in serum, as well as Pr in follicular fluid, were positively correlated with clinical pregnancy (Supplementary Figure S2A and Supplementary Figure S3A, available in www.besjournal.com); however, according to the individual element's univariate exposure-response curves with pregnancy, only Pr in follicular fluid showed a nonlinear relationship (Supplementary Figure S3B). In the bivariate exposure-response function of serum REEs, interactions were found between Ce and Sm (Supplementary Figure S2C). Regarding the follicular fluid, interactions were found between Nd and La, Ce, and Pr (Supplementary Figure S3C). Ultimately, in both the serum and follicular fluid, BKMR mixed exposure to REEs showed a positive association with clinical pregnancy outcomes (Supplementary Figure S2D, Supplementary Figure S3D). The results of the WQS model analysis also suggest a positive association between mixed exposure and clinical pregnancy (Supplementary Figure S2E, Supplementary Figure S3E).

DISCUSSION

In this study, the relationship between the REE concentration in samples from both partners and assisted reproductive outcomes was investigated using a prospective cohort design. We found that after adjusting for factors such as age, BMI, infertility type, fertilization method, and smoking, high concentrations of La in semen may be associated

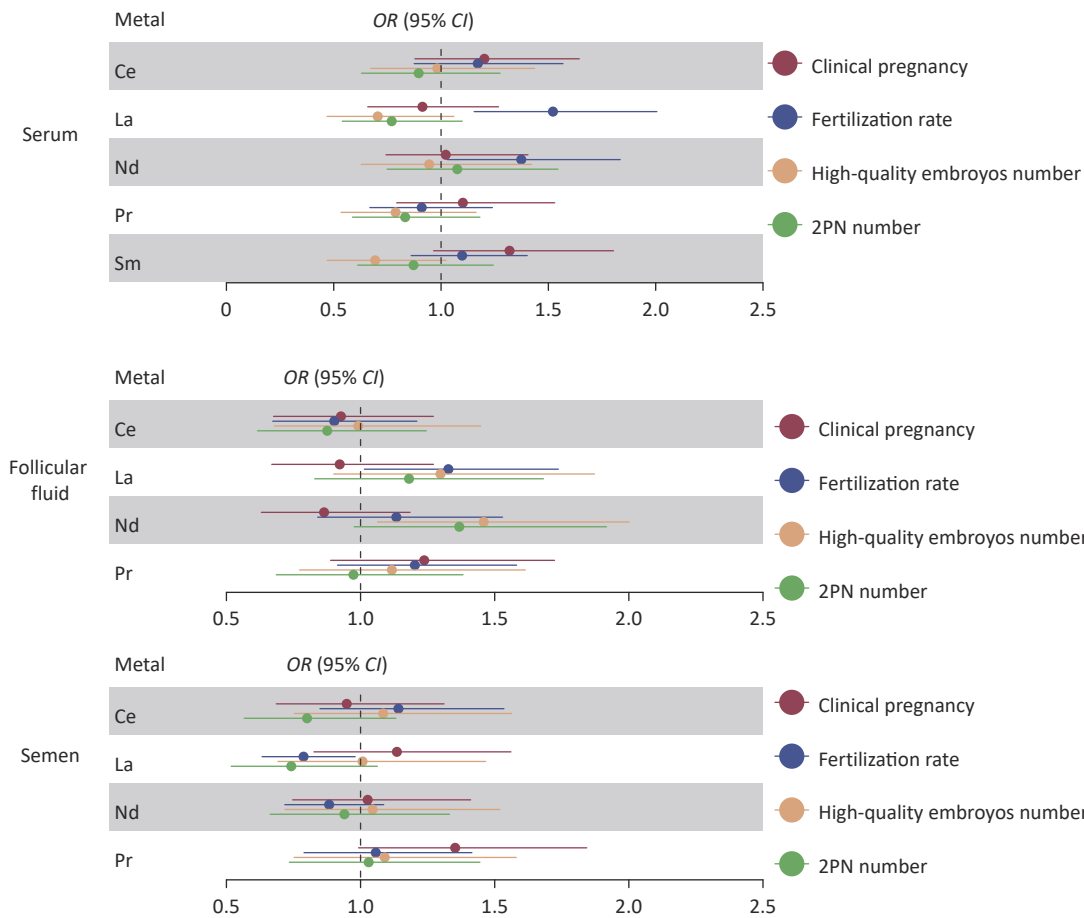

Figure 1. Relative risk of LREE concentrations associated with reproductive characteristics in IVF-ET couples. Results of the associations of REE with the number of 2PN, the number of good-quality embryos, fertilization rate, and clinical pregnancy (adjusted by age, BMI, fertilization mode, infertility type, and smoking status). Blue represents a statistically significant negative association; red represents a statistically significant positive association. LREE, light rare earth elements; IVF-ET, *in vitro* fertilization-embryo transfer; 2PN, two pronuclei.

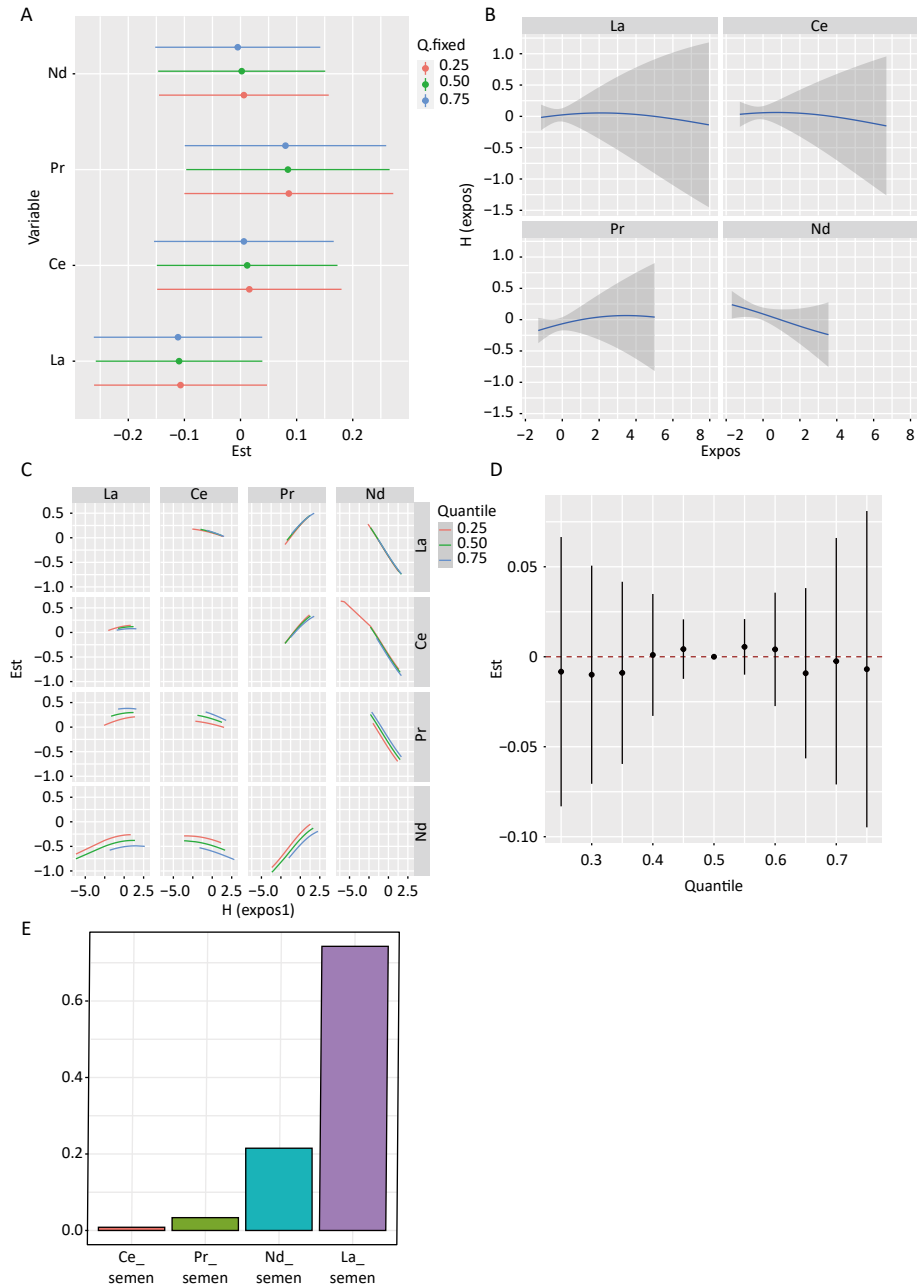

Figure 2. Mixture exposure analysis of WQS model regression models and BKMR Models for clinical pregnancy in semen. (A) Single REEs association. The plot compares the changes in clinical pregnancy [h(expo)] when the LREE is at its 75th and 25th percentiles, while the other elements are fixed at their 25th, 50th, and 75th percentiles. (B) Univariate exposure-response analysis for the associations between each REE and clinical pregnancy. Univariate exposure-response functions and 95% confidence intervals are calculated for the associations between each REE and clinical pregnancy, with other elements fixed at their median concentrations. (C) Bivariate exposure-response functions for a type of REE were fixed at either the 25th, 50th, or 75th percentile, while the remaining REEs were fixed at the median. (D) Association between mixed REE exposure and clinical pregnancies. The plot compares the estimated risk change [h(expo)] when all REEs are at their specific quantiles to those at their 50th percentile. (E) The weights of REEs in clinical pregnancy based on WQS regression analysis. Models were adjusted for age, BMI, fertilization mode, infertility type, and smoking status. LREE, light rare earth elements.

with a decrease in fertilization rate. However, existing levels of rare earth element exposure do not have adverse effects on clinical pregnancies. The elements primarily ingested in daily life are predominantly light REEs[31], which is consistent with the high detection rates of REEs we observed. Previous studies have reported positive correlations among REEsin serum[16,20]. Our research conducted correlation analyses on the detected rare earth elements from three sample sources, revealing significant positive correlations, suggesting similar intake, transport, or metabolic pathways among these elements.

The follicular fluid consists of secretions from follicular cells and blood serum[32]. All REEs in follicular fluid originate from the environment and enter the follicles through the bloodstream. The blood-follicle barrier (BFB), acting as a selective "molecular sieve" between follicles and blood, controls molecular exchange based on size and charge. It is composed of endothelial cells, basement membrane, inner layer, follicle basal membrane, and granular membranes[33,34]. Previous research has indicated that the elemental content in follicular fluid better represents long-term exposure levels[35]. Our study detected rare earth element concentrations in the follicular fluid of the same women and found that the concentrations were significantly lower (La: median 0.016 ng/mL) than those in serum and showed no correlation with the rare earth element concentrations in serum. This suggests that serum levels of REEs do not represent levels in follicular fluid, indicating that these elements may not easily accumulate in the reproductive system. The follicular fluid is a crucial microenvironment during oocyte maturation and development, playing a key role in oocyte quality, subsequent fertilization, and embryo development potential[36]. The direct effect of the follicular fluid environment on oocytes may have a greater impact on assisted reproductive outcomes and related indicators. There have been no previous reports on the detection of REEs in follicular fluid, and our study provides preliminary evidence for the transport and metabolism of REEs in the female reproductive system.

When analyzing the concentrations of REEs in semen samples from male partners undergoing assisted reproduction, we found that an increase in La concentration in semen was associated with a decrease in the fertilization rate, which is consistent with previous results from zebrafish and sea urchin studies[37-39]. Short-term exposure to La (III) (10^{-4}–10^{-5} mol/L) showed a decrease in the sperm fertilization rate[37-39]. Additionally, a decrease in fertilization rate has been found in sea urchins[37,38], zebrafish[39], and humans[40,41], albeit without inducing embryotoxic effects. Our findings align with those of previous studies, indicating that elevated La exposure is associated with reduced fertilization rates, yet seemingly exerts no significant influence on the likelihood of achieving clinical pregnancy. Previous studies have detected the presence of REEs in semen, with findings indicating that La and Ce concentrations do not affect sperm quality and that there is an association between rare earth element concentrations in semen and Ca^{2+} concentrations[11]. Some studies have reported that La can compete for binding sites with Ca^{2+}, potentially altering cell function and disrupting mitochondrial membrane potential and electrolyte gradients[42]. Furthermore, after stratifying the study cohort based on the fertilization technique employed (IVF and ICSI) after artificial selection, the relationship between La concentration and fertilization rate was no longer statistically significant in couples using ICSI. ICSI procedures may correct the effect of La on the fertilization rate, suggesting that La may inhibit intracellular Ca^{2+} influx by competing for binding sites with Ca^{2+}, leading to fertilization failure and decreased fertilization rates.

In the correlation analysis of pregnancy outcomes, only high La exposure in semen was associated with a decrease in the fertilization rate in the univariate analysis, with no statistically significant correlations found between various elements in other samples and clinical pregnancy. Additionally, both the BKMR and WQS analyses revealed no correlation between mixed exposure to REEs in semen and clinical pregnancy. The BKMR mixed exposure analysis found a positive correlation between Pr and clinical pregnancy, whereas Nd showed a negative correlation with clinical pregnancy, suggesting inconsistent effects among elements as a possible reason for the lack of an effect in the mixed exposure analysis. However, mixed exposure in females, whether in the follicular fluid or serum, showed a protective effect on clinical pregnancy. Previous studies have reported that REEs exhibit redox behavior, acting as antioxidants at low concentrations/short exposure times, stimulating or protecting biological processes, whereas at high concentrations and long exposure times, oxidative stress may occur, inducing adverse reactions[43]. This indicates that an increase in rare earth element concentration may cause pregnancy toxicity. However, our results indicated that the currently

detected concentrations in both partners did not reach toxic thresholds. Additionally, previous research has reported that high Pr (median 0.030 ng/mL) exposure in the maternal serum may increase the risk of premature rupture of membranes, and high La (median 0.072 ng/mL) exposure may increase the risk of neural tube defects. The detected concentrations of REEs in the serum (Pr: median 0.026 ng/mL; La: median 0.080 ng/mL) in our study were similar to those reported in the literature to affect offspring, suggesting that current rare earth element exposure may not have significant toxic effects on pregnancy but may have adverse effects on offspring, necessitating further follow-up and monitoring.

CONCLUSION

Our study revealed a possible association between high La exposure in semen and a decline in fertilization rates. Our study is the first to report REEs concentrations in follicular fluid; La, Ce, Pr, and Nd were found at significantly lower concentrations than in female serum, suggesting that these four REEs may not accumulate in the reproductive system. However, at the currently detected levels ofREEs exposure, mixed exposure did not exhibit reproductive toxicity.

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The authors declare that there are no conflicts of interest.

AUTHOR CONTRIBUTIONS

Yutong Wang and Jing Li contributed equally. Yutong Wang completed the collection and analysis of experimental samples and data, as well as the writing of the manuscript. Jing Li completed the collection of experimental samples and data and the revision of the manuscript. Shirong Xu, Yue Sun, and Yali Huang participated in the collection of samples and clinical data. Lailai Yan completed the mass spectrometry detection. Shengli Lin, Wei Guo, and Linlin Wang completed the collection and analysis of clinical data. Zhenchen Hou participated in the writing and revision of the manuscript. Ying Wang guided the clinical sample collection and data analysis. Chan Tian designed and guided the study.

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