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Hair mercury concentrations and in vitro fertilization (IVF) outcomes among women from a fertility clinic

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Abstract

Total hair mercury (Hg) was measured among 205 women undergoing in vitro fertilization (IVF) treatment and the association with prospectively collected IVF outcomes (229 IVF cycles) was evaluated. Hair Hg levels (median=0.62 ppm, range: 0.03-5.66 ppm) correlated with fish intake (r=0.59), and exceeded the recommended EPA reference of 1ppm in 33% of women. Generalized linear mixed models with random intercepts accounting for within-woman correlations across treatment cycles were used to evaluate the association of hair Hg with IVF outcomes adjusted for age, body mass index, race, smoking status, infertility diagnosis, and protocol type. Hair Hg levels

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were not related to ovarian stimulation outcomes (peak estradiol levels, total and mature oocyte yields) or to fertilization rate, embryo quality, clinical pregnancy rate or live birth rate.

Keywords

Mercury (Hg); oocyte; fertilization; in vitro fertilization (IVF); implantation; human

1. Introduction

Mercury (Hg) exists in three different chemical forms; elemental, inorganic, and organic (methylmercury or MeHg). While Hg occurs naturally in the environment with sources such as volcanoes and geothermal vents, the predominant source is coal combustion emissions in the atmosphere [1, 2]. Inorganic mercury is released into the environment from industrial processes, and subsequently metabolized into organic mercury by aquatic organisms. MeHg, in turn, bioaccumulates within fish and biomagnifies up the food chain [1]. The primary source of Hg exposure among humans is through seafood consumption, with inhalation of elemental mercury vapors (from dental amalgams, button cell batteries, broken thermometers, and compact fluorescent light bulbs) being a minor route of exposure. Due to the ease of incorporation into biological tissue, MeHg is the most toxic form of environmental mercury exposure for humans [1].

Mercury is a known neurotoxicant but its potential reproductive effects have not been well studied. In 2004, based on its neurotoxicity, the United States Environmental Protection Agency (EPA) and United States Food and Drug Administration (FDA) jointly issued consumer advice regarding fish consumption for women who might become pregnant, pregnant women, nursing mothers and young children [3]. In 2002, a study in Hong Kong [4] compared 157 infertile couples to 26 fertile couples, and observed a positive correlation of Hg levels with seafood consumption, and higher Hg levels among infertile couples than fertile couples. Also noted was the association of unexplained infertility diagnosis and abnormal semen analyses with higher levels of Hg. In a second study in 2008, among 619 women undergoing IVF [5], 18.7% of the women had blood Hg levels greater that the EPA safety reference (≥5.8 μg/L); however, no relationship with fertilization rates or pregnancy outcome was observed. Finally, a small cohort study [6-10] evaluated blood mercury concentrations in up to 50 female and 33 male IVF patients, depending on the covariate of interest, finding no association between female Hg levels and fertilization rates. Embryo fragmentation was noted to be significantly higher as Hg levels increased in the men.

Based on the limited study of the impact of mercury exposure on early reproductive measures and potential risks, as shown in limited epidemiologic studies [4-10], we evaluated hair mercury levels and gamete, embryo, and clinical outcomes in an assisted reproductive technologies (ART) population seeking treatment through In Vitro Fertilization (IVF) due to a history of inability to conceive. This unique study cohort allows evaluation at the very earliest stages of conception through birth and provides an opportunity to study the impact of mercury exposure at stages otherwise unobservable in humans.

2. Methods

2.1. Study Participants and data collection

The cohort for this study included female patients from the Massachusetts General Hospital (MGH) Fertility Center (Vincent Obstetrics and Gynecology Service, Division of Reproductive Medicine and IVF) undergoing IVF treatment using their own oocytes who enrolled in an ongoing prospective cohort study on environmental risk factors for reproductive health. All female patients over 18 years of age and less than 45 seeking infertility evaluation or treatment at the MGH Fertility Center were eligible to participate. Women were eligible to be included in this analysis if they had completed at least 1 fresh IVF cycle between December 2004 and May 2013 and provided a hair sample for the measurement of Hg (n=295 women with 429 corresponding fresh IVF cycles). Women were excluded from the analysis if they had missing hair mercury levels (n=4) or BMI (n=1). Cycles were excluded if hair mercury samples were taken >5 months after cycle initiation (n=84 women, 192 cycles), or hair mercury samples were taken >1 year before cycle initiation (n=1) leaving a total of 205 women and 229 cycles in our final analytic dataset. The study was approved by the Human Studies Institutional Review Boards of the MGH and Harvard School of Public Health (HSPH). Participants signed an informed consent after the study procedures were explained by a research nurse and all questions were answered.

Upon entry into the study, subjects completed a brief questionnaire that collected data on demographic characteristics, medical history and lifestyle. Subjects also completed two extensive take-home questionnaires with one relating to lifestyle factors, occupation, and medical history, and the second a validated food frequency questionnaire (FFQ) (introduced in April 2007) [11]. Total fish intake was assessed in a subset of the subjects (n=157) with the FFQ. Clinical information was abstracted from the patient's electronic medical record. Subsequent to an infertility evaluation, each patient was assigned infertility diagnoses by a physician at the MGH Fertility Center according to the Society for Assisted Reproductive Technology (SART) definitions as previously described [12].

2.2. Clinical Protocols and Endpoints

Analysis of blood hormone values for Day 3 FSH and peak estradiol as well as oocyte retrieval details have been previously described [12]. All study participants were treated per clinic protocols for gonadotropin-induced ovarian stimulation also described previously [12]. Briefly, one of three stimulating protocols was used for each subject: 1) Gonadotropin releasing hormone (GnRH)- antagonist protocol, 2) follicular phase GnRH-agonist/flare protocol, or 3) luteal phase GnRH agonist protocol using low, regular and high-dose leuprolide (Lupron). Selection of protocol for the subject is based on their expected response to the stimulating agents with the antagonist and flare protocols typically used for women with expected or demonstrated diminished ovarian response. The flare protocol is often reserved for the subgroup of low responder women above the age of 40, while the antagonist protocol to the low responder group, that is, under 40 years of age.

Retrieved oocytes were subjected to either conventional insemination or intracytoplasmic sperm injection (ICSI) using the male partner's sperm. Evaluation of oocyte maturation,

pronuclear development and embryo scoring are as described previously [13]. Briefly, oocytes were classified as germinal vesicle, metaphase I, metaphase II (MII) or degenerated. Fertilized oocytes were checked for fertilization and graded as either normally fertilized (two pronuclei, referred to as 2PN thereafter) or abnormally fertilized (one, three or more pronuclei). Fertilization rate was determined after 17-20 hours after insemination as the number of oocytes with two pronuclei divided by the number of MII oocytes inseminated. Embryos were evaluated for cell cleavage rates on day 2 and 3 of culture with a division from 2-4 and 6-8 cells, respectively, considered normal. Values below 2 cells (Day 2) and 6 cells (Day 3) were considered slow and greater than 4 cells (Day 2) and 8 cells (Day 3) were designated accelerated. Cleavage stage embryos were designated with a grade based on blastomere size and degree of cytoplasmic fragmentation, described as follows: Grade 1, blastomeres of equal size with no cytoplasmic fragmentation; Grade 2, blastomeres of equal size, with ≤ 20% cytoplasmic fragmentation; Grade 3, blastomeres of distinctly unequal size with ≤ 20% cytoplasmic fragmentation; Grade 4, blastomeres of equal or unequal size with significant fragmentation ranging from 20-50%; Grade 5, few blastomeres of any size with fragmentation exceeding 50% of entire embryo. An embryo grade of 1 or 2 was categorized as high quality, 3 of intermediate quality and grades of 4 or 5 were grouped as poor quality. For analysis we classified embryo grade as “best quality” if they had 4 cells on day 2, 8 cells on day 3, and a morphologic quality score of 1 or 2 on days 2 and 3. Clinical outcomes were evaluated per initiated cycle. We defined successful implantation as a serum β-hCG level > 6 mIU/mL typically measured 17 days (97% measured by day 17, range 15–20 days) after egg retrieval, clinical pregnancy as the presence of an intrauterine pregnancy confirmed by ultrasound, and live birth as the birth of a neonate on or after 24 weeks gestation.

2.3. Hair Collection and Mercury Analysis

Hair was chosen as the preferred biomarker for Hg levels in our subjects since it reflects exposure over several months [14]. The hair sample was collected at (or as close as possible to) the time of recruitment into the study. Hair samples were collected between June 2005 and March 2013. The whole hair sample was cleaned before analysis to remove extraneous contaminants by sonication for 15 minutes in a 1% Triton X-100 solution. After sonication, samples were rinsed with distilled deionized water and dried at 60°C for 48 hours. Total Hg in parts per million (ppm) was measured in the proximal 2 cm of hair (where 1 cm of length would represent 1 month of exposure) using a Direct Mercury Analyzer 80 (Milestone Inc, Monroe, CT) with a matrix matched calibration curve. Certified reference material GBW 07601 (human hair; Institute of Geophysical and Geochemical Exploration, China) containing 360 ppm mercury was used as the quality control standard. The limit of detection (LOD) for Hg was 0.5 ppm with the percentage recovery for quality control standards ranging from 90 to 110 percent.

2.4. Dietary assessment

Participants completed a previously validated 131-item FFQ at home [11]. They were asked to report how often, on average, they consumed specific foods during the previous year. The FFQ had nine categories for intake frequency options that ranged from never to six or more times per day. The nutrient content of each food and the specific portion size was calculated by the nutrient database from the US Department of Agriculture [15] with additional

information from manufacturers when necessary. Assessment of fish intake using this questionnaire has been validated against prospectively collected diet records representing 1 year of a diet in a different study [16]. In that study, the de-attenuated correlation of fish intake assessed with the FFQ and the 1 year average of prospectively collected dietary records was 0.66 [16]. Fish intake was defined as the sum of dark meat fish (e.g. canned tuna, salmon), white meat fish (e.g. cod, haddock), and shellfish (e.g. shrimp scallops).

2.5. Statistical analysis

Women were classified into quartiles based on hair Hg concentrations. Demographic characteristics of the study participants, as well as the distribution of outcome measures and potential covariates, were reported (median and interquartile range (IQR), as appropriate). We summarized the distribution of hair Hg exposures using the median and IQR, and used analysis of variance, chi-square test, and Fisher's exact test, to test for associations across quartiles. Among participants (n=62) with repeated hair Hg measurements (2 or 3) due to high concentrations, the Hg concentration from the first sample was used in all statistical analyses.

We used multivariate generalized linear mixed models with random intercepts to evaluate the association between hair mercury and ART outcomes. We used Poisson models to analyze the association between quartiles of Hg concentration with total number of oocytes retrieved (count) and binomial distribution and logit link function were specified for fertilization, embryo quality, and clinical outcomes. In addition, comparisons were made between those above vs. below the EPA reference of 1 ppm [17]. Tests for linear trend were conducted across quartiles of Hg using the median Hg concentration in each quartile as a continuous variable in the regression models. We also used Hg as a continuous variable to test for trend, but the results were similar to those using the median Hg concentration (results not shown). Outcomes of interest included oocyte maturation (total MII/total oocytes retrieved), fertilization (total 2PN/total MII), day 3 cleavage (fast vs. normal and no/slow vs normal), and embryo quality (poor vs not poor) (proportions). For example, for oocyte maturation, the outcome was total MII and the offset was total oocytes retrieved.

Confounding was evaluated using prior knowledge on biological relevance (such as age [18], BMI [19], and smoking [20]) or descriptive statistics from our study population through the use of directed acyclic graphs [21]. Covariates considered in this way in all models included: age (continuous), BMI (continuous), race (white vs. other), smoking status (ever smoker vs other), infertility diagnosis (male factor, female factor, and unexplained infertility), and treatment protocol type (Luteal phase agonist, flare, and GnRH antagonist). We calculated the Spearman correlation coefficient for hair Hg concentrations with total fish consumption per week. In a sensitivity analysis, clinical outcomes were evaluated per embryo transfer. We considered the possibility that previous IVF or IUI outcomes might influence Hg exposure, given the persistent nature of the latter. We conducted all statistical analyses using SAS version 9.2 (SAS Institute Inc., Cary, NC) and considered two-sided significance levels less than 0.05 as statistically significant.

3. Results

3.1. Demographics, hair mercury, and dietary characteristics

The study included 205 women with 229 completed IVF cycles (median age at egg retrieval=35 years; range 27 to 43 years). Excluded women did not differ significantly from those included in the analysis in terms of age, hair Hg, smoking, BMI and other characteristics but had lower percentage of previous infertility exam, previous IUI, and previous IVF; and a longer time interval between cycle initiation and hair mercury sampling. In addition, included women had higher percentage of day 5 embryo transfer (Supplemental Table 1). Most women were Caucasian (82%) and non-smoking (74%). Approximately a third of couples were categorized to 3 different primary SART diagnoses; female factor, male factor or unexplained infertility. In a subgroup of patients (n=157) the median fish consumption was 1.5 serving per week. The majority of cycles were low dose luteal phase stimulation (73%) with 11.2 oocytes retrieved on average per cycle. Of total oocytes retrieved 86% were considered mature prior to ICSI or at fertilization check after conventional insemination. Of mature oocytes, 69% fertilized normally. Fifty seven percent of cycles experienced an embryo transfer on day 3 of culture and 30% had a day 5 transfer with an overall successful implantation per retrieved cycle of 54%.

The median total hair mercury level was 0.62 ppm (IQR=0.35 - 1.24), ranging from 0.03 to 5.66 ppm (Table 1). Thirty-three percent of the women exceeded the hair Hg EPA reference level of 1ppm. Hair Hg was strongly correlated with total fish consumption per week (r=0.59, p=0.0001). Age and alcohol consumption were also positively associated with hair mercury concentrations (Table 1).

3.2. Association between Hg and IVF outcomes

Peak estradiol and the proportion of mature (MII) oocytes were not associated with hair Hg concentration (Table 2). However, there were suggestions of inverse associations of hair Hg levels with total oocyte yield and MII oocyte yield. After adjustment for age, race, BMI, smoking status, infertility diagnosis and protocol type, the (95% CI) mean total oocyte yield was 9.6 (8.5, 10.8) among women whose hair mercury concentration was above the EPA reference of 1ppm and 11.2 (10.4, 12.1) among women whose hair mercury concentration was below 1ppm (p=0.04) (Supplemental Table 2). The adjusted (95% CI) mean MII oocyte yields were 8.3 (7.3, 9.3) and 9.4 (8.7, 10.2) among women whose hair mercury concentration was above and below the EPA reference of 1ppm, respectively (p=0.08) (Supplemental Table 2). There was no association of hair Hg with fertilization rate or embryo cleavage (Tables 3 and 4). Hair Hg was, however, associated with lower % of best quality embryos. Hair mercury concentrations were not associated with clinical outcomes per initiated cycle (Table 5) or per transfer (Supplemental Table 3). Apart from total oocyte yield, none of the outcomes evaluated (peak E2 levels, MII yield, proportion of MII/total oocytes, fertilization rate, embryo quality measures) differed between women above compared to those below the EPA safety reference (results not shown). Hair Hg did not vary by those who had prior IUI/IVF vs no prior IUI/IVF and previous IUI or IVF did not modify the association between hair Hg and IVF outcomes (results not shown).

4. Discussion

We evaluated the potential impact of Hg exposure, assessed via hair Hg measurements, in a cohort of women undergoing infertility treatment with IVF in eastern Massachusetts. Hair Hg levels were considerably higher in this cohort compared to women of reproductive age in a nationally representative sample [22] (0.62 ppm vs. 0.19 ppm) and one third of women in this cohort had hair Hg concentrations that exceeded the EPA reference of 1ppm. The wide range of Hg exposure in this population, coupled with a design focused on studying women undergoing IVF, allowed us to investigate how Hg may impact very early measures of reproductive success that cannot be observed among couples trying to conceive naturally. Nevertheless, we found no evidence that Hg has an impact, detrimental or beneficial, on measures of ovarian response to gonadotropins, fertilization, early embryonic development or the probabilities of achieving a pregnancy or having a live birth following infertility treatment with IVF.

While environmental exposure to methylmercury is widespread, evaluation of its impact on reproductive outcomes has been limited. There is some information on the impact of Hg exposure on early pregnancy outcomes in the accidental mass population exposures that occurred in Minamata, Japan [23] in 1956 and Iraq in 1971 [24]. Very few epidemiological studies have evaluated the association between Hg exposure and reproductive health outcomes, in part due to the difficulty in assessing early reproductive outcomes in naturally conceiving women. Some have studied these outcomes by comparing Hg levels in infertile and fertile couples. One of the earliest studies to evaluate the influence of Hg exposure included 155 infertile females, 150 infertile males and a fertile control group of 26 couples in China [4]. In addition to blood Hg concentrations, a FFQ was conducted to determine dietary intake, with emphasis on fish and shellfish. The infertile group had significantly higher blood Hg levels than the fertile controls and the levels correlated with the quantity of seafood consumed ($r=0.21$, $P < 0.001$).

A second study in a Saudi Arabian IVF cohort detected Hg in 79% of follicular fluid samples collected during egg retrieval and 88% of blood samples collected from 619 women, with a median blood Hg level of 3.19 μg/L. Within this cohort, 19% had elevated (≥ 5.8 μg/L) blood Hg levels [5] compared to 33% elevated (>1 ppm) hair Hg in our study population. Analysis of variables in the questionnaire from the Saudi Arabian study found that 88% of women reported regular consumption of seafood, possibly an indication that fish was a main source of Hg in this cohort. Elevated blood Hg levels were also noted in 14% of women reporting use of skin-lightening creams (commonly containing inorganic Hg). As in our study, the authors found no correlation between Hg levels (in both blood and follicular fluid) and fertilization rate or pregnancy rate. Another small cohort of 25 women and 15 men has been studied extensively in San Francisco, CA [6-10, 25]. This cohort consisted of a higher proportion of women of Asian ethnicity (30%) [9] as compared to the US general population (4.8%) and our study (10%). Similarly, seafood consumption was positively associated with Hg levels within the subjects, with 84% reporting consumption of at least one seafood item in a week and the strongest association being found with “mollusks” consumption [26]. Bloom et al. [6] found detectable levels of Hg in follicular fluid, however at lower levels than in blood collected from these women. Follicular fluid Hg levels were

higher for Asian women (0.92 μg/L) than other ethnic groups of their cohort (non-Asian = 0.79 μg/L). Blood Hg levels were positively associated with the number of mature oocytes for use with ICSI [8] whereas follicular fluid Hg levels were positively associated with normally fertilized oocytes [6]. In this same cohort, while blood Hg was not associated with pregnancy rates, when other heavy metals (lead and cadmium) were taken into account in the model, blood Hg was associated with reduced pregnancy rates [25]. Another small cohort in the United Kingdom of 30 women undergoing IVF reported a negative correlation between hair Hg and oocyte yield and follicle number. However, there was no correlation noted with other measures such as fertilization, cleavage rate or average embryo quality [27]. While the San Francisco and UK cohorts found a relationship between Hg and oocyte yield, these studies may have been limited due to sample size. The biomarkers of exposure used in both studies were also different (blood verses hair). Blood and hair Hg have been shown to be well correlated, offering valid measures of exposures with blood levels demonstrating more immediate exposure and hair levels offering an averaged exposure over time [28].

One criticism of these studies and ours is that women undergoing IVF may not be representative of the general population. Findings from occupational studies in naturally conceiving women have also found associations between elemental Hg exposure and adverse reproductive health outcomes. A study in dental professionals found an association between hair Hg and menstrual cycle disorders [29]. Two additional studies in dental professionals demonstrated delayed time to pregnancy in the higher exposure groups [30, 31] and a linear increase in prevalence of adverse pregnancy outcomes was observed in women occupationally exposed (industrial lamp factory) to Hg vapors [32]. In a recent prospective cohort study, higher maternal blood Hg was associated with increased time to pregnancy in couples trying to conceive naturally at environmentally relevant exposure levels of Hg (highest quartile of total mercury ≥ 1.2 μg/L) [33].

While we found limited associations with reproductive health outcomes in our study population we cannot exclude the possibility that different results would be demonstrated in a population with much higher Hg levels, for instance as found in populations with high consumption of fish such as in the Faroes Islands [34] and Seychelles [35]. We are also not able to evaluate the influence of an acute exposure during the actual IVF stimulation cycle. Furthermore, hair Hg concentration was strongly correlated with total fish consumption per week, an important proxy for Omega-3 fatty acid intake [4, 26, 36, 37]. A recent study in the mouse model demonstrated that a diet rich in Omega-3 fatty acids resulted in improved reproductive outcomes in older female mice (>10 months) and increased oocyte quality as measured by percent reaching the mature metaphase II stage and by mitochondrial distribution throughout the oocyte [38]. It is therefore possible that hair Hg concentration is acting as a biomarker of fish intake for the individuals studied and the dietary benefits from consuming seafood outweigh any detrimental effects of mercury at the levels detected in our study subjects. Our analysis is also limited because it does not include the male component of the couple or the male/female interactions that may exist related to Hg exposure and outcomes.

In conclusion, we found very limited evidence of associations of hair mercury with adverse reproductive outcomes among women undergoing IVF. In our study population, fish consumption was the primary source of Hg exposure. Therefore, disentangling the beneficial reproductive health effects of fish intake from any potential adverse effects of mercury is difficult given the strong correlation between hair Hg and fish consumption in our study population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Hair Hg among 205 women undergoing in vitro fertilization was evaluated.
- One-third of women had hair Hg that exceeded the EPA reference of 1ppm.
- There was no evidence that hair Hg levels impacted early reproductive outcomes.

Table 1

Demographic and dietary characteristics of 205 women in the EARTH Study by quartile of hair mercury.

	Quartile of hair mercury concentration				p[a]
Quartile (Range, ppm)	Q1 (0.03 - 0.34)	Q2 (0.35 - 0.61)	Q3 (0.62 - 1.24)	Q4 (1.27 - 5.66)	
N	51	51	52	51	
	Median (IQR) or N (%)				
Personal Characteristics					
Age, years	35.0 (34.0, 39.0)	35.0 (31.0, 39.0)	34.5 (32.0, 37.5)	36.0 (33.0, 39.0)	0.08
White/Caucasian, N (%)	40 (78.4)	40 (78.4)	43 (82.7)	44 (86.3)	0.77
Body Mass Index, kg/m^2	24.3 (21.6, 29.2)	23.0 (21.0, 25.8)	22.7 (21.1, 24.9)	23.0 (20.5, 25.2)	0.15
Ever smoker, N (%)	7 (13.7)	11 (21.6)	21 (40.4)	15 (29.4)	0.12
Time enrolled from start of study months	58.5 (36.7, 77.7)	62.3 (41.2, 73.4)	54.8 (36.6, 71.4)	47.4 (18.2, 74.7)	0.29
Baseline Reproductive Characteristics					
Initial infertility diagnosis, %					0.89
Male factor	17 (33.3)	22 (43.1)	21 (40.4)	20 (39.2)	
Female factor					
Endometriosis	3 (5.9)	2 (3.9)	2 (3.9)	4 (7.8)	
Tubal factor	6 (11.8)	3 (5.9)	4 (7.7)	2 (3.9)	
Diminished ovarian reserve	5 (9.8)	4 (7.8)	4 (7.7)	3 (5.9)	
Ovulation disorders	2 (3.9)	4 (7.8)	4 (7.7)	6 (11.8)	
Uterine disorders	0 (0.0)	0 (0.0)	0 (0.0)	2 (3.9)	
Unexplained	18 (35.3)	16 (31.4)	17 (32.7)	14 (27.5)	
Initial treatment protocol, %					0.94
Antagonist	8 (15.7)	5 (9.8)	6 (11.5)	5 (9.8)	
Flare[b]	8 (15.7)	8 (15.7)	8 (15.4)	8 (15.7)	
Luteal phase agonist[c]	35 (68.6)	38 (74.5)	38 (73.1)	38 (74.5)	
Day 3 FSH, IU/L (n=181)	6.5 (5.5, 7.7)	6.8 (6.2, 8.1)	7.4 (5.6, 9.6)	6.3 (5.2, 8.5)	0.17
Previous infertility exam, n%	44 (86.3)	44 (86.3)	47 (90.4)	49 (98.0)	0.16
Previous IUI, n%	26 (51.0)	21 (41.2)	33 (63.5)	31 (60.8)	0.09
Previous IVF, n%	19 (37.3)	13 (25.5)	17 (32.7)	17 (33.3)	0.64
Embryo Transfer Day, n (%)*					0.14
No embryos transferred	3 (5.9)	3 (5.9)	8 (15.4)	9 (17.7)	
Day 2	5 (9.8)	1 (2.0)	1 (1.9)	1 (2.0)	
Day 3	26 (51.0)	31 (60.8)	31 (59.6)	25 (49.0)	
Day 5	17 (33.3)	16 (31.4)	12 (23.1)	16 (31.4)	
Number of Embryos Transferred, n (%)					0.27
No embryos transferred	3 (5.9)	3 (5.9)	8 (15.4)	9 (17.7)	
1 embryo	10 (19.6)	5 (9.8)	5 (9.6)	3 (5.9)	
2 embryos	27 (52.9)	31 (60.8)	27 (50.0)	30 (64.8)	
3+ embryos	11 (21.6)	12 (23.5)	12 (23.1)	8 (15.7)	
Dietary Characteristics					

	Quartile of hair mercury concentration				p[a]
(n=157)	38	40	44	32	
Total energy intake, kcal/day	1761 (1514, 2213)	1631 (1357, 2377)	1845 (1286, 2360)	1856 (1504, 2146)	0.97
Caffeine intake, mg/day	64.2 (11.5, 195.4)	96.1 (51.1, 184.9)	111.9 (32.7, 252.9)	158.8 (89.7, 217.3)	0.11
Alcohol intake, g/day	1.9 (0.6, 9.9)	3.4 (1.5, 12.2)	10.2 (3.6, 17.6)	14.2 (7.4, 18.8)	0.0002
Saturated fat, % energy	9.9 (8.4, 11.3)	10.2 (8.8, 12.0)	10.6 (9.2, 12.5)	10.9 (9.7, 12.5)	0.49
Mono unsaturated fat, % energy	11.8 (9.8, 14.4)	11.9 (10.5, 13.7)	12.5 (10.9, 14.8)	12.3 (11.3, 14.0)	0.36
Polyunsaturated fat, % energy	5.9 (4.9, 7.6)	6.2 (5.2, 7.0)	6.3 (5.4, 7.0)	6.5 (5.6, 7.3)	0.59
Trans fat, % energy	1.0 (0.8, 1.2)	0.9 (0.7, 1.1)	1.0 (0.7, 1.1)	1.0 (0.9, 1.2)	0.66
Fish intake, servings/wk	0.7 (0.0, 1.1)	1.4 (1.1, 1.8)	2.2 (1.3, 2.7)	1.8 (1.7, 2.9)	<0.0001
Dark meat fish, servings/wk	0.3 (0.0, 0.6)	0.6 (0.3, 0.7)	1.1 (0.6, 1.1)	1.0 (0.7, 1.1)	<0.0001
White meat fish, servings/wk	0.1 (0.0, 0.4)	0.4 (0.1, 0.7)	0.6 (0.3, 0.7)	0.6 (0.6, 1.0)	<0.0001
Shellfish, servings/wk	0.1 (0.0, 0.4)	0.6 (0.1, 0.6)	0.6 (0.1, 1.0)	0.6 (0.1, 0.6)	<0.0001
Omega 3 intake from foods, g/day	0.1 (0.0, 0.1)	0.2 (0.1, 0.2)	0.3 (0.2, 0.4)	0.3 (0.2, 0.4)	<0.0001

[a] From Kruskal-Wallis test for continuous variables and chi-squared tests and fisher exact tests (when one or more cell counts were ≤ 5) for categorical variables.

[b] Follicular phase GnRH-agonist/Flare protocol.

[c] Luteal phase GnRH-agonist protocol.

Table 2

Association between hair mercury and controlled ovarian hyper-stimulation outcomes in 205 women (214 fresh cycles) from the EARTH Study [a].

		Mean (95% CI)		
	N cycles	Crude model[b]	Adjusted model excluding protocol and diagnosis[c]	Adjusted model[d]
Hair mercury [range, ppm]			Peak E2 level, ng/dL	
Q1 [0.03 - 0.34]	55	2134 (1909, 2361)	2107 (1885, 2330)	2115 (1894, 2336)
Q2 [0.35 - 0.61]	51	2129 (1902, 2365)	2094 (1869, 2320)	2076 (1853, 2300)
Q3 [0.62 - 1.24]	52	2053 (1828, 2298)	2101 (1870, 2332)	2107 (1879, 2335)
Q4 [1.27 - 5.66]	54	1940 (1708, 2172)	1943 (1717, 2170)	1929 (1705, 2153)
P-trend[e]		0.18	0.25	0.21
Hair mercury [range, ppm]			Oocyte total yield, n	
Q1 [0.03 - 0.34]	56	11.2 (9.8, 12.8)	10.9 (9.5, 12.6)	10.8 (9.5, 12.3)
Q2 [0.35 - 0.61]	51	11.8 (10.3, 13.6)	11.7 (10.2, 13.5)	11.5 (10.1, 13.1)
Q3 [0.62 - 1.24]	53	10.7 (9.3, 12.2)	10.9 (9.4, 12.6)	10.9 (9.6, 12.5)
Q4 [1.27 - 5.66]	54	9.7 (8.4, 11.3)	9.8 (8.5, 11.3)	9.5 (8.3, 10.9)
P-trend		0.07	0.13	0.07
Hair mercury [range, ppm]			MII oocyte yield, n	
Q1 [0.03 - 0.34]	56	9.2 (8.0, 10.5)	9.0 (7.8, 10.3)	8.9 (7.8, 10.2)
Q2 [0.35 - 0.61]	51	10.1 (8.8, 11.6)	10.0 (8.7, 11.5)	9.9 (8.6, 11.2)
Q3 [0.62 - 1.24]	53	9.1 (7.9, 10.4)	9.2 (8.0, 10.6)	9.2 (8.1, 10.6)
Q4 [1.27 - 5.66]	54	8.4 (7.2, 9.6)	8.4 (7.3, 9.7)	8.2 (7.1, 9.4)
P-trend		0.13	0.21	0.15
Hair mercury [range, ppm]			MII/Total oocytes	
Q1 [0.03 - 0.34]	56	0.83 (0.78, 0.87)	0.83 (0.78, 0.88)	0.84 (0.79, 0.88)
Q2 [0.35 - 0.61]	51	0.86 (0.82, 0.90)	0.86 (0.82, 0.90)	0.87 (0.83, 0.90)
Q3 [0.62 - 1.24]	53	0.85 (0.81, 0.89)	0.86 (0.81, 0.89)	0.86 (0.81, 0.90)
Q4 [1.27 - 5.66]	54	0.87 (0.83, 0.91)	0.87 (0.83, 0.91)	0.88 (0.84, 0.92)
P-trend		0.29	0.31	0.26

Abbreviations: CI, confidence interval; E2, estradiol; IVF, *in vitro* fertilization; MII, mature oocytes.

[a] All analyses were run using generalized linear mixed models with random intercepts, linear (for peak E2) or Poisson distribution (for oocyte counts), identity (for peak E2) or log (for oocyte counts) link function, and compound symmetry correlation structure.

[b] Data are presented as predicted marginal means adjusted for age.

[c] Data are presented as predicted marginal means adjusted for age, BMI, race, and smoking.

[d] Data are presented as predicted marginal means adjusted for age, BMI, race, smoking, infertility diagnosis, and protocol type.

[e] Test for trend were performed using the median level of hair mercury in each quartile as a continuous variable in the model.

Table 3

Association between hair mercury and fertilization rates stratified by insemination and ICSI among 205 women (213 fresh IVF cycles) from the Environment and Reproductive Health Study.[a] (89 women with 91 IVF cycles; 110 women with 121 ICSI cycles)

		Mean (95% CI)		
	N cycles	Crude model[b]	Adjusted model excluding protocol and diagnosis[c]	Adjusted model[d]
Hair mercury [range, ppm]			Fertilization Rate, all cycles	
Q1 [0.03 - 0.34]	56	0.70 (0.62, 0.76)	0.71 (0.64, 0.78)	0.72 (0.64, 0.78)
Q2 [0.35 - 0.61]	51	0.67 (0.59, 0.74)	0.67 (0.60, 0.74)	0.67 (0.60, 0.74)
Q3 [0.62 - 1.24]	53	0.74 (0.67, 0.80)	0.73 (0.66, 0.80)	0.74 (0.66, 0.80)
Q4 [1.27 - 5.66]	54	0.69 (0.61, 0.75)	0.69 (0.61, 0.75)	0.69 (0.61, 0.76)
P-trend[e]		0.98	0.75	0.71
Hair mercury [range, ppm]			Fertilization Rate, IVF cycles	
Q1 [0.03 - 0.34]	25	0.66 (0.47, 0.80)	0.68 (0.49, 0.82)	0.67 (0.41, 0.86)
Q2 [0.35 - 0.61]	17	0.71 (0.49, 0.87)	0.71 (0.49, 0.86)	0.70 (0.39, 0.89)
Q3 [0.62 - 1.24]	23	0.71 (0.52, 0.85)	0.70 (0.50, 0.84)	0.72 (0.45, 0.89)
Q4 [1.27 - 5.66]	27	0.65 (0.47, 0.80)	0.66 (0.48, 0.81)	0.67 (0.42, 0.85)
P-trend		0.76	0.72	0.86
Hair mercury [range, ppm]			Fertilization Rate, ICSI cycles	
Q1 [0.03 - 0.34]	31	0.73 (0.64, 0.81)	0.74 (0.64, 0.81)	0.74 (0.64, 0.82)
Q2 [0.35 - 0.61]	34	0.64 (0.56, 0.72)	0.64 (0.56, 0.72)	0.64 (0.55, 0.73)
Q3 [0.62 - 1.24]	29	0.78 (0.70, 0.85)	0.78 (0.70, 0.85)	0.78 (0.69, 0.85)
Q4 [1.27 - 5.66]	27	0.71 (0.61, 0.79)	0.71 (0.60, 0.79)	0.71 (0.60, 0.80)
P-trend		0.77	0.94	0.88

Abbreviations: ICSI, intra-cytoplasmic sperm injection; IVF, in vitro fertilization.

[a] All analyses were run using generalized linear mixed models with random intercepts, binomial distribution, logit link function

[b] Data are presented as predicted marginal means adjusted for age.

[c] Data are presented as predicted marginal means adjusted for age, BMI, race, and smoking

[d] Data are presented as predicted marginal means adjusted for age, BMI, race, smoking, infertility diagnosis, and protocol type.

[e] Test for trend were performed using the median level of hair mercury in each quartile as a continuous variable in the model.

Table 4

Association between hair mercury and embryo quality among 179 women with 192 cycles from the Environment and Reproductive Health Study.[a]

	Mean Percentage (95% CI)		
	Crude model[b]	**Adjusted model excluding protocol and diagnosis[c]**	**Adjusted model[d]**
Hair mercury [range, ppm]		Poor quality embryos %	
Q1 [0.03 - 0.34]	17 (11, 25)	16 (11, 24)	16 (11, 24)
Q2 [0.35 - 0.6l]	19 (13, 27)	19 (13, 27)	19 (13, 27)
Q3 [0.62 - 1.24]	16 (10, 23)	15 (10, 23)	15 (10, 23)
Q4 [l.27 - 5.66]	16 (11, 24)	16 (11, 24)	17 (11, 25)
P-trend[e]	0.75	0.75	0.85
Hair mercury [range, ppm]		Accelerated Cleavage, %	
Q1 [0.03 - 0.34]	5.9 (3.3, 10.2)	4.6 (2.5, 8.3)	4.3 (2.3, 8.1)
Q2 [0.35 - 0.61]	6.8 (4.0, 11.3)	5.5 (3.2, 9.4)	5.1 (2.9, 9.0)
Q3 [0.62 - 1.24]	6.7 (3.9, 11.3)	6.8 (4, 11.4)	6.3 (3.6, 10.8)
Q4 [1.27 - 5.66]	4.1 (2.1, 8.0)	4.3 (2.2, 8.3)	3.8 (1.8, 7.5)
P-trend	0.27	0.71	0.58
Hair mercury [range, ppm]		Slow embryo cleavage %	
Q1 [0.03 - 0.34]	22 (15, 31)	22 (15, 31)	22 (15, 31)
Q2 [0.35 - 0.61]	29 (21, 39)	31 (23, 40)	31 (23, 41)
Q3 [0.62 - 1.24]	31 (23, 42)	30 (22, 40)	30 (22, 41)
Q4 [1.27 - 5.66]	22 (15, 31)	21 (14, 29)	21 (14, 30)
P-trend	0.48	0.30	0.36
Hair mercury [range, ppm]		% With ≥ 1 Best Quality Embryo on Day 2 & 3	
Q1 [0.03 - 0.34]	80 (64, 90)	80 (64, 90)	80 (63, 90)
Q2 [0.35 - 0.61]	63 (47, 77)	62 (45, 76)	62 (45, 77)
Q3 [0.62 - 1.24]	56 (39, 71)*	56 (39, 72)*	57 (39, 72)*
Q4 [1.27 - 5.66]	64 (48, 78)	65 (48, 79)	65 (47, 79)
P-trend	0.34	0.40	0.44

[a] All analyses were run using generalized linear mixed models with random intercepts, binomial distribution, logit link function

[b] Data are presented as predicted marginal means adjusted for age.

[c] Data are presented as predicted marginal means adjusted for age, BMI, race, and smoking

[d] Data are presented as predicted marginal means adjusted for age, BMI, race, smoking, infertility diagnosis, and protocol type.

[e] Test for trend were performed using the median level of hair mercury in each quartile as a continuous variable in the model.

* Indicates a p-value < 0.05 comparing that quartile vs. first quartile.

Table 5

Association between hair mercury and clinical outcomes per initiated cycle in 205 women (229 cycles) from the EARTH Study[a] (157 women and 170 cycles with FFQ)

	Adjusted Mean Proportion (95% Confidence Interval)		
	Crude model[b]	**Adjusted model excluding protocol and diagnosis[c]**	**Adjusted model[d]**
Hair mercury [range, ppm]		Proportion with Successful Implantation (y/n)	
Q1 [0.03 - 0.34]	0.50 (0.35, 0.64)	0.49 (0.34, 0.64)	0.50 (0.35, 0.65)
Q2 [0.35 - 0.61]	0.55 (0.40, 0.69)	0.55 (0.40, 0.69)	0.54 (0.39, 0.69)
Q3 [0.62 - 1.24]	0.52 (0.38, 0.66)	0.54 (0.39, 0.68)	0.54 (0.39, 0.69)
Q4 [1.27 - 5.66]	0.61 (0.46, 0.73)	0.60 (0.45, 0.73)	0.59 (0.44, 0.73)
P-trend[e]	0.30	0.32	0.41
Hair mercury [range, ppm]		Proportion with Clinical Pregnancy	
Q1 [0.03 - 0.34]	0.46 (0.32, 0.60)	0.45 (0.31, 0.61)	0.46 (0.31, 0.62)
Q2 [0.35 - 0.61]	0.50 (0.35, 0.64)	0.50 (0.35, 0.64)	0.49 (0.34, 0.64)
Q3 [0.62 - 1.24]	0.45 (0.31, 0.60)	0.46 (0.32, 0.61)	0.46 (0.32, 0.61)
Q4 [1.27 - 5.66]	0.55 (0.41, 0.69)	0.54 (0.39, 0.68)	0.54 (0.39, 0.68)
P-trend	0.36	0.42	0.48
Hair mercury [range, ppm]		Proportion with Live Birth	
Q1 [0.03 - 0.34]	0.34 (0.22, 0.49)	0.35 (0.22, 0.50)	0.34 (0.21, 0.50)
Q2 [0.35 - 0.61]	0.38 (0.25, 0.52)	0.37 (0.24, 0.53)	0.36 (0.23, 0.52)
Q3 [0.62 - 1.24]	0.39 (0.26, 0.53)	0.39 (0.26, 0.54)	0.38 (0.25, 0.54)
Q4 [1.27 - 5.66]	0.42 (0.29, 0.57)	0.41 (0.27, 0.55)	0.40 (0.26, 0.55)
P-trend	0.44	0.59	0.61

[a] All analyses were run using generalized linear mixed models with random intercepts, binomial distribution, logit link function

[b] Data are presented as predicted marginal means adjusted for age.

[c] Data are presented as predicted marginal means adjusted for age, BMI, race, and smoking, infertility diagnosis, protocol type, fish and total calorie intake.

[d] Data are presented as predicted marginal means adjusted for age, BMI, race, smoking, infertility diagnosis, and protocol type.

[e] Test for trend were performed using the median level of hair mercury in each quartile as a continuous variable in the model.