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# Toxic metals in seminal plasma and in vitro fertilization (IVF) outcomes

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ABSTRACT

We measured toxic metals in seminal plasma collected from 30 men using vitro fertilization (IVF), to evaluate associations with semen quality and IVF outcomes. A doubling in Hg-adjusted Pb concentration was associated with 47% lower total motile sperm. Positive associations were suggested for Hg with pregnancy and live birth, adjusted for Cd or Pb. A negative association was suggested for Hg-adjusted Cd with pregnancy. These data add to evidence indicating that toxic metals impact IVF.



## 1. Introduction

Exposure to toxic metals, including mercury (Hg), cadmium (Cd), and lead (Pb), is widespread and may lead to male reproductive toxicity. Adverse reproductive outcomes have been established at high doses, such as those experienced in the workplace (Sheiner et al., 2003), but conflicting results have been reported for the low levels of exposure most individuals receive from 'background' environmental sources (CDC, 2013). Investigator have detected these toxic metals in human seminal plasma (SP), corroborating the potential for reproductive toxicity; however, the results of studies assessing associations with semen quality and reproductive outcomes have been inconsistent to date (Benoff et al., 2003, 2009; Mendiola et al., 2011; Telisman et al., 2000; Wu et al., 2008). Though exposure levels were generally low, we previously reported negative associations for male blood Hg and Pb with embryo quality parameters among couples undergoing in vitro fertilization (IVF), indicative of biologically relevant doses (Bloom et al., 2011). Our previous work also suggests inverse associations between male urine Cd and oocyte fertilization (Bloom et al., 2010) and pregnancy (Bloom et al., 2012). Given that few data are available to characterize male reproductive risks posed by low level exposures, and to expand upon our prior studies, we conducted a pilot analysis of associations between Hg, Cd, and Pb measured in SP, and semen quality parameters and IVF outcomes.

## 2. Methods

Sample selection and clinical protocols were previously described in detail (Bloom et al., 2010, 2011, 2012). Briefly, 58 female patients undergoing IVF treatment at the University of California at San Francisco (UCSF) and 36 male partners were recruited to the Study of Metals and Assisted Reproductive Technologies (SMART) between March 12th, 2007 and April 29th, 2008. Participants provided biologic specimens and completed a dietary and health behaviors questionnaire. Thirty male partners agreed to SP analysis, comprising the sample for the current study. On the day of oocyte retrieval, fresh semen was collected by masturbation into a specimen cup without lubricant and centrifuged through isolate according to clinical protocol. The SP layer was aliquoted into two 1.8 mL cryovials for analysis (this is usually discarded). Men were advised to abstain from ejaculating for two to five days prior to semen collection. Semen quality parameters were measured using sperm counting chambers and a standard microscope at 400 × (Nikon, Inc. Melville, NY). We calculated total motile sperm count (TMC) as semen volume (mL) × sperm concentration (million/mL) × the proportion of motile sperm (%). Collected oocytes were fertilized using fresh sperm by conventional insemination or by intracytoplasmic sperm injection (ICSI).

Outcomes were assessed in the context of usual clinical IVF protocols. Oocyte fertilization was identified by the appearance of two pronuclei approximately 16–18 h after insemination. The embryo fragmentation score (EFS), a negative predictor of implantation, was examined 48 h post-fertilization, and embryo cell

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number (ECN), a positive predictor of implantation, was assessed on the day of transfer (48–72 h post-fertilization). Implantation was assessed by quantitative serum β-hCG ELISA 14 days after embryo transfer, followed by a second confirmatory test 2–3 days later if positive. After two weeks, pregnancy was confirmed by ultrasound visualization of one or more gestational sacs. Live birth was captured by contacting obstetrical offices nine months later. Informed consent was obtained prior to enrollment and the study protocol was approved by the UCSF Committee on Human Research.

We analyzed SP for Hg, Cd and Pb using a modification of a method recently developed and validated for the analysis of trace elements in follicular fluid (Kruger et al., 2012). Specimens (diluted 1+99) were analyzed in duplicate using an Element 2 sector field inductively coupled plasma-mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). The limits of detection (LODs) were 0.1 μg/L for Hg, 0.1 μg/L for Cd, and 0.23 μg/L for Pb.

We used multivariable linear regression to evaluate associations between SP metals and semen quality parameters adjusted for confounding variables, following a log transformation. We identified age and cigarette smoking as confounding variables, defined as predictors of both exposures and outcomes, using directed acyclic graphs (Greenland et al., 1999). Influential observations were excluded when DFBeta $\geq |1.96|$. We used Poisson regression with robust variance estimation by generalized estimating equations (Zou, 2004), to assess associations between SP metals and IVF endpoints including oocyte fertilization, 'good' vs. 'bad' embryo (i.e., ECN=6–8 and EFS=1–2 vs. 'other' among day three transfers), implantation, pregnancy, and live birth. We estimated relative risks (RR) and their 95% confidence intervals (95% CI) by exponentiating regression coefficients. In 'single-metal models,' an individual SP metal was entered as the predictor of interest and adjusted for age and cigarette smoking (SP Cd only). In 'Hg+Cd models,' SP Hg and SP Cd were entered as the predictors of interest and adjusted for age and cigarette smoking. 'Hg+Pb models' were defined as SP Hg and SP Pb adjusted for age. We were unable to include SP Cd and SP Pb in the same model due to collinearity. We defined statistical significance as $P<0.05$ for a two-tailed test, and used SAS v.9.3 (SAS Institute, Cary, NC) for statistical analysis.

## 3. Results

The median age of study participants was 37 years (range 32–45), and most were white (90%) and never-smokers (82.8%). None reported occupational exposure to toxic metals. Approximately 13% of men had male factor infertility as the primary diagnosis, whereas 33.3% was unexplained and 45.3% was attributed to female factor infertility. The median (25th %tile, 75th %tile) total sperm concentration was 56 million/mL (25, 106) and 49.5 million (31, 64) for TMC. On average, 66.7% of cases used ICSI and 66.0% of collected oocytes were fertilized successfully. Approximately 6.4 embryos were obtained from couples with 72.4% transferred on day three. The mean ECN and EFS were 5.8 and 2.2, respectively. Of 28 couples with embryos transferred, 43% had an implantation, 39% achieved pregnancy, and 36% succeeded in live birth.

The median (25th %tile, 75th %tile) concentration of SP Hg (0.12 μg/L; <0.1, 0.26) was lower than for our previously reported blood Hg (3.35 μg/L; 1.95, 5.12). Similarly, SP Pb (0.66 μg/L; <0.23, 1.51) was substantially lower than our prior report for blood Pb (1.26 μg/dL; 1.04, 1.68). On the other hand, SP Cd was higher (0.25 μg/L; 0.20, 0.34) than earlier described blood Cd (<0.20 μg/L; <0.20, 0.28) and urine Cd (0.18 μg/L; 0.06, 0.31). Approximately 53% of SP Hg and 67% SP Pb exceeded LODs, whereas 100% exceeded the LOD for SP Cd. We detected strong correlations between SP Hg and blood Hg ($r=0.87$; $P<0.0001$), and between SP Cd and SP Pb ($r=0.81$; $P<0.0001$), although none for the other metals.

The results of the multivariable linear regression analysis of SP metals on semen quality parameters are presented in Table 1. We detected a significant association between SP Pb and TMC ($\beta=-0.89$; 95% CI −1.74, −0.05). In addition, adjusted for Hg, Pb was negatively associated with TMC ($\beta=-0.92$; 95% CI −1.79, −0.05), indicative of a 47% lower TMC per doubling in SP Pb concentration. Adjusted for covariates, Cd was positively associated with sperm concentration ($\beta=5.49$; 95% CI −0.53, 11.51), although not in a significant fashion.

Table 2 provides the results of the multivariable Poisson regression of SP metals on IVF outcomes. Effect estimates were positive for SP Hg and good embryo, implantation, and pregnancy, although not significant. We detected a positive association between Hg and live birth (RR=13.18; 95% CI 1.13, 153.21), although highly imprecise, and not significant after adjustment for Cd or Pb. Effect estimates were strong and negative for Cd with implantation and pregnancy; however, these were also imprecise and not of significance.

## 4. Discussion

In the current study, a doubling in the level of SP Pb was associated with a 47% lower TMC, whereas no significant association was indicated for total sperm concentration. Previous studies reported negative correlations between SP Pb and progressively motile sperm count (Telisman et al., 2000) and sperm motility (Benoff et al., 2003), and a positive association with the percent immotile sperm (Mendiola et al., 2011). Developing sperm are thought to be protected by the blood–testis-barrier (BTB), unless compromised by high-dose exposure to toxicants (Kusakabe et al., 2008). However, SP Pb concentrations were low in our study compared to prior reports (Benoff et al., 2003; Mendiola et al., 2011; Telisman et al., 2000). Still, experimental results suggested Pb accumulation in the epididymis (Marchlewicz et al., 1993), a male accessory organ which is not protected by the BTB, and

**Table 1**
Linear regression models of semen quality parameters on seminal plasma (SP) mercury (Hg), cadmium (Cd), and lead (Pb) measured in male partners of IVF patients; the Study of Metals and Assisted Reproductive Technology (SMART).

| Semen quality parameters[a] | Metals (μg/L) | Single metal model | | | | Hg+Cd model | | | | Hg+Pb model | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $n$ | $\beta$[b] | 95% CI | | $n$ | $\beta$[c] | 95% CI | | $n$ | $\beta$[d] | 95% CI | |
| Total sperm concentration (millions/mL) | Hg | 29 | 0.74 | −1.94 | 3.41 | 27[e] | 1.60 | −1.42 | 4.62 | 28[f] | 1.11 | −1.34 | 3.56 |
| | Cd | 27[e] | 4.91 | −1.03 | 10.84 | – | 5.49 | −0.53 | 11.51 | – | – | – | – |
| | Pb | 28[f] | 0.55 | −0.28 | 1.37 | – | – | – | – | – | 0.61 | −0.23 | 1.46 |
| TMC | Hg | 30 | −0.18 | −3.47 | 3.11 | 29 | 0.63 | −2.95 | 4.21 | 30 | −0.64 | −3.77 | 2.48 |
| (millions) | Cd | 29 | −2.08 | −6.25 | 2.09 | – | −1.95 | −6.27 | 2.36 | – | – | – | – |
| | Pb | 30 | −0.89 | −1.74 | −0.05 | – | – | – | – | – | −0.92 | −1.79 | −0.05 |

CI, confidence interval.

[a] Log-transformed sperm concentration and total motile sperm count (TMC).
[b] Regression coefficient for a one unit increase in log-transformed SP metal concentration, adjusted for age (Hg, Pb) and cigarette smoking (Cd).
[c] Regression coefficient for a one unit increase in log-transformed SP metal concentration, adjusted for age, cigarette smoking, and another metal.
[d] Regression coefficient for a one unit increase in log-transformed SP metal concentration, adjusted for age and another metal.
[e] After excluding $n=1$ influential observation and $n=1$ missing cigarette smoking.
[f] After excluding $n=1$ influential observation.