Reproductive Toxicology 77 (2018) 43–52

Contents lists available at ScienceDirect

Reproductive Toxicology

journal homepage: www.elsevier.com/locate/reprotox

Toxic elements in hair and in vitro fertilization outcomes: A prospective cohort study

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ARTICLE INFO

Article history:
Received 13 October 2017
Received in revised form 29 January 2018
Accepted 2 February 2018
Available online 10 February 2018

Keywords:
Arsenic (As)
Cadmium (Cd)
Hair
Lead (Pb)
Mercury (Hg)
In vitro fertilization
Reproductive outcomes
Prospective cohort study

ABSTRACT

We analysed the association between the concentration of four toxic elements (As, Cd, Hg and Pb) and diverse reproductive outcomes in a cohort of 194 women with fertility disorders undergoing IVF in a public hospital. Concentration in hair specimens was explored as biomarker of exposure during the three months prior to oocyte retrieval. The proportion of negative results, especially regarding pregnancy and birth outcomes, is remarkable. However, we found that the probability of mature oocytes was inversely associated with the concentration of Hg in hair (RR = 0.81, 95% CI: 0.70-0.95) and directly associated with that of Pb (RR = 1.18, 95% CI: 1.03–1.35). These findings provide insights for future research on the links between heavy metal concentrations and IVF outcomes.

1. Introduction

Exposure to toxic elements such as arsenic (As), cadmium (Cd), mercury (Hg) or lead (Pb) is considered a major public health problem [1]. Exposure to these elements has been associated with reduction of fecundity and fertility [2–5], as well as other health disorders [6] including carcinogenicity to humans (Group 1) due to exposure to As and Cd [7,8]. Heavy metal exposure was negatively associated with poor ovarian follicular health, reduced fecundity, and adverse pregnancy outcomes in women [9]. Low intensity, long-lasting exposure (not only in relation to industrial [10] and mining [11] activities, but also through the ingestion of food and water [12,13], the use of body care products [14] and domestic chemical products, or drug use [15]), could have effect on fertility at four levels: on gonads by altering their normal functioning (acting on both oogenesis and spermatogenesis [16]), on gametes (by altering the genetic information mediated by increase in DNA fragmentation), on the endocrine system (acting as endocrine disruptors [17], able to determine low response to stimulation with gonadotropins, which could be treated by adjusting FSH doses and administering drugs with LH action), and on the body in general (increasing oxidative stress and apoptosis [18] or by altering the expression of telomerase and telomere length [19], which could determine a low ovarian reserve due to premature follicular depletion). Exposure can also result in epigenetic alterations in germ cells [20,21] with effects on embryonic development [22,23] (described, in particular, following exposure to Hg and Pb in patients undergoing in vitro fertilization [24]), which may confer susceptibility to diseases later in life[25–29]. On the other hand, the antagonistic metabolic interaction of Cd with essential metals such as selenium (Se) and zinc (Zn) is known, such elements providing protection

Abbreviations: FF, follicular fluid; IVF, In vitro fertilization; ICSI, Intracytoplasmic sperm injection; hCG, Human chorionic gonadotropin; MII, Metaphase-II; FSH, Follicle-stimulating hormone; LH, Luteinizing hormone; BMI, Body mass index; RR, Risk ratio.

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https://doi.org/10.1016/j.reprotox.2018.02.001
0890-6238/

against Cd toxicity [30–32]), as does Se with Hg [33,34], so that Se supplementation in the procreation period was suggested [35,36].

Concentrations in blood and urine are the most commonly used biomarkers to document metal and metalloid adverse health effects. In addition to the association of heavy elements with a coupleís fertility [37–44], for women undergoing in vitro fertilization (IVF) associations between blood and urine trace concentrations of Cd, Hg and Pb and reproductive outcomes were described [12,45–51]. Follicular fluid (FF) and hair samples present practical advantages as biomarkers as they provide additional information on the risk of infertility (considered as a couple of childbearing age that fail to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse [52]). Regarding IVF outcomes, the association between FF trace amounts of Pb and Cd and oocyte fertilization had been described [48,53], and between blood Pb levels and embryo cleavage [47]. However, since FF specimens are not easily accessible, the use of other biomarkers should be considered, especially when sampling is non-invasive (not posing risk to patients, able to be performed at a lower cost) [54].

Hair presents many advantages over FF, blood and urine, providing retrospective information on exposure to drugs, and it can differentiate between chronic and acute or recent consumption [55]. Also, it is easy to obtain, has considerable stability over long periods, and validated analytical procedures provide reliable results. Thus, measuring the concentrations of environmental pollutants in human hair has been widely used to assess individual exposure [56] (including the effect of toxin exposure on reproductive processes [57–59] and the analysis of subtle effects during development [60,61]) and as a biomarker of the monitoring of this exposure during critical stages of development (which could be validated through other sources of information and more specific biomarkers [62,63]), when the concentrations of these elements in hair are correlated with their internal concentrations (as described for Hg [64,65] and As [66,67]). Results published previously by our group have shown a correlation between the concentrations of four toxic elements (As, Cd, Hg, Pb) in samples of hair and FF obtained from 205 women undergoing IVF, finding that the As concentration in hair is positively correlated with observed levels of As, Hg and Pb in FF [68], which led us to consider the potential utility of hair samples to estimate concentrations in FF and for a preliminary assessment of the effect of exposure to these elements on the oocyte, embryo and foetus in women undergoing IVF in a cohort study. Previously, the concentration of elements in hair had been used to assess the degree of exposure to heavy metals [69] (in particular, in workers and residents of areas with intensive electronic waste recycling activities [70]), so as to provide primary clues for birth defect risk factor study [71], and also to evaluate the impact of heavy metal exposure on the gonadal response to hormones applied during the stimulation protocol for IVF [50].

To further explore the use of hair as a biomarker of exposure to toxic elements, the objective of this study was to explore the associations between the concentrations of four toxic elements (As, Cd, Hg, Pb) in the hair and reproductive outcomes in a cohort of women undergoing IVF.

2. Materials and methods

2.1. Study design and participants

This study was designed as a prospective cohort study. The study population consisted of women who underwent a cycle of IVF in the unit of reproductive health of the Mother and Children Hospital of Malaga, Spain. This centre serves as a reference for health care for people with sterility disorders in the province of Malaga, in accordance with the general criteria set out in the public health system of Andalusia [72]. Annually, approximately 450 cycles of IVF are performed in the clinic. The clinic reported a total oocyte fertilization rate of 63%, an implantation rate of 33% and a clinical pregnancy rate of 28.9%, with an average of 1.8 embryos transferred per patient during the study period. On the other hand, 22.1% of IVF cycles were cancelled, mainly due to an inadequate follicular response.

Between February 3, 2014 and on March 10, 2015, a consecutive sampling of all patients treated in our unit was performed (n = 450). Therefore, all patients who came with infertility and patients of 38 to 40 years of age wishing to have children without a partner, were evaluated to participate (n = 450). As part of the routine treatment, an initial evaluation of infertility in women who came for IVF treatment was carried out, including medical and reproductive history. Patients with an indication for oocyte retrieval were eligible for this study (n = 349). All patients who agreed to participate in the study (n = 216), provided informed consent during the period of cycle preparation. Participants completed a questionnaire to determine their lifestyles and basic demographic information, and agreed to provide samples of FF and hair for analysis. The collection of samples, interviews and a review of clinical histories were carried out by the authors of this study. The Provincial Research and Ethics Committee of Malaga approved the study.

2.2. Clinical protocol and collection of samples

Briefly, the patients underwent ovarian stimulation induced by gonadotropins according to the established clinical protocol. When two or more follicles exceeded 17 mm in diameter measured by transvaginal ultrasonography, human chorionic gonadotropin (hCG) was administered and oocytes were retrieved 35–37 h later. The oocytes retrieved at the maturation stage of metaphase II (MII) were fertilized mostly through intracytoplasmic sperm injection (ICSI) or in a few cases through conventional insemination, using spermatozoa from the partner or a donor obtained the day of oocyte retrieval. Approximately 16–18 h after microinjection/insemination, zygotes were identified by the appearance of two pronuclei. A single embryologist, blinded to the exposure data, examined all embryos produced. The embryo cell number was evaluated on the transfer day, and the cleavage or growth rate was characterized as a positive predictor of the success of IVF. The embryo quality was assessed between 48 and 72 h after fertilization in accordance with the consensus scoring system for the embryo cleavage stage [73]. As a predictor of IVF success, the embryo classification system was designed according to the implantation potential, resulting in three quality grades of the embryos (G1-Grade 1 or good: fragmentation <10%, stage-specific cell size, and no evidence of multinucleation; G2-Grade 2 or fair: fragmentation 10–25%, stage-specific cell size for majority of cells, and no evidence of multinucleation; and G3-Grade 3 or poor: fragmentation >25%, cell size not stage specific, and evidence of multinucleation). The embryos were transferred on the second or third day after fertilization. The pregnancy outcome was assessed using quantitative chemiluminescent serum tests for beta hCG (human chorionic gonadotropin beta subunit) using the BHCG Flex® reagent cartridge in the Siemens Dimension view 500 autoanalyzer [74], performed 14 days after embryo transfer, followed by a second test which confirmed biochemical pregnancy 2–3 days later if the first was positive (≥5 mUI/mL). Clinical pregnancy was confirmed 2 weeks after the beta hCG test by transvaginal ultrasonography showing one or more gestational sacs [52].

On the FF collection day, a trained nurse collected a sample of hair from every woman's scalp in the occipital area, using surgical stainless steel scissors, taking a lock approximately 0.5 cm in diameter (at least 100 mg) and marking the root ends and the tip of the