Rapid Communication

Correlation of Four Toxic Elements Concentrations in Hair and Follicular Fluid Collected from Women Undergoing *in vitro* Fertilization

Pedro García-Fortea, Isaac Cohen-Corcia[*]**, Alberto Reche-Rosado and Ernesto González-Mesa**

Obstetric and Gynecology Research Group, Malaga Regional and University Hospital (IBIMA), Malaga, Spain

[*]**Corresponding author:** Isaac Cohen-Corcia, Obstetric and Gynecology Research Group, Malaga Regional and University Hospital (IBIMA), C/Montes de Oca 18, piso 1, puerta 5, Málaga (CP 29007) Spain, Tel: +0034626990522; Fax +34952393768; E-mail: icohenc@gmail.es

Abstract

We assess the correlation between four trace elements concentrations (As, Cd, Hg, Pb) in hair and follicular fluid (FF) specimens collected from 205 women undergoing *in vitro* fertilization (IVF), and analysed using high resolution inductively coupled plasma-mass spectrometer. We find that hair arsenic concentration was positively correlated with FF arsenic, mercury and lead concentrations. We conclude that trace element determination in hair specimens would be of interest to clarify their effect on outcomes of women undergoing IVF in a prospective cohort study.

Keywords: Arsenic (As); Cadmium (Cd); Follicular fluid (FF); Hair; *in vitro* fertilization (IVF); Lead(Pb); Mercury (Hg)

Introduction

It has been reported that the association between persistent inorganic pollutants (such as arsenic -As-, cadmium -Cd-, mercury -Hg- and lead -Pb-) and increased oxidative stress, cell apoptosis, endocrine disruption and epigenetic damage [1]. Through these mechanisms of toxicity, such elements could adversely affect outcomes of patients undergoing *in-vitro* fertilization (IVF), which could be assessed by determining their concentrations in the body tissues. Then, controlling exposure to such toxic elements could be of interest to treat couples with reduced fertility, and increase the likelihood of success in assisted reproduction techniques.

Previous studies have conducted preliminary evaluations of the association between trace metal (Hg, Cd and Pb) concentrations measured in follicular fluid (FF), blood and urine specimens collected from women undergoing IVF, with their IVF outcomes [2,3]. Also, a method for the measurement of elements in FF at trace concentrations was developed and validated [2]. Nevertheless, those specimens would be suitable to assess for acute effects, when IVF patients could be rather exposed under long-term and low intensity conditions. On the other hand, hair is considered an accessible biological specimen of interest to study bioaccumulation of potentially toxic compounds [4], and, therefore, widely used in analysis with clinical, legal and occupational interest. In fact, it was confirmed that hair testing is suitable to verify illicit drugs consumption in couples undergoing assisted reproductive technology [5].

This is a preliminary study to assess the correlation of trace elements (As, Cd, Hg and Pb) concentrations in hair samples and FF collected from women undergoing *in vitro* fertilization, to explore their effect on IVF outcomes in a future study.

Materials and Methods

This study was designed as a cross-sectional study, prior to a subsequent prospective cohort study to assess these trace elements concentrations effect on IVF outcomes. Study population consisted of female patients undergoing an IVF cycle at the Malaga "Hospital Materno-Infantil" Center for Reproductive Health (performing annually approximately 450 1st IVF cycles). The provincial ethics and research committee approved the study.

Between February 3rd, 2014 and March 10th, 2015, a consecutive sampling was performed on all female patients assessed for eligibility. Informed consent was obtained during the pre-cycle preparation period from all study participants, providing FF and hair specimens for analysis. Of the 452 eligible patients, 205 agreed to participate and were recruited. Sample collection, interviews and the review of clinical records, were performed by the study authors.

Clinical protocol and FF samples preparation was made according to a previously detailed procedure [2]. For hair samples, approximately 0.5 cm (at least 100 mg) was collected from the occipital area of scalp roots from each woman, using stainless steel surgical scissors. Whole specimens were shipped to the Malaga University Central Services for Research Support (SCAI-UMA, Spain). This laboratory was blinded to clinical data for women. Specimens were analyzed for 111 Cd, 208 Pb, 202 Hg, and 75 As using a method optimized for an Element XR high resolution inductively coupled plasma-mass spectrometer (HR-ICP-MS, Thermo Fisher Scientific, Bremen, Germany), equipped with a Conikal Concentric Nebulizer (Thermo Fisher) and a Helix Twinnabar cyclonic spray chamber (Thermo Fisher). The HR-ICP-MS was operated according to a previously detailed procedure [2]. The correlation between each element's concentration in FF and hair were analysed by the Spearman's rho correlation statistic. The value of P<0.05 was used to judge the significance for this parameter. The statistical analysis was performed with the SPSS (Statistical Packages for Social Sciences, IBM SPSS, Armonk-NY USA) 15.0 software package for Windows.

Results

Table 1 shows the distributions for measured elements in follicular fluid and hair specimens. Correlations amongst elements concentrations in FF and hair are presented in Table 2. Statistically significant correlations have been detected for hair As and FF As

(rho=0.326, P<0.0001), FF Hg (rho=0.371, P<0.0001), FF Pb (rho=0.342, P<0.0001) and hair Cd (rho=0.164, P=0.049), and also for FF As and FF Cd (rho=0.503, P<0.0001), FF Hg (rho=0.389, P<0.0001), and FF Pb (rho=0.176, P=0.022). Finally, FF Hg was correlated with FF Pb (rho=0.354, P<0.0001), and hair Cd with hair Pb (rho=0.604, P<0.0001).

Metals	n	LOD	%>LOD	Mean	SD	Min.	P25	Median	P75	Max.
Follicular fluid As (µg/g)	205	0.003	97.1%	0.072	0.054	0.000	0.038	0.054	0.090	0.301
Follicular fluid Cd (ng/g)	205	0.04	68.8%	0.650	1.000	0.000	0.004	0.338	0.921	8.819
Follicular fluid Hg (µg/g)	205	0.005	47.3%	0.008	0.046	0.000	0.000	0.005	0.008	0.664
Follicular fluid Pb (µg/g)	205	0.001	68.8%	0.017	0.057	0.000	0.001	0.004	0.012	0.602
Hair As (µg/g)	205	0.02	39.0%	0.104	0.299	0.000	0.000	0.013	0.048	2.418
Hair Cd (µg/g)	205	0.04	96.6%	0.073	0.295	0.000	0.012	0.026	0.061	3.778
Hair Hg (µg/g)	205	0.09	95.1%	1.231	1.171	0.000	0.552	0.911	1.567	8.577
Hair Pb (µg/g)	205	0.008	96.6%	2.014	4.286	0.000	0.407	0.900	1.945	36.414

Table 1: Distribution of elements concentrations measured in follicular fluid and hair from *in vitro* fertilization patients on the day of oocyte collection (LOD: Limit of Detection; %>LOD: Percentage above the LOD; SD: Standard Deviation; Min: Minimum Value; P25: 25th Percentile; P75: 75th Percentile; Max: Maximum Value).

Discussion

So far, no correlation was described between trace elements concentrations in hair and FF samples. We found that hair as concentration was positively correlated with FF As, Hg and Pb concentrations. If subsequent studies confirm arsenic effect on IVF outcomes, its hair concentration could be considered as a prognostic biomarker in patients undergoing IVF.

	FF As	FF Cd	FF Hg	FF Pb	Hair As	Hair Cd	Hair Hg	Hair Pb
FF As	-	0.517	0.453	0.307	0.404	0.039	0.144	-0.065
	-	(<0.0001)	(<0.0001)	(<0.0001)	(<0.0001)	(0.579)	(0.04)	(0.356)
FF Cd		-	0.393	0.296	0.017	0.022	0.024	-0.064
		-	(<0.0001)	(<0.0001)	(0.803)	(0.759)	(0.729)	(0.364)
FF Hg			-	0.504	0.355	0.062	0.223	0.032
			-	(<0.0001)	(<0.0001)	(0.378)	(0.001)	(0.647)
FF Pb				-	0.336	-0.015	0.009	-0.081
				-	(<0.0001)	(0.826)	(0.898)	(0.251)
Hair As					-	0.061	0.140	-0.080
					-	(0.385)	(0.045)	(0.905)
Hair Cd						-	0.054	0.632
						-	(0.445)	(<0.0001)
Hair Hg							-	0.185
							-	(0.008)
Hair Pb								-
FF As								-

Table 2: Correlation (P-value) among elements concentrations in follicular fluid (FF) and hair in 202 *in vitro* fertilization (IVF) patients (P<0.05 in bold type).

Our sample was representative for the Spanish population with fertility disorders. Our centre is part of the national health system and reference for healthcare to this pathology in Malaga province (Spain) by the general criteria defined in Andalusia [6]. Of the 452 eligible patients, 247 were excluded according to the criteria in the referred prospective cohort study to assess these trace element concentrations effect on IVF outcomes, due to the following reasons: clinical factors n=101, refused to participate n=122, the follicular pick-up was cancelled n=13, or follicular fluid was blood stained n=11. This non-recruited group was matched to the study sample in terms of demographic and clinical factors, but not in terms of primary infertility diagnosis and response to ovarian stimulation, although no association was observed between both variables and trace element concentrations. The proportion of those with diminished ovarian reserve was lower in recruited cases than in excluded cases (Chi^2=11.953, 4 g.l. p= 0.018), and the ovarian response was lower in excluded cases than in recruited cases (Chi^2=15.773, 2 g.l. p= 0.0004).

Conclusions

A direct correlation exists between hair As concentration and FF As, Hg and Pb concentrations. Compared with FF, hair specimens are easily accessible and show long-term and low intensity exposition. Then, we conclude that trace elements determination in hair specimens would be of interest to clarify their effect on outcomes of women undergoing IVF in a prospective cohort study.

Acknowledgements

References

1. Anderson K, Nisenblat V, Norman R (2010) Lifestyle factors in people seeking infertility treatment - A review. Aust N Z J Obstet Gynaecol 50: 8-20.

2. Bloom MS, Kim K, Kruger PC, Parsons PJ, Arnason JG, et al. (2012) Associations between toxic metals in follicular fluid and *in vitro* fertilization (IVF) outcomes. J Assist Reprod Genet 29:1369-1379.

3. Al-Saleh I, Coskun S, Mashhour A, Shinwari N, El-Doush I, et al. (2008) Exposure to heavy metals (lead, cadmium and mercury) and its effect on the outcome of in-vitro fertilization treatment. Int J Hyg Environ Health 211:560-579.

4. Gil F, Hernández AF, Márquez C, Femia P, Olmedo P, et al. (2011) Biomonitorization of cadmium, chromium, manganese, nickel and lead in whole blood, urine, axillary hair and saliva in an occupationally exposed population. Sci Total Environ 409:1172-1180.

5. Pichini S, De Luca R, Pellegrini M, Marchei E, Rotolo MC, et al. (2012) Hair and urine testing to assess drugs of abuse consumption in couples undergoing assisted reproductive technology (ART). Forensic Sci Int 218: 57-61.

6. Guía de Reproducción Humana Asistida en el Sistema Sanitario Público de Andalucía (2015) Sevilla (Spain): Dirección General de Asistencia sanitaria y Resultados, Servicio Andaluz de Salud.