

**Heavy metals analysis in human follicular fluid
from patients undergoing IVF.**

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The purpose of this study was first to establish a suitable method to detect heavy metal HM found either in body fluids (bioaccumulation) or as exogenous toxicants added to granulosa cells or human spermatozoa cultures (in vitro cytotoxicity tests).

For the first time HM like cadmium, lead, chromium and nickel were measured in human follicular fluid (FF) collected from 20 patients undergoing IVF treatment at the University Women's hospital in Tübingen, Germany.

For analyses we used a Perkin-Elmer 1100B atomic absorption spectrometer with an HGA 500 electrothermal atomiser. For all experiments we used pyrolytic graphite coated tubes and solid stabilized temperature platform furnace (STPF) .The STPF concept was then consequently adapted and performed for the follicle fluid problematic.

Among the 20 patients randomly selected for HM analysis, no particular bioaccumulation of cadmium and lead could be assessed in FF comparison to standard blood concentrations (**Cd** 0,45 µg/l vs 0,7 µg/l; **Pb** 12,26 µg/l vs. 81,5 µg/l).

However for all FF samples, the mean concentration of **chromium** was more than **300 times higher** than in blood, serum, or plasma (75,8µg/l vs 0,23µg/l). The mean concentration for nickel was also 20 times higher than in blood (39,7µg/l vs 2,0 µg/l) and 40 times higher than in serum or plasma (39,7µg/l vs 1,0 µg/l).

As we suspected an exogenous contamination is possibly originating from the metallic puncture cannula (stainless steel). We could recover by washing out the cannula the same high observed concentrations of chromium and **nickel**.These concentrations were progressively decreasing with the different washing steps (from 2300µg/l to 31 µg/l in 5 washing steps each 2ml volume).

Our data clearly show that heavy metals can be detected with atomic absorption spectrometry using an adapted STPF concept.

The high detected concentrations of chromium and nickel were probably due to exogenous contamination caused by the puncture cannula. A protocol for washing out the cannula and reducing the contamination to the basal levels before each follicular puncture is proposed.

Further investigations concerning analysis of molybdenum, vanadium and iron are now undertaken to confirm this hypothesis. Moreover cytotoxicity of chromium and nickel acting in synergism or not is under current investigations using alternative in vitro cytotoxicity assay (see abstract U.Vallon et al. ESHRE 95)