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24, 25(OH)2D3: A NOVEL ACCURATE INDICATOR OF OVARIAN VITAMIN D STATUS IN WOMEN AT REPRODUCTIVE AGE.

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OBJECTIVE: The impact of vitamin D in reproductive outcomes is still an unresolved issue, mainly because of lack of accuracy measuring vitamin D metabolites. 24,25-dihydroxyvitamin D (24,25 (OH)2D) is the main product of the catabolism of 25-hydroxyvitamin D (25(OH)D), so it has been proposed as an effective systemic indicator of vitamin status D. However, its presence and impact at reproductive level, especially in the follicular fluid, has not been studied yet. We aimed to determinate the concentrations and correlations of 24,25(OH)2D3 with other vitamin D metabolites in serum (S) and follicular fluid (FF) from mature oocytes, in order to evaluate its potential implication in the ovarian physiology.

DESIGN: Prospective, non-interventional study.

MATERIALS AND METHODS: Thirty-five egg donors were included in the study. Following controlled ovarian hyperstimulation using an antagonist protocol and standard doses of subcutaneous FSH, oocytes retrieval was done 36 hours after a bolus of GnRH agonist. S samples and pooled FF from mature follicles were obtained. 24,25(OH)2D3, 25(OH)D3 and 1,25(OH)2D3 concentrations were measured through liquid chromatography- tandem mass spectrometry (LC-MS/MS) using a UPLC -TQ-S Xevo Waters system with a Waters Acquity BEH C18 (1,7mm 2,1 x 100mm) column. A paired design was implemented in all statistical approaches performed. Pearson correlation test was used to evaluate S and FF vitamin D metabolites correlations. Mean differences between each vitamin D metabolite in S and FF were evaluated using Wilcoxon test.

RESULTS: Mean values for 25(OH)D3 concentrations were 91.56_39.01 nM in S, and 58.12_19.54 nM in FF. For 24,25(OH)2D3 the mean values in S concentrations were 156.16_109.96 nM, and 112.63_60.86 nM in FF. 1,25(OH)2D3 was not detected in any sample. 24,25(OH)2D3 and 25(OH) D3 concentrations in S and FF showed to be highly correlated ($r=0.92$, $p\text{-value}=2.713e-14$ for S and $r=0.91$, $p\text{-value}=5.031e-14$ for FF). After analyzing each metabolite separately, the strongest correlation between S and FF concentrations was observed for 24,25(OH)2D3 ($r=0.77$, $p\text{-value}=1.121e-07$), followed by 25(OH)D3 ($r=0.69$, $p\text{-value}=6.902e-06$).

CONCLUSIONS: This is the first study evaluating 24,25(OH)2D3 concentrations and correlations with other vitamin D indicators in FF and S. Our results suggest 24,25(OH)2D3 as an accurate indicator of vitamin D status in the ovary, and

represents an important step in understanding the specific role of vitamin D in the ovarian physiology and reproductive outcomes. Supported by: IVI RMA Fundaci_ on IVI.