**Title:**
Association between Serum and Follicular Fluid 25- Hydroxy Vitamin levels and Pregnancy Rate in Women Undergoing IVF/ICSI

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**Key Words:**
Vitamin D, 25-hydroxy vitamin D, IVF, ICSI, pregnancy rate

**Study Question:** Is there any association between follicular fluid or serum 25-hydroxy vitamin D (25OH-D) levels and pregnancy rates in women undergoing IVF / ICSI cycles?

**Summary Answer:** Follicular fluid (25OH-D) levels <30ng/ml is associated with diminished pregnancy rates in the patients undergoing IVF/ICSI cycles. Serum (25-OHD) status is unrelated to pregnancy outcomes.

**What is known already?** According to current studies, an epidemic Vitamin D deficiency has been deemed among most of ethnic groups in the world. The presence of vitamin D receptor in female reproductive tissues suggests the regulatory roles in reproductive system. The recent evidence indicates the potential effects on ovarian function, endometrial receptivity, and embryo quality. The results regarding the effect of vitamin D on clinical outcomes in assisted reproductive technologies are conflicting.

**Study design, size, and duration:** This was a prospective cohort study on 160 subfertile couples in an academic tertiary care center (IVF Unit, Yas Hospital, Tehran University of Medical Sciences, Tehran, Iran) between March 2015 and March 2016.
**Participants, material, setting, methods:** The study included 160 subfertile women younger than 40 years old and undergoing IVF / ICSI cycles. Serum samples were collected on triggering days. The follicular fluids were collected on oocyte retrieval days. Vitamin D status was measured by assessing 25OH-D levels in using Enzyme-linked immunoassay (ELISA, Accu-bind, Monobind Inc, Lake Forest, USA). Vitamin D insufficiency was defined as Serum 25OH-D<30ng/ml. Follicular fluid concentration of 30ng/ml was as cut off point for study evaluation.

**Main Results:** Among all patients, 28.8% (46/160) were vitamin D insufficient whereas 71.3 % (114/160) had normal vitamin D levels. In term of follicular fluid levels, 24.8 % (40/160) were less than 30 ng/ml and 75.1% (120/160) were equal or more than 30 ng/ml. The data regarding to baseline characteristics including age, parity, type and cause of infertility, stimulation protocol, endometrial thickness, and number of transferred embryos were similar between women with different serum and follicular fluid 25OH-D levels. The chemical and clinical pregnancy rates were detected in 49 (30.6%) and 39(24.4%) women, respectively. The clinical pregnancy rate was lower among those women who had follicular fluid <30ng/ml, compared with those women with follicular fluid ≥ 30 ng/ml (12.5% vs. 28.3%, respectively, ρ= 0.04). The chemical pregnancy rates also varied by follicular fluid 25OH-D concentration being lower in cases with follicular fluid 25OH-D <30ng/ml and higher in cases with follicular fluid ≥30ng/ml (17.5% vs. 35%, respectively, ρ= 0.03). No statistically significant differences in chemical and clinical pregnancy rates were detected in women with normal serum vitamin D and the women with insufficient levels (28.8%, 21.7% vs. 32.3%, 25.4%, ρ= 0.23, ρ=0.14, respectively).

**Limitation, reasons for caution:** The sample collections were obtained on the days of ovulatory triggering and ovum pick up during the stimulated cycles. The effects of nonphysiological hormonal milieu (particularly high estrogenic hormones level) induced by exogenous gonadotropins on serum and follicular fluid vitamin D levels might be a confounding factor.

Wider implications of the findings:
The study has not evaluated mechanism by which follicular fluid vitamin D impacts on cycle outcomes. It might be attributed to the effect of vitamin D at level of ovarian functions and/or oocyte/embryo quality. Further study designs are needed to elucidate the potential underling mechanisms, relevant factors, and therapeutic implications.

**Competing interest:** The authors report no conflict of interest